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Abstract about this Special Issue

This special issue is highly related to Microbiology and Bioinformatics application for pharmacology and its usage in medical and health care systems towards providing leads for molecular development and an initiative in Basic science to perform advanced research in areas like the virtual patient and CRISPER Human. Also, it focuses on the integration of machine learning algorithms to mankind. In addition to the basic science involved in Microbiology, Bioinformatics and Pharmacology, the advancements in the current stage of applying the schemas of artificial intelligence (AI) in basic sciences are also focused in this issue. Artificial intelligence in the diagnosis of health care is the requirement for the current era of Digital Genomics. This special issue focuses on both basic science and advanced developments in the current era of Digital Genomics. The articles published in this special issue will certainly bring a positive effect for the development of health care systems and scientific leads to develop molecules with the available resources enhance the maximum utilization of scientific knowledge to potentiate diagnosis and therapy in health care.

CONTENT

SP-1

RANDOM FOREST ALGORITHM FOR PREDICTING CHRONIC DIABETES DISEASE

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ABSTRACT

The Random Forest Algorithm is an Ensemble learning method for classification or regression problems. It can be used for building predictive problems. In recent years, Chronic Diabetes Disease is keeping on increasing. There are many reasons like changes in our life style, our food habits. It roots an increase in blood sugar levels. If Diabetes Disease remains untreated or unidentified many different types of other problems may be happened. The doctors are difficult to identify these kinds of diseases easily. The machine learning algorithms support the doctor to solve these types of problems. In this paper, we implemented the Random Forest algorithm to predict diabetes disease at an early stage. Experiments are performed using the R tool on Pima Indians Diabetes Dataset which is from the UCI machine learning repository. The performance of the algorithm is evaluated using measures on Accuracy. Results obtained in Random Forest Algorithm displays accuracy is 80.08%.

KEYWORDS*: Diabetes, Random Forest, Machine learning, Classification*

I. INTRODUCTION

Diabetes is a type of disease in which our blood glucose (sugar) level is too high. The glucose comes from the food that we eat. Insulin is a hormone, it helps the glucose to reach our cells and give energy. There are two types of diabetes. One is Type 1 and another one is Type 2. In Type 1, the body does not make insulin. In type 2, the body does not use insulin well. Type 2 is a common one. Without enough insulin, the glucose remains stays in blood. If it stays in our blood, it causes many problems like damage in the eyes, kidneys and also causes heart disease. Pregnant women also affect diabetes disease called gestational diabetes. Exercise, weight control and meal plan can control diabetes.Chronic diabetes disease is called a silent killer. It makes the body produce less insulin level and causes increased blood sugar, which leads to many difficulties and disturbs the normal operation of many organs, such as eyes, kidneys, and nerves. In recent years diabetes disease prediction has concerned high attention in research[1.] Diabetes is a serious threat to health development because diabetes is a disease that caused most other diseases (complications). Diabetic retinopathy is the greatest important manifestation of diabetic micro angiopathy and is also one of the most common complications in people with diabetes[2].Classification algorithms are applied mostly in the medical field for classifying data into a different number of classes based on constraints.Diabetes is one of the illnesses which affect the body in producing the insulin and levels of glucose in the blood. Intensify hunger, frequent urination is some symptoms for diabetes. It caused due to high blood pressure. Many problems occur if diabetes disease remains untreated. Diabetic keto acidosis and non ketotic hyperosmolar coma are the most complications include in diabetes[3].If sugar substance cannot be controlled means Diabetes is analyzed seriously. Diabetes disease is affected due to specific factors like height, weight, hereditary and insulin but the major factor is considered is sugar absorption among all these factors. The initial forecast of chronic disease is only one method to recover from these problems[4]. A maximum number of Researchers are conducting tests for diagnosing chronic diabetes diseases. There are using different kinds of classification algorithms of machine learning approaches. They used their experiments like SVM, KNN, Decision tree, Naivebayes, etc. They proved that the machine learning algorithms work superior for diagnosing different kinds of diseases[5-7]. Data mining and machine learning algorithms increase in power. They are handling the bulk of the amount of data to combine data from dissimilar sources and mixing the information[8].In this research, works focus on the Random Forest algorithm. This algorithm is used and evaluated on Pima Indians Diabetes Dataset to predict the diabetes disease. The performance of the algorithm is discussed and

achieved good accuracy.

II. RELATEDWORK

Xiang Gao[9]et al implemented the Random Forest algorithm for predicting Employee Turnover. In this method, they offer a new analytic method that can help human resource departments predict employee turnover more accurately and its experimental results provide further insights to reduce employee turnover intention. They received 92.65% as Accuracy value. Katherine Ellis et al[10] implemented a random forest classifier for the prediction of energy expenditure and type of physical activity from wrist and hip accelerometers.A random forest classifier is used to predict activity type and a random forest of regression trees to estimate METs. Predictions were evaluated using leave-one-user-out cross-validation. The hip accelerometer acquired an average accuracy of 92.3% in predicting four types of activity. They are household, stairs, walking, and running. The wrist accelerometer got an average accuracy of 87.5%. Through all eight activities are combined laundry, window washing, dusting, dishes, sweeping, stairs, walking, running. The hip and wrist accelerometers got average accuracies of 70.2% and 80.2% respectively[10].

Harsh Valecha[11] et al used Random forest algorithm for predicting consumer behavior. Consumer behaviour in this competitive world as trends is unstable. In this, the author examines the relationship between consumer behaviour parameters and willingness to buy. First we examine to find the relationship between consumer behaviour to buy products on changing parameters such as environmental factor, organizational factor, individual factor and interpersonal factor. In this paper, the authors suggest that a time-evolving random forest classifier is best to forecast the behaviour of consumer. It helps that interrupts the choice of purchasing the product. Results of random forest classifiers are more accurate than other types of machine learning algorithms. S. Karthick et al mentioned one of the greatest challenges that the meteorological department faces are to predict the weather accurately. The predictions mentioned here are important as they influence daily life and also affect the economy of a state or even a nation.

Weather predictions are also essential since they form the first level of preparation against natural disasters which may make a difference between life and death. It reduces the loss of resources and minimizes the mitigation steps which are expected to be taken after a natural disaster happens. This work focuses on analyzing algorithms that are suitable for weather prediction and highlights the performance analysis of C4.5 with Random Forest algorithms. After a comparison between the data mining algorithms and corresponding ensemble technique used to boost the performance, a classifier is obtained that will be further used to predict the weather[12].

III. METHODOLOGY USED

A. Model Diagram

We build a Random Forest (RF) model to predict chronic diabetes disease. Figure.1 explains the series of the process involved in this Random Forest model.

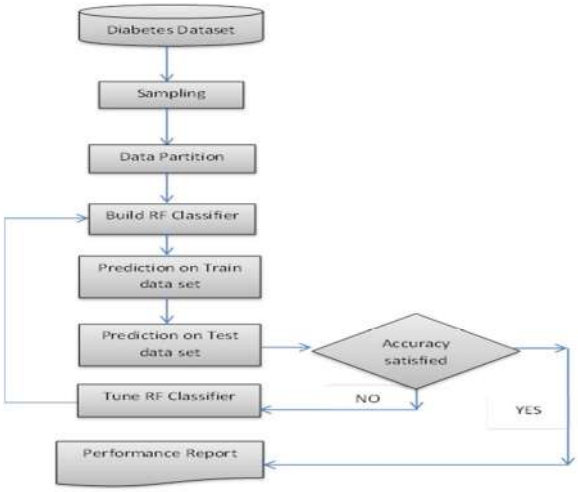

Figure 1: Proposed Model Diagram

B. Brief Description of Algorithm Used

Random Forest

The Random Forest tree is developed by aggregating trees. It can be used for solving classification or regression problems. If y variable is factor value applies the classification or y value is continuous apply the regression. This algorithm avoids fitting. It can be used in the situation to handle a large number of features in the dataset. The feature selection is done on the importance of the feature value. It is very user-friendly method. It has only two free parameters. One is known as Trees that are called ntree. By default ntree value is 500. The second parameter is the mtry. Here variables are randomly sampled as candidates at each split. The default value for mtry is for classification sq.root(p) and regression p/3. p represents a number of features or number of variables.

The Random Forest algorithm was performed by three steps. They are

1. Find ntree bootstrap samples.
2. For each bootstrap sample, grow aun-pruned tree by choosing the best split based on a random sample of mtry predictors at each node.
3. Predict new data using the majority of votes for classification and average for regression based on ntree trees.

In Random Forest algorithm Out Of Bag (OOB) is calculated. For each bootstrap iteration and related tree prediction error using data, not in the bootstrap sample (also called Out Of Bag or OOB) is estimated.

C. Dataset Used

The Diabetes dataset has 768 instances and 9 different types of features. It is taken from the Pima Indian diabetes Dataset from UCI Machine Laboratory. It is originally taken from the National Institute of Diabetes and Digestive and Kidney Diseases. The main aim of the dataset is to predict whether or not a patient has diabetes, based on some diagnostic measurements comprised in the dataset. The dataset has 768 instances and 9 features.

The proposed methodology is assessed on Diabetes Dataset namely (PIDD) [13], which is taken from the UCI Repository. This dataset which are feminine patients. The dataset also contains numeric-valued 9 attributes where the value of one class '0' preserved as tested negative for diabetes and value of another class '1' is preserved as tested positive for diabetes. The Dataset description is defined in Table-2.In this experiment, 70% (532) of the data from Pima Indians Diabetes Dataset(PIDD) used for the training process and 30% (236) of the data used for the testing process.

Table 2. Attribute Description

ID	Feature Name	Description
V1	Pregnancies	Number of times pregnant
V2	Glucose	Plasma glucose concentration a 2 hours in an oral glucose tolerance test
V3	Blood Pressure	Diastolic blood pressure (mm Hg)
V4	SkinThickness	Triceps skinfold thickness (mm)
V5	Insulin	2-Hour serum insulin (mu U/ml)
V6	BMI	Body mass index (weight in kg/(height in m)^2)
V7	Diabetes PedigreeFunction	Diabetes pedigree function
V8	Age	Age (years)
V9	Outcome	Class variable (0 or 1) 268 of 768 are 1, the others are 0

IV. RESULTS AND DISCUSSION

The main goal of this work is the prediction of the patient affected by diabetes using the R tool by using the medical database PIDD. In this work, R tool is used for experimenting. R is free software with a collaborative project with many contributors. It includes a collection of various machine learning techniques for data classification, clustering, regression, visualization, etc.In the first step we read the dataset then view the dataset values. Then saw the structure of the data set. It displays the following values.

'data.frame': 768 obs. of 9 variables:$ V1: int 6 1 8 1 0 5 3 10 2 8 ...$ V2: int 148 85 183 89 137 116 78 115 ...$ V3: int 72 66 64 66 40 74 50 0 70 96 ...$ V4: int 35 29 0 23 35 0 32 0 45 0 ...$ V5: int 0 0 0 94 168 0 88 0 543 0 ...$ V6: num 33.6 26.6 23. 3 28.1 43.1 25.6 ...$ V7: num 0.627 0.351 0.672 0.167 2.288 ...$ V8: int 50 31 32 21 33 30 26 29 53 54 ...$ V9: Factor w/ 2 levels "no","yes": 2 1 2...

Here in V9 feature is the factor variable. "1" represents the first level called "No" that is not affected by the disease , "2" represents the second level called "Yes" that is patient is affected by the diabetes disease. From Table 3, dataset shows that 268 patients affected by the diabetes disease and 500 patients did not have diabetes disease. In the next step, the dataset is partitioned in two sets that are train data set and test dataset. The dataset is divided into 70% of the dataset is called the train dataset and 30% of the data set is called the test data set. In this, the training dataset contains 532 data are train dataset and 236 data are test dataset.Then build the random forest classifier model. If print the random forest model the following result will be displayed.Call:randomForest(formula = V9 ~ ., data = train) . Type of random forest: classificationNumber of trees: 500➔➔ntree No. of variables tried at each split: 2➔➔mtry OOB estimate of error rate: 24.67% Confusion matrix: no yesclass.error no 250 46 0.1554054 yes 67 95 0.4135802. In the next step prediction is performed on the training data set using random forest model. The confusion matrix for train data set will be displayed as follows:Confusion Matrix and Statistics: Reference-Prediction noyesno296 0yes 0 162Accuracy: 195% CI : (0.992, 1)No Information Rate : 0.6463P-Value [Acc> NIR] : < 2.2e-16 Kappa: 1 Mcnemar's Test P-Value: NASensitivity: 1.0000Specificity: 1.0000PosPredValue: 1.0000NegPredValue: 1.0000Prevalence: 0.6463Detection Rate: 0.6463 Detection Prevalence: 0.6463 Balanced Accuracy: 1.0000 'Positive' Class: no. For training data set 100% prediction accuracy value is obtained. After that prediction is performed on the testing dataset using a random forest classifier. The confusion matrix is displayed as follows for the testing dataset, the Confusion Matrix and Statistics: Reference-Prediction no yesno170 37yes34 69Accuracy: 0.771 95% CI: (0.7201, 0.8166)No Information Rate: 0.6581P-Value [Acc> NIR]: 1.016e-05 Kappa: 0.4876 Mcnemar's Test P-Value: 0.8124 Sensitivity: 0.8333Specificity: 0.6509PosPredValue: 0.8213NegPredValue: 0.6699 Prevalence: 0.6581 Detection Rate: 0.5484Detection Prevalence: 0.6677Balanced Accuracy: 0.7421 'Positive' Class: no. For the testing dataset, we got 77.1% of accuracy value. So the Out Of Bag error is plotted in Figure.2.

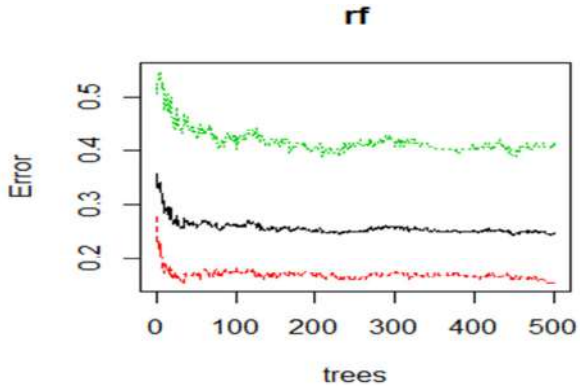

Figure 2: OOB Error

As number trees grow the OOB error rate will decrease initially after that more or less constant. So here, it is not able to improve the error after 300 trees. In the next step, the random forest value will be tuned.So mtry value is 3. Then again build the random forest model using ntree=300 and mtry=3.Then, Call:randomForest(formula = V9 ~ ., data = train, ntree = 300, mtry = 3,importance = TRUE, proximity = TRUE), Type of random forest: classification, Number of trees: 300, No. of variables tried at each split: 3OOB estimate of error rate: 22.27%.

So, Confusion matrix:no yes class.errorno 249 47 0.1587838yes 55 107 0.3395062.Now the error rate will be minimized from 24.67% to 22.27%. Again run the training dataset using tuned Random forest model. We got an accuracy is100%. Then run the test dataset using the tuned RF model. We received an 80.08% accuracy value.

So the Confusion Matrix and Statistics is ReferencePrediction no yesno 128 23yes 24 61Accuracy: 0.8008 95% CI: (0.7441, 0.8498)No Information Rate: 0.6441 P-Value [Acc> NIR] : 1.078e-07 Kappa : 0.5668 Mcnemar's Test P-Value : 1Sensitivity : 0.8421 Specificity : 0.7262 PosPredValue : 0.8477NegPredValue : 0.7176Prevalence : 0.6441Detection Rate : 0.5424Detection Prevalence : 0.6398Balanced Accuracy : 0.7841

'Positive' Class : no . Then the importance of features is plotted.

V. CONCLUSION

In early stage, disease prediction is more important in real world. It is the main problem. In this work, we put into efforts to modeling a system that result in the prediction of diabetes disease. During this work, the Random Forest machine learning classification algorithm is studied and evaluated on various measures. Experiments are performed on the Pima Indians Diabetes Database. Experimental results determine the adequacy of the designed system with an achieved accuracy of 80.08 % using the Random Forest classification algorithm. In the future, the designed system with the used machine learning classification algorithms can be used to predict or diagnose other diseases. The work can be extended and improved for the automation of diabetes analysis including some other machine learning algorithms.

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SP-2

CLOSED SEQUENTIAL PATTERN MINING IN BIOLOGICAL DATA

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Abstract: Sequential pattern mining is a technique which efficiently determines the closed patterns from very large datasets. The traditional sequential pattern mining algorithms can mine short sequences efficiently but these algorithms are inefficient for mining long sequences. The biological data DNA sequences have long sequences with small alphabets and long sequences. These biological data can be mined for finding the co-occurring biological sequence. These co-occurring sequences are important for biological data analysis and data mining. To mine long sequences efficiently closed sequential pattern mining is used. The mined patterns have less number of closed sequences. Most of the algorithms are based on candidate generation method which leads to more search space and greater running time. This paper proposes an efficient Closed Sequential Pattern Mining (CSPM) algorithm for mining closed sequential patterns. The CSPM algorithm mines closed patterns without candidate generation. This algorithm uses two pruning methods namely, BackScan pruning, and frequent occurrence check methods. The former method prunes the search space and latter detects the closed sequential pattern in early run time. The proposed algorithm is compared with PrefixSpan algorithm and better scalability and interpretability is achieved. The experimental results are based on DNA datasets which outperform the other algorithms in terms of efficiency, memory and running time.

KEYWORDS*: S*equential pattern mining, DNA, closed sequential patterns, prefixspan, CSPM

I. INTRODUCTION

Sequential pattern mining is a technique which identifies the interesting complete set of sub-sequences from huge dataset[1] The Sequential pattern is a sequence of item sets that frequently occur in a specific order; all sequence items in the datasets are based on time value operation or within a time gap. Each large sequence corresponds to a temporary ordered list of events, where every event is a collection of items occurring simultaneously. The temporary ordering of the list of events is induced among the entire timestamps associated with the events[8]. Usually, the customer transaction events are viewed together as an interesting sequence, called customer-sequence, where each customer transaction is expressed as an itemset in that sequence and all the customer transactions are listed in an ordered list with respect to the transaction-time[6].

Closed sequential pattern mining is related to sequential mining and closed mining techniques. Sequential pattern mining was first introduced by R. Agrawal and R. Srikanth[1], and it has become an important data mining task. Vast sequential algorithms were proposed for pattern mining, the efficient algorithms are SPADE[3], PrefixSpan[4] and SPAM[5]. SPADE algorithm uses breadth-first search process whereas PrefixSpan and SPAM algorithms use depth-first search method. SPADE algorithm implements a vertical data format method and mines the sequential patterns using simple join. PrefixSpan algorithm implements a horizontal data format method and mines the sequential patterns using the pattern growth method. SPAM algorithm mines sequential patterns using vertical bitmap representation method and it outperforms PrefixSpan and SPADE algorithms on large datasets. However, SPAM algorithm requires more memory than PrefixSpan and SPADE methods.

Closed sequential pattern mining was first proposed by X.Yan et al.[2] to overcome the limitations of sequential pattern mining algorithms. The closed multidimensional sequential pattern mining is the combination of closed itemset pattern mining and closed sequential pattern mining. This method can mine

more useful information than the sequential pattern. The closed sequential patterns can be mined in two ways,

1. The first step is to find final closed sequential patterns and ,
2. The second step is to find the closed sequential pattern set and post-prune it.

The author's contributions can be summarized as follows:

1. Introduced the problem of mining closed sequential patterns in biological sequence.
2. Proposed an efficient mining algorithm CSPM which mines closed sequential patterns without candidate generation.
3. Proposed two pruning methods namely BackScan method and frequent occurrences method to prune search space.
4. The varied minimum support threshold increased the efficiency.
5. The efficiency of CSPM algorithm is evaluated with two pruning methods which outperform in terms of memory and running time.

The rest of this paper is structured as follows. In Section 2, the related works of sequential mining algorithms are given. In Section 3, problem definition is discussed. In Section 4, the extension of closed sequential pattern mining is explained in detail. In Section 5, performance evaluation report is discussed. Finally, we conclude the work in Section 6.

II. RELATEDWORKS

Many sequential mining algorithms have been proposed in recent years, these approaches cover various data mining problems. In general two research problems are concerned in mining sequential patterns.

1. *Improve the efficiency of the mining process*[9]*:* This mining process mainly focuses on improving the efficiency of sequential patterns.
2. *Extracting the time-related patterns using the mining process*[10]*:* This pattern extraction method can find other time-related patterns from various databases such as weblog patterns, cyclic patterns etc for finding frequent patterns in time-related databases.

III. PROBLEM STATEMENT

In this section, some preliminary concepts are introduced and then closed sequential pattern mining problem is formalized. Let A ={a_1,a_2,….,a_m} be a set of all alphabets. A subset of A is called an alphabet item set. A sequence S=(S_1,S_2, …, S_n) ($S_i \subseteq$ A) is an ordered list of sequence item sets. The items in each sequence itemset are sorted in ordered list. The sequence item set length is defined as the total number of the item et in the given sequence. A sequence alphabet itemset SI_1 =(X_1,X_2,…..,X_m) is a subsequence of another sequence item set SI_2 =(Y_1,Y2,….,Y_n), denoted as $SI_1 \sqsubseteq SI_2$, if there exists integers $1 \leq a_1 < a_2 < \ldots < a_m \leq n$ and $X_1 \subseteq Y_{i1}$, $X_2 \subseteq Y_{i2}$, . . . , and $X_m \subseteq Y_{im}$. SI_2 represent a super-sequence of SI_1 and SI_2 contains SI_1.

A sequence database, SDB={SI_1,SI_2,…,SI_n}, is a set of sequences and each sequence has an id. The size, |SDB|, of the sequence database SDB is the total number of sequences in the SDB. The support of a sequence X in a sequence database SDB is the no of sequences in SDB which contain X.

(a) Definition-1 (Sequential Patterns): A sequence is an ordered list of itemsets. Given a minimum support threshold min_sup, a sequence α is a sequential pattern on sequence database only if support (α) is greater than min_sup.

(b) Definition-2 (Closed Sequential Patterns): A sequential pattern α is a closed sequential pattern if there does not exist a sequential pattern β , such that support (α) = support (β) and α ⊏ β.

Table I.Sequence Database Example

Sequence ID	Sequence data
Sequence X	a,c,a,a,g,a,t,g,c,c
Sequence Y	g,c,c,g,a,g,a,c,a,g
Sequence Z	c,t,c,c,t,g,c,a,a,a

Table I represents sequence database example which contains sequenceID and sequence data. The closed sequential pattern mining is used to mine the complete set of closed patterns which satisfies the given minimum support value min_sup for a given input sequence item set. The table represents the sample sequence database with sequence ID and sequence data.

IV. PROPOSED METHOD

In this section, the proposed algorithm CSPM is described for mining closed sequences. This algorithm uses the depth first search technique for mining closed sequential patterns. The breadth-first search technique is mostly used but they are inefficient for mining closed sequence. Closed pattern mining is used to overcome the limitations of sequential pattern mining. The closed sequential patterns can be mined in two different ways namely:

1. Finding the closed sequential patterns without verifying the discovered patterns.
2. Finding candidate set for the closed sequential pattern and post-pruning it.
3.

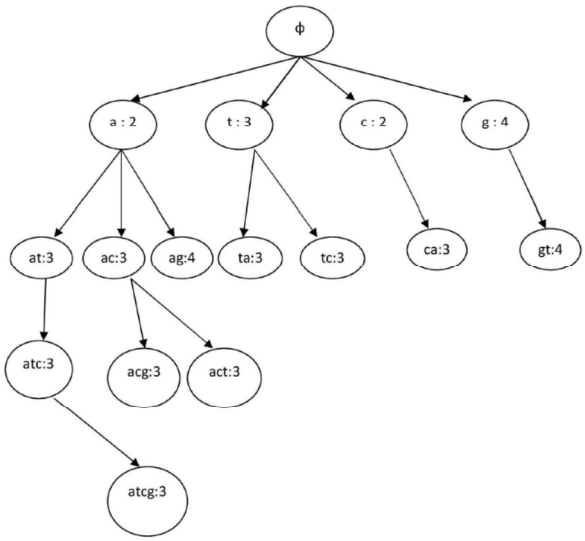

Fig. 1. Mining Closed Sequential Patterns in Lexicographic Order

The mining of closed sequential patterns adopts the structure of lexicographic order which is shown in Fig. 1. The tree can be constructed by using following steps:

1. The root contains 'φ' symbol at the top of the tree which represents the empty string.
2. All the nodes in the tree represent the closed sequential patterns.
3. Calculate the index value for each node.
4. Remove the non-closed sequences in the tree.

(a)Definition: The problem of closed sequential pattern mining is to find the complete set of closed sequential patterns which satisfies the minimum support threshold, min_sup for an input sequence database SDB.

***(b)Algorithm*: CSPM**

(c)Input:

a) An input Sequence database, SDB and

b) Minimum support Threshold, min_sup.

(d)Output:

a) The complete set of closed sequential patterns.

V. EXPERIMENTAL RESULTS

In this section, the experimental reports of proposed CSPM and PrefixSpan algorithms are verified on the following claims: 1) The set of discovered closed sequential patterns 2) Proposed CSPM algorithm shows better efficiency with various support threshold 3) CSPM algorithm has better scalability for biological sequence databases in terms of efficiency, memory and running time.

To evaluate the various aspects of the algorithm CSPM an extensive performance study is performed. In the experimental results CSPM and PrefixSpan algorithms are compared for various parameters. The DNA dataset is

used in performance study which can be downloaded from the National Center for Biotechnology Information (NCBI) website. For the experimental test randomly sampled 500 sequences with a length-10 is formed for the dataset.

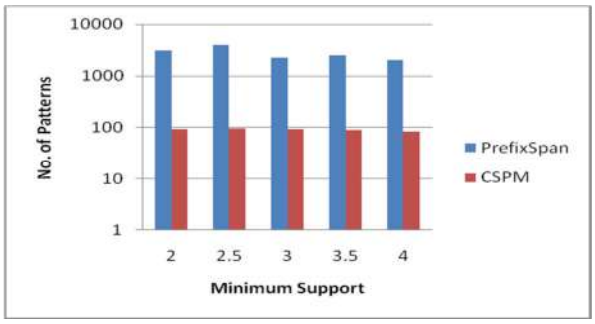

Fig. 2 No. of patterns in DNA dataset

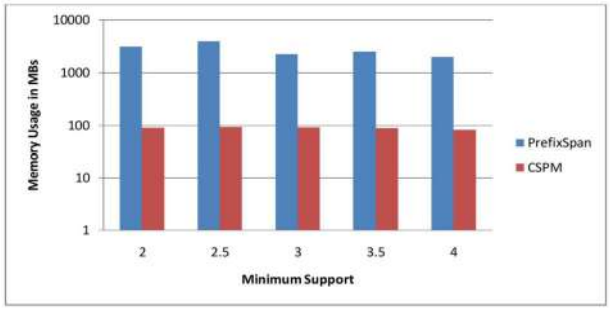

Fig. 3. Memory Usage for DNA dataset

In Fig. 2 the number of pattern generation generally affects the run time of the dataset. The CSPM and PrefixSpan algorithm are compared with varied minimum support value and a number of patterns generated in closed sequential mining. The CSPM algorithm generates less number of patterns then PrefixSpan which reduces the running time.
In Fig. 3 the CSPM and PrefixSpan are compared for memory usage with different minimum support values. The various minimum support values are 2, 2.5, 3, 3.5 and 4 respectively. The proposed CSPM algorithm consumes less memory than PrefixSpan.

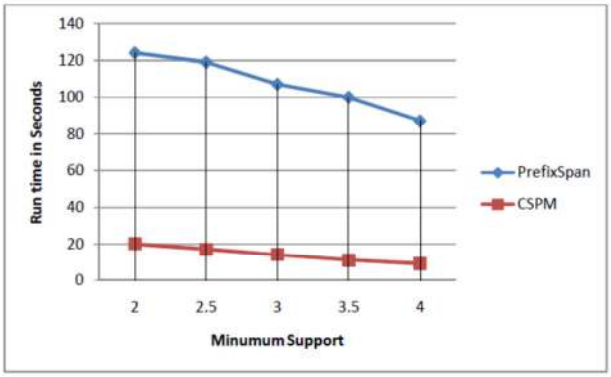

Fig. 4.Runtime for DNA dataset

In Fig. 4 the two pruning methods BackScan search pruning and checking occurrence method greatly reduce the search space in the given dataset. The CSPM and PrefixSpan are compared with randomly sampled DNA dataset. The CSPM algorithm consumes less run time than PrefixSpan for varied minimum support values.

VI .CONCLUSION

In this research paper, the problem of mining closed sequential pattern in the biological sequence is introduced and studied in detail with different experimental results. An efficient algorithm named CSPM is implemented for closed sequences. The CSPM algorithm has following features: 1) It mines closed sequences without candidate generation which greatly reduces the search space, 2) Two pruning methods are used which is very effective in speeding up the mining time, 3) varied minimum length constraint is used to further prune search space. The experimental study includes DNA dataset for the performance study of CSPM algorithm. The CSPM algorithm is more efficient than PrefixSpan in terms of efficiency, memory and running time which includes various pruning techniques.

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SP-3

EFFICIENTLY MINING CLOSED SEQUENCE PATTERNS IN DNA WITHOUT CANDIDATE GENERTION

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ABSTRACT

Sequential pattern mining is a technique which efficiently determines the frequent patterns from small datasets. The traditional sequential pattern mining algorithms can mine short-term sequences efficiently, but mining long sequence patterns are in efficient for these algorithms. The traditional mining algorithms use candidate generation method which leads to more search space and greater running time. The biological DNA sequences have long sequences with small alphabets. These biological data can be mined for finding the co-occurring biological sequence. These co-occurring sequences are important for biological data analysis and data mining. Closed sequential pattern mining is used for mining long sequences. The mined patterns have less number of closed sequences. This paper proposes an efficient Closed Sequential Pattern Mining without Candidate Generation (CSPMCG) algorithm for efficiently mining closed sequential patterns. The CSPMCG algorithm mines closed patterns without candidate generation. This algorithm uses two pruning methods namely, BackScan pruning, and frequent occurrence check methods. The former method prunes the search space and latter detects the closed sequential pattern in early run time. The proposed algorithm is compared with PrefixSpan and SPADE algorithms, better scalability and interpretability is achieved for proposed algorithm. The experimental results are based on sample DNA datasets which outperform the other algorithms in efficiency, memory and running time.

KEYWORDS: ***Text Mining, Clustering, Semi supervised Learning, Constrained Clustering, Co-Clustering***

I. INTRODUCTION

Sequential pattern mining (SPM) is a technique which identifies the interesting complete set of sub-sequences from huge dataset[7]. The SPM is a sequence of item sets that occurs frequently in a specific order. The sequence items in the item sets are based on two factors either on time value operation or within a time gap. The ordered list is represented in large sequence, where every event is a collection of item-set occurring simultaneously. The entire timestamps associated with the events is the ordering of the list of events[8]. In customer transaction, the events are viewed together as an interesting sequence known as customer sequence. Each customer transaction is expressed as individual item set in the sequence and all the customer transactions are listed in an ordered list with respect to the transaction-time[6].

Sequential pattern mining was first introduced by R. Agrawal and R. Srikanth[1] and it has become an essential data mining task. In past years more sequential algorithms were proposed for mining from which SPADE[3], PrefixSpan[4] and SPAM[5]was efficient. In SPADE algorithm, the breadth-first search process is used whereas in PrefixSpan and SPAM algorithmsuses depth-first search method for mining process. The vertical data format method is implemented in SPADE algorithm and mines the sequential patterns using simple join. The horizontal data format method is implemented in PrefixSpan algorithm which mines sequential patterns using the pattern growth method. The SPAM algorithm uses vertical bitmap representation method for mining sequential patterns. This algorithm outperforms PrefixSpan and SPADE algorithms in mining large datasets. However, this algorithm requires more memory space when compared to PrefixSpan and SPADE mining algorithms.

The closed sequential pattern mining was first proposed by X.Yan et al.[2] to overcome the limitations of sequential pattern mining algorithms. This method can mine more useful information than the sequential pattern. The closed sequential patterns can be mined in two steps, (i) To find final closed sequential patterns and (ii) To find the closed sequential pattern set and post-prune it.

The author's contributions can be summarized as follows: (i) introduced the problem of mining closed sequential patterns in biological sequence; (ii) The CSPMCG algorithm is proposed which mines closed sequential patterns efficiently without candidate generation, (iii) Proposed two pruning methods namely BackScan method and frequent occurrences method to prune search space, (iv) The varied minimum support threshold increased the efficiency and (v) The efficiency of CSPMCG algorithm is evaluated with two pruning methods which outperform in terms of memory and running time.

II. RELATED WORKS

Recently many sequential mining algorithms have been proposed and these approaches cover various data mining problems. In general two research problems are concerned in mining sequential patterns.

Improve the efficiency of the mining process[9]: This mining process mainly focuses on improving the efficiency of sequential patterns.

Extracting the time-related patterns using mining[10]: This pattern extraction method can find other time-related patterns from various databases such as weblog patterns, cyclic patterns etc for finding frequent patterns in time-related databases. The sequential pattern mining methods are classified into two types: (i) Apriori Algorithmsand (ii) Pattern Growth Algorithms.

A. Apriori Algorithms

The Apriori[1] and AprioriAll[2] are the algorithms which were implemented for frequent item set mining. The apriori property is used in this algorithm and generates candidate sequences using apriori-generate join procedure. All the non-empty subsets of a frequent item set must also be frequent which belongs to a category of properties. This property is knows as ant-monotonic property (or) downward-closed property. This algorithm reduces the search space of the algorithm. It scans the data item set for generating candidate item and generates frequent item set by removing infrequent data item set. In this algorithm two steps are involved, first it joins two data item sets and in the second step, the algorithm calculates the occurrence of each candidate set and the search space is reduces by pruning the infrequent data item set.

B. Pattern-Growth Algorithms

The pattern growth method is the solution to the problem of generate-and-test which is on based the algorithms sequential pattern mining. By using this method candidate generation step is avoided and it focuses on the search space of the database. In this algorithm 3 steps are involved namely, building the database for mining, dividing the database search space and finally generating candidate sequences by frequent growth method.

III. PROBLEM STATEMENT

The closed sequential pattern mining problem is formalized and explained here.

Let $A = \{a_1, a_2, \ldots, a_m\}$ be a set of all alphabets. The subset of A is called an alphabet data item set. A sequence $S=(S_1, S_2, \ldots, S_n)$ ($S_i \subseteq A$) is an ordered list of sequence data item sets. The data items in each sequence item set are sorted in ordered list. The sequence data item set length is defined as the total number of the data item set in the given sequence. The sequence alphabet data item set $SI_1 = (X_1, X_2, \ldots, X_m)$ is a subsequence of another sequence data item set $SI_2 = (Y_1, Y2, \ldots, Y_n)$, denoted as $SI_1 \sqsubseteq SI_2$, if there exists integers $1 \leq a_1 < a_2 < \ldots < a_m \leq n$ and $X_1 \subseteq Y_{i1}$, $X_2 \subseteq Y_{i2}$, . . . , and $X_m \subseteq Y_{im}$. SI_2 represent a super-sequence of SI_1 and SI_2 contains SI_1.

A sequence database, SDB={SI_1,SI_2,…,SI_n}, is a set of sequences and each sequence has an ID. The size, |SDB|, of the sequence database SDB is the total number of sequences in the SDB. The support of a sequence X in a sequence database SDB is the no of sequences in SDB which contain X item sets.

(i) Definition 1 (Sequential Patterns): A sequence is an ordered list of data item sets. Given a minimum support threshold min_sup, a sequence α is a sequential pattern on sequence database only if support (α) is greater than min_sup.

(ii) Definition 2 (Closed Sequential Patterns): A sequential pattern α is a closed sequential pattern if there does not exist a sequential pattern β, such that support (α) = support (β) and α ⊏ β.

The closed sequential pattern mining is used to mine the item set of closed sequential patterns which satisfies the minimum support value min_sup for a given input sequence data item set. The table 4.1 represents the sample sequence database with sequence ID and sequence data.

IV. PROPOSED METHOD

The CSPMCG algorithm is proposed for mining closed sequential patterns. This algorithm uses the depth-first search method for mining closed sequential patterns. The breadth-first search technique is mostly used but they are inefficient for mining closed sequence. The CSPMCG algorithm is used to overcome the limitations of sequential pattern mining. The closed sequential patterns can be mined in two different ways namely: (i) Finding the closed sequential patterns without verifying the discovered patterns and (ii) Finding closed sequential pattern and post-pruning it.

(a) Definition: The CSPMCG is used to solve the problem of mining the item set of closed sequential patterns which satisfies the minimum support threshold, min_sup for an input sequence from database SDB.

***(b) Algorithm*:** Closed Sequential Pattern Mining Without Candidate Generation (CSPMCG)

Input*:*

- An input Sequence database, SDB and
- Minimum support Threshold, min_sup.

(c)Output*:*

a. Closed sequential patterns without candidate generation.

The flow diagram Fig.4.2 represents the proposed CSPMCG algorithm. The method includes various steps such as constructing segment tree, verifying the minimum threshold value, mining frequent n-sequences, applying pruning methods namely BackScan search method and occurrence check method, identifying the closed sequential patterns.

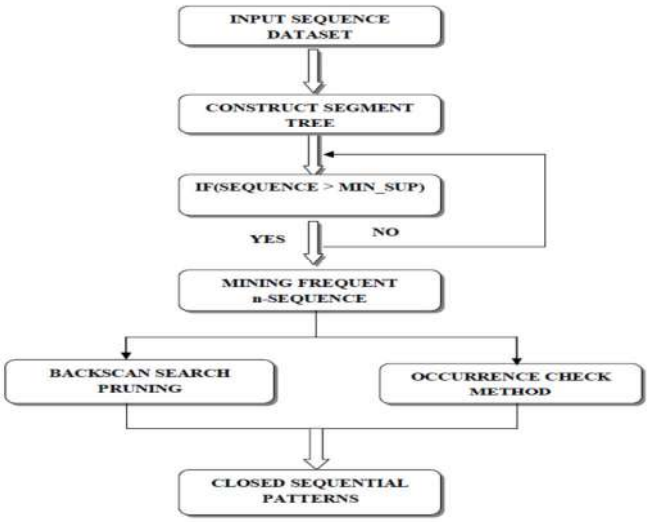

Figure. 1 CSPMCGalgorithm

V EXPERIMENTAL RESULTS

In this section, the experimental reports of proposed CSPMCG, PrefixSpan and SPADE algorithms are

verified on the following steps:
1) Finding the closed sequential patterns,
2) Proposed CSPMCG algorithm shows better efficiency by finding lesser closed frequent patterns, and
3) CSPMCG algorithm has better scalability for biological sequence databases in terms of efficiency, memory and running time.

Table 1 Sequence Database

Sequence ID	Sequence
1	CGAAC
2	TGCGA
3	CGAC
4	ACGGA

Table 5.1 represents sequence database example which contains sequenceID and sequence data. To evaluate the various aspects of the algorithm CSPMCG an extensive performance study is performed. In the experimental resultsCSPMCG,PrefixSpan and SPADE algorithms are compared for various parameters.

Table.2 Comparison of two forms of frequent patterns

Sequence Form	Frequent Patterns	Pattern Length
PrefixSpan	A:4,C:4,G:4,AA:2,AC:3, CA:4,CC:3,CG:3,GA:4,GC:3, CAC:2,CGA:3,CGC:2, GAC:2,CGAC:2	**15**
SPADE	A:4,C:4,G:4,AC:3, CA:4,CC:3,CG:3,GA:4,GC:3, CAC:2,CGA:3,CGC:2, GAC:2,CGAC:2	**13**
CSPMCG	AA:2,AC:3,CA:4, CC:4,GA:4,GC:3,CGA:3, CGAC:2	**8**

The above experiments were conducted on a machine with Intel Core i3 2.0 Ghz CPU, 4GB memory and Windows 7 system implemented in net beans IDE 8.2. In the experiment we compared PrefixSpan, SPADE and CSPMCG algorithms for given sequence database. The number of patterns in PrefixSpan is 15 in length, patterns in SPADE is 13 in length whereas CSPMCG mines 8 closed frequent patterns for the given dataset. The frequent patterns and closed sequential patterns are shown in the table.2. The pattern length for CSPMCG is lesser than PrefixSpan and SPADE algorithms which also decreases the memory space and increases the efficiency.

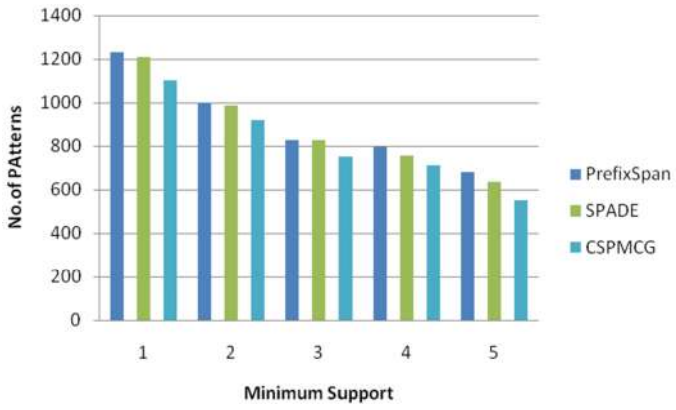

Fig.2 No. of patterns in DNA dataset using various algorithms

In Fig.2 minimum support and total number of pattern is represented which generally affects the process time of the algorithm. The PrefixSpan, Spade and CSPMCG algorithms are compared with different

parameters for closed sequence patterns without candidate generation. The CSPMCG algorithm mines lesser number of closed patterns than PrefixSpan and SPADE algorithms which reduces the running time of the system.

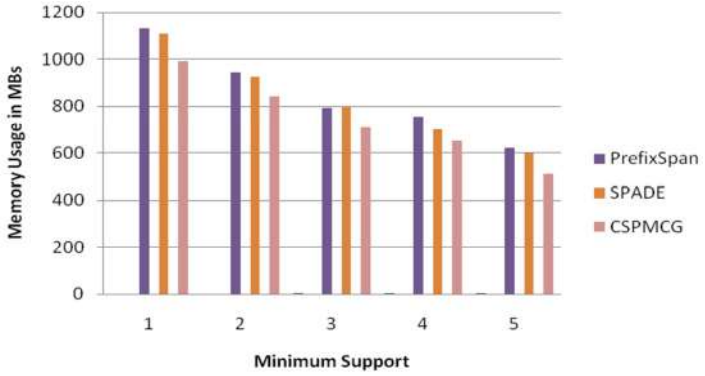

Fig.3Memory Usage for DNA dataset for various algorithms

In Fig.3 the PrefixSpan, SPADE and CSPMCG algorithms are compared for calculating memory usage with varied minimum support values. The various minimum support values are 1,2,3,4 and 5 respectively. The proposed CSPMCG algorithm consumes less memory space than PrefixSpan and SPADE algorithms.

VI CONCLUSION

The problem of mining closed sequential pattern in the biological sequence is introduced and studied in detail with different experimental results. An efficient algorithm named CSPMCG is implemented for closed sequences. The CSPMCG algorithm has following features: (1) It mines closed sequential patterns without candidate generation which greatly reduces the search space and (2) Two pruning techniques are used which is very efficient in mining time. The experimental study includes sequence dataset for the performance study of CSPMCG algorithm. The proposed algorithm is more efficient than PrefixSpan and SPADE in terms of efficiency, memory and running time which includes various pruning techniques.

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SP-4

A COMPUTATIONAL APPROACH TO IDENTIFY THE CO EXPRESSION BASED MICRORNA (MIRNA) REGULATION IN THE DISEASE PATHOLOGY OF PSORIASIS

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ABSTRACT

In the era of post genomics, identification of a potential miRNA to regulate gene and transcription factors by applying a computational approach is a challenging task to execute. The challenge was addressed by identifying the associated transcription factors (TFs) and genes from Mogrify along with micro RNAs from miRTarBase. On the basis of association between Genes-miRNAS-TFs a network of transcription factor based miRNA was constructed. Finally, on the basis of statistical studies and miRNA based compatibility the network was analyzed to identify a potential miRNA to be utilized as a biomarker to treat psoriasis.

KEYWORDS: **Post genomics, Mogrify, miRNA, miRTarBase, biomarker**

I. INTRODUCTION

Psoriasis is a disorder caused due to certain faulty signals in the human body mediated by immune system by making. It's still a belief that psoriasis can be developed under the specified condition i.e, accelerated growth of skin cells signaled by the immune system. In case of psoriasis, the skin cells mature in 3-6 days. Instead of being in shed, the cells in skin get pile up to cause the visible lesions. It was also found that the genes that cause psoriasis can determine the reaction of a person's immune system. These genes can either cause psoriasis or Type-I Diabetesor rheumatoid arthritis which are immune-mediated. Pathophysiology of psoriasis involves the understanding of the occurrence of prominent pathologies in the major components of skin i.e. the epidermis and the dermis. There are two well established hypotheses about the process that occurs in the development of the disease. The first hypothesis considers psoriasis as a disorder with excessive growth and reproduction of skin cells. Here, the problem is viewed as a fault of the epidermis and its keratinocytes. In second hypothesis, the disease is viewed as an immune-mediated disorder. Here, the factors produced by the immune system are secondary to excessive reproduction of skin cells[1-2].

Micro RNA consist of 19-25 nucleotides and is a family of non coding RNA (ncRNA) which was discovered in 1993, and regulates the expression of approximately 30% of protein-coding miRNAs in humans[3].Base pairing at the position of 2-8 nucleotides were relative to the 5′ end of the small RNA to be termed as the "seed" region and it appears to be important for target recognition (Xiaetal 2013). Mature miRNAs undergoes multiple steps and initially produced sequentially two intermediate forms of miRNAs, namely primary (pri-) and precursor (pre-) miRNAs. In this process, Drosha (RNase III enzyme) and the double-stranded RNA (dsRNA) binding protein Dgcr8 cleaves the pri-miRNAs to produce a hairpin-shaped pre-miRNAs that are recognized by Exportin5 and they are subsequently transported from the nucleus to cytoplasm[3]. There is another RNase III enzyme called Dicer which cleaves the pre-miRNAs to release ~22-nt double-stranded RNA duplexes (namely miRNA/miRNA* duplexes) with ~2-nt 3' overhangs[4]. One strand of a RNA duplex is termed as a mature miRNA which is further loaded into an Argonaute protein in the RNA-induced silencing complex (RISC) to exert its regulatory function on the basis of its binding with the target transcripts[5].

II. RELATED WORKS

A unique miRNA can regulate the expression of hundreds of proteins and the expression of a specific protein may be controlled by several miRNAs[6]. The sequence conservation of most miRNAs lies between the distantly related organisms to suggest the impact of a strong evolutionary pressure[7] and they have been shown to participate in many fundamental life processes like cell differentiation, organogenesis, development, growth control and cell death. Accordingly, deregulation of miRNA expression has been shown to contribute to cancer, heart diseases, infectious diseases and inflammatory diseases making them potential targets for medical diagnosis and therapy[8]. Initially, Lee had found lin-4 as a regulator of developmental timing in nematode Caenorhabditis elegans[9]. After several years, Reinhart had discovered lethal-7 (let-7) gene in Caenorhabditis elegans[10]. At present, 2500 miRNAs are discovered in the human genome and Majority of them are intragenic[11]. Micro RNAs are initially transcribed as a part of an RNA stem-loop that in turn forms part of a several hundred nucleotides long miRNA precursor miRNA (pri-miRNA)[12].

III. MATERIALS AND METHODS

II. Mogrify

Mogrify identifies the key transcription factors and associated genes involve in the Cell differentiation of keratinocytes. Mogrify was tested with 173 cell types and 134 tissues of human[13].

III. miRTarBase

miRTarBase[14]is a platform to identify the experimentally validated miRNAs of associated genes. miRTarBase is one of the largest repositories of gene-miRNA associations in humans and mouse.

IIII. Cytoscape

Cytoscape[15]software is used for network construction, visualization and analysis in bioinformatics with an open source platform for visualizing the interactions in molecular networks and integrating them with the profiles of gene expression. Additional features in cytoscape are available as plugins for network and molecular profiling.

IIV. Cytohubba

Cytohubba[16]is a cytoscape plugin for performing the analyses of gene regulation & protein-protein interaction involved in the process of cellular pathways in the process of signal transduction. Cytohubba ranks the nodes of network by topological methods like Radiality, Betweenness, Closeness, Bottleneck, EcCentricity and etc.

IV. miRmap

miRmap[17]software addresses the challenges in post transcriptional repression of miRNAs in human genome by evolutionary, probabilistic thermodynamic and sequence-based features.

IVI. Triplex RNA

Triplex RNA[18] is a database of cooperating micrRNAs with their mutual targets. In this database miRNA target prediction is based on the analysis of predicted miRNA triplex with molecular dynamics simulations and differential modeling procedures in mathematics.

IVII. DAVID

The database for Annotation, Visualization and Integrated discovery (DAVID) contain complete information about functional annotation of genes. The current version of DAVID[19]is 6.8 and it provides a set of comprehensive tools for functional annotation of genes.

IV. METHODOLOGY (Computational Approach of Transcription factor based miRNA regulation)

1. Identify the disease associated genes and Transcription Factors from Mogrify.
2. Obtain the associated list of experimentally validated miRNAs from miRTarBase.
3. Construct and analyze the network in Cytoscape.
4. Identify the miRNA based hub genes and transcription factors from cytohubba.
5. Identify the implication of miRNA in Regulatory network in miRmap & miRNA Triplex.
6. Identify and analyze the gene associated pathways in DAVID.

V. RESULTS AND DISCUSSION

A. Construction of Transcription Factor based Regulatory Network in Bottom-Up Approach (Cytoscape)

In case of transcription factor based miRNA regulation (bottom up approach), the regulatory network was constructed with 48 genes, 221 miRNAs and 4 TFs. Network was initiated by the mining of 225 regulators from Mogrify, miRtarbase and target scan (i.e. 4 TFs & 221 miRNAs) to interact with the 48 target genes in such a way to form 273 nodes and 491 edges.

B. Analysis of Transcription Factor based Regulatory Network in Bottom-Up Approach (Cytohubba)

The genes and their regulators (Micro RNAs & Transcription Factors) in Top-down approach were subjected to the analysis in cytohubba by various global based statistical methods like Edge Percolated Component, Bottleneck, EcCentricity, Closeness, Radiality, Betweenness and Stress along with local based statistical methods like Maximal Clique Centrality, Density of Maximum Neighborhood Component, Maximum Neighborhood Component and degree to identify their connectivity. Among the various methods of analysis for transcription factor based miRNA regulation in bottom up approach only a local based statistics of Edge Percolated Component method in cytohubba resulted in obtaining a regulatory network of gene-miRNA-TFs in top-down approach. The details of regulatory network were given in Figures 1-5.

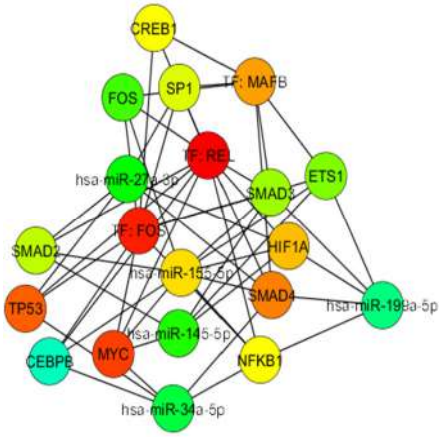

Fig.1 EPC method for top 20 hubs.

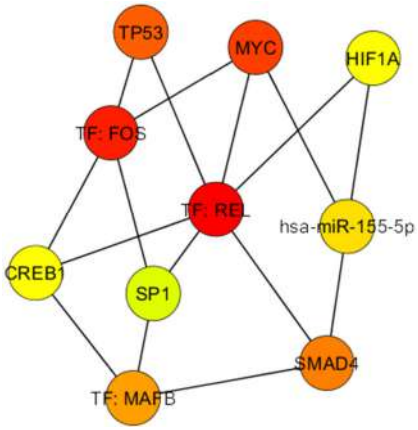

Fig.2 EPC method for top 10 hubs.

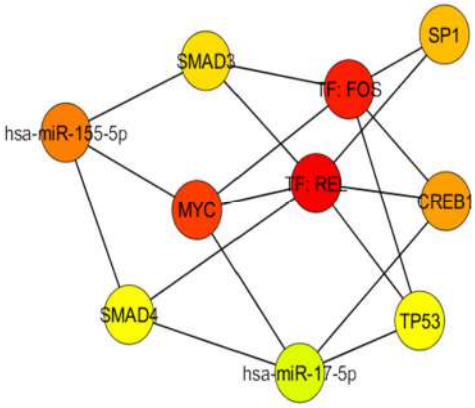

Fig.3 Radiality method for top 10 hubs

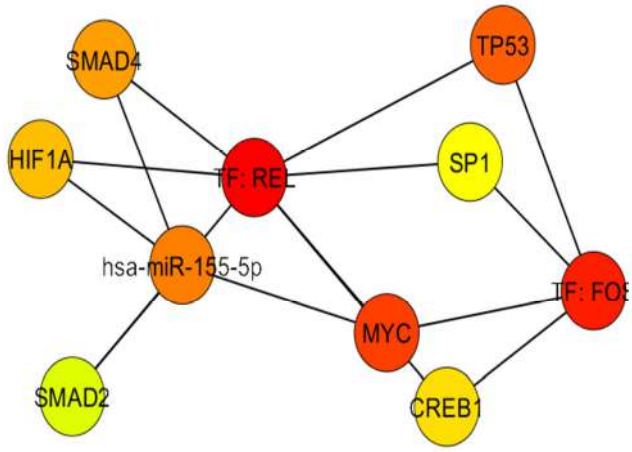

Fig. 4 Eccentricity method for top 10 hubs

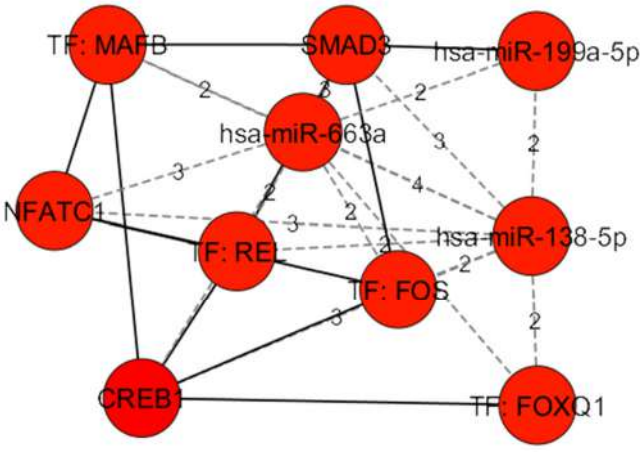

Fig. 5 Closeness method for top 10 hubs

The implication of miRNAs in the regulatory network of transcription factor based bottom-up approach was analyzed on the basis of compatibility with respect to gene-miRNA seed pairing and gene-miRNA-miRNA triplex with respect to nature of binding and the details were given in Table.1.

Table.1 Implication of miRNAs in the regulatory network of Transcription factor based miRNA regulation

S. No.	Genes	Micro RNAs	Binding Score in % (miRmap)	Paired miRNA (Triplex RNA)	Binding Energy in Kcal/mol. (Triplex RNA)	Nature of Binding (Triplex RNA)
1	TP53	hsa-miR-155-5p	NIL	NIL	NIL	NIL
2	MYC	hsa-miR-155-5p	NIL	NIL	NIL	NIL
3	HIF1A	hsa-miR-155-5p	75.79	hsa-miR-653	-9.58	Canonical Triplex
4	CREB1	hsa-miR-155-5p	55.85	NIL	NIL	NIL
5	SP1	hsa-miR-155-5p	82.33	hsa-miR-296-3p	-35.96	Canonical Triplex
6	SMAD4	hsa-miR-155-5p	9.49	NIL	NIL	NIL

In case of miRNAs implication in transcription factor based bottom-up approach hsa-miR-155-5p is highly compatible with SP1 on the basis of seed pairing.

C. Scope & Significance of transcription factor based miRNA regulatory network

Combinatorial Analysis of transcription factor based miRNA regulatory network in Bottom-up approach indicate the fact that has-miR-155-5p is involved in the repression of Transcription factors FOS, REL and MAFB and activation of gene SP1 and the probable miRNA based regulatory networks[20] are (i) Gene: SP1, miRNA: hsa-miR-155-5p & TF: FOS; (ii) Gene: SP1, miRNA: hsa-miR-155-5p & TF: REL and (iii) Gene: SP1, miRNA: has-miR-155-5p & TF: MAFB.

D. Regulatory Analysis of transcription factor based miRNA regulatory network

The suppressor of cytokine signaling 1 along with the IFN-gamma-dependent factors promotes the positively regulation of IFN regulatory factor-1 and Sp1[21]. Increased expression of miR-155-5p in dermal mesenchymal stem cells of psoriatic patients targets cytokines[22-23]. Fos protein is specifically expressed during cell differentiation of human karatinocytes[24]. NF-κB p65 and c-Rel control epidermal development in skin[25]. MAFB is involved in the regulation of epidermal differentiation[26].

E. Pathway Analysis

The obtained associated genes from Mogrify were subjected to pathway analysis in DAVID on the basis of P value and Benjamini statistic and the result is given in Table. 2

Table.2 Annotation of Kegg Pathways

S. No.	Pathway	P value	Benjamini
1.	HTLV-1 infection	6.0E-18	6.6E-16
2.	Osteoclast differentiation	5.8E-11	1.10E-09
3.	Hepatitis B	1.80E-10	6.40E-09
4.	MAPK signaling pathway	4.10E-10	1.10E-08
5.	Transcriptional misregulation in cancer	8.60E-10	1.90E-08
6.	TNF signaling pathway	3.80E-09	6.80E-08
7.	B cell receptor signaling pathway	7.20E-08	1.10E-06
8.	Adipocytokine signaling pathway	2.10E-03	1.20E-02
9.	Pathways in cancer	6.80E-07	9.30E-06

The genes associated with Psoriasis follows the hierarchy of HTLV1 infection, Osteoclast differentiation, Hepatitis B and MAPK signaling pathway, in case of Pathway Analysis in transcription factor based regulation.

VI. CONCLUSION

Computational analysis of Transcription factor based miRNA regulation gave us a different view point to focus the disease pathology of Psoriasis. In future the pathways associated with miRNA needs to be reconstructed to understand the complete role of has-miR-155-5p in the pathogenesis of Psoriasis.

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SP-5

COMPUTATIONAL ANALYSIS OF MIRNA BASED TRANSLATIONAL REPRESSION AND PROTEIN-PROTEIN INTERACTION NETWORK IN PSORIASIS BY TOP DOWN APPROACH

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ABSTRACT

Psoriasis is an autoimmune disorder characterized by the activation of hyper proliferative keratinocytes in the human skin. In the era of post genomics, miRNA have been identified to play a significant role in the signaling pathways associated with the pathogenesis of autoimmune disorders. In this study, we focus on identifying a lead towards the involvement of specific miRNAs in the transcriptional and translation repression by a computational approach called "Top down approach" to establish miRNA as a diagnostic and therapeutic biomarker for Psoriasis. In case of the identification of specific miRNAs as a biomarker in the signaling pathways associated with pathogenesis of Psoriasis. Initially, the genes associated with Psoriasis were retrieved from the catalog of Pubmed, OMIM and DisGeNET. Then the associated miRNAs along with transcription factors and translation factors were retrieved from miRTarBase, RAIN and RegNetwork. Then the network profiling of miRNA based gene regulation were analyzed using cytoscape. Finally the vital pathways, miRNAs, transcriptional and translational factors were analysed with respect to the properties of undirected network with correlation to biological network motif for identifying the miRNA based transcriptional and translational repression in the disease pathology of Psoriasis.

KEYWORDS: ***Keratinocytes, Pathophysiology, miRNA, Psoriasis, RegNetwork, RAIN, Cytoscape***

I. INTRODUCTION

Events in gene regulation play a vital role in a various processes of in a cell with respect to development and physiology. Macromolecules such as DNAs, RNAs and Proteins coordinate to operate various responses under diverged conditions. Hence, more efforts were made to reveal a structure of a gene regulatory network from the profile of transcriptomic datasets generated by ChIP-Seq[1], RNA-Seq[2] and Microarray[3]. Though there are a number of data-driven techniques in reverse engineering to identify regulatory relationships between targets and their regulators, the accuracy of existing methods were very low due to the curse of limitation in significance of dimensionality in their applications[4]. However, some recent studies have suggested a promising alternative for identifying a structure of a regulatory network by combining the high-throughput profiling data (transcriptome) with the knowledge on well existing or predicted relationships on regulation from various literature and databases[5]. For example, the framework can significantly improve the accuracy of regulatory relationship by identifying a prior knowledge to the profile a transcriptomic data. The results from other studies suggest that the incorporation of prior knowledge can identify the context-specific interactions in regulation to correspond certain phenotypes.

II. RELATED WORKS

In case of comparative analysis in the regulation of gene data, there exist two possibilities which are vital for analysis. In the Initial step, comparison must be done across species to allow us to infer a better relationship in functional regulation; it can be either due to the conservation in functional elements through a process of purified selection or due to the ability to link genetic changes across the strains or species with respect to their functional impact[3]. The inference must result in an accurate model of a regulatory network. In the next step, the inferred networks were analyzed across species from the reconstructed models with respect to their evolutionary and phylogenetic lens to shed a light on the processes of evolution and associated adaptations. The level of analyses can help us to address the questions about the constraints that operate on quantitative

traits such as level of expression on individual genes, modules, and entire pathways. Methods in computational analysis for comparative analysis of gene regulation must tackle two major challenges: the inference of gene regulation because it receives an additional power for consideration in phylogenetic analysis of network evolution. In practice, these challenges can be tackled in the context of the different entities and components in the models of gene regulatory network i.e. "one line of research focuses on regulatory interactions with DNA was most prominently involved in the identification and analysis across species with a binding site for TFs. In this mechanism the clustered sites in a genomic region and form a *cis*-regulatory module (CRM)[4]. The focus in next level involves the understanding of evolution in epigenomic or transcriptional states in the gene-module or single-locus level. Finally the emerging approaches consider an entire pathway or a sub-network which includes regulators, targets and interactions.In each level of analysis, the computational methods need (*a*) To identify relevant entities (e.g., regulatory DNA or gene module or sub-network)[5] in the species under study to essentially infer a regulatory model; (*b*) To compare the entities across species or strains for estimating the level of conservation and divergence; (*c*) To assess the rate of change in the evolutionary model. An entity is said to evolve neutral, if the change across the species is linear with respect to the divergence time i.e. neither purifying nor diversifying selection[6,7].

III. MATERIALS AND METHODS

In Top-down approach[8], initially the miRNA with a maximum degree of association with Psoriasis was obtained from RAIN database and finally, the enrichment analysis of biological process was analyzed in STRING database.

A. RAIN

RAIN[9] is a database of interactions between a ncRNA–RNA and a ncRNA–protein along with the integration of STRING database for protein–protein interactions. These ncRNA associations were established from curated examples, automatic literature mining experimental data and interaction predictions.

IV. RESULTS AND DISCUSSION

A. Scope and significance of miRNA in the Translational Regulatory Network of Psoriasis in Top down Approach

The association of miRNA and Protein interactions was analyzed in RAIN database[10]to identify the translational regulatory network of Psoriasis in Top-down approach. The translational network of hsa-miR-186-5p is given in Figure 1.

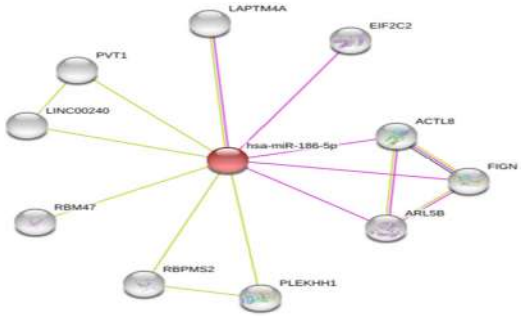

Fig.1 Translational regulatory network of hsa-miR-186-5p

The miRNA hsa-miR-186-5p is associated with proteins LAPTM4A, EIF2C2, ACTL8 and ARL5B on the basis of experimental studies and the probable translational regulatory network of Psoriasis in top-down approach were (i) Gene: STAT2; miRNA: hsa-miR-186-5p Protein: LAPTM4A; (ii) Gene: STAT2; miRNA: hsa-miR-186-5p; Protein: EIF2C2; (iii) Gene: STAT2; miRNA: hsa-miR-186-5p; Protein: ACTL8 and (iv) Gene: STAT2; miRNA: hsa-miR-186-5p; Protein: ARL5B. The lysosomal protein of transmemberane (LAPTM4A) is associated with the signaling of IL17A in Psoriasis[11]. The protein EIF2C2 was involved in the epigenome wide association of Psoriasis[12].The protein ARL5B[13] is associated with Psoriasis by triggering the sensing responses[14] in the dentritic cell[15]. The regulation of ACTL8 is yet to be explored in Psoriasis (Fig.1).

B. Protein-Protein interaction network of Psoriasis in Top-down Approach

Protein-Protein interaction network of Psoriasis in Top-down approach was anlaysed in STRING database[13]along with the significance of Biological Process, Molecular Function and Cellular Component with respect to the studies on Gene Onthology (Fig. 2). The interactions between multiple proteins were obtained on the basis of a high level of confidence with a score of 0.7 in a scale of 1 and the protein-protein interaction from various sources like text mining, experiments, databases, co expression, co occurance, neighborhood and gene fusion were analysed and the results are illustrated in Figure 2.

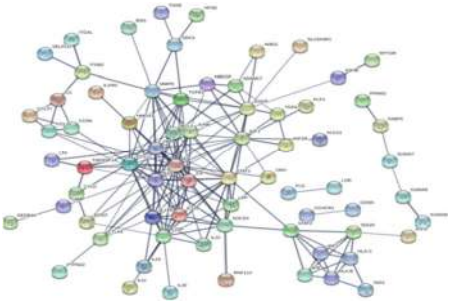

Fig.2 Protein-Protein interaction network of Psoriasis in Top-down approach

V. CONCLUSION

In case of the Protein-Protein interaction network of Psoriasis in Top-down approach, there were 91 nodes and 194 edges with an average node degree of 4.26. The functional enrichment of Protein-Protein interactions in Top-down approach with respect to the analysis of gene onthology (GO) is highly associated with the pathway of immune response (GO: 0006955) in the biological process and the association was followed by the pathway of receptor binding (GO: 0005102) in molecular function and the localization of extracellular space in the pathway associated with the cellular component (GO: 0005615).In case of the domain analysis of protein-protein interaction of Psoriasis (Top-down approach) in PFam and Interpro, there was an association of two pathways (Four helical cytokine like pathway :IPR009079 and TNF pathway : PF00229).

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SP-6

COMBINATION OF AQUATIC WEED EXTRACT AND PANCHAGAVYA AS BIOSTIMULANTS OF PLANT GROWTH

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ABSTRACT

The combination of biofertilizer- panchagavya, weed extract (*Eichhornia crassipes)* and weed based panchagavya along with *phosphobacter* sp and *Rhizobium* sp were used for microbial analysis, enzyme activity, seed germination, plant biometric and chlorophyll content of the plant *Arachis hypogea* (groundnut). The weed based panchagavya formulation increased the levels of all the parameters in the experimental plant. It was highly effective at 10% concentration.

KEYWORDS: ***Eichhornia crassipes, biofertilizer, panchagavya, Arachis hypogaea***

INTRODUCTION

Fertilizers are any organic or inorganic material of either natural or synthetic origin which is added to the soil to supply nutrients that are essential for the growth of plants[1]. The use of fertilizer has its own advantages and disadvantages in the factors of nutrient supply, crop growth and quality of the environment. The advantages should be unified in order to make the best use of all types of fertilizers and achieve balanced nutrient management for crop growth[2]. With the agro-based products, the fertilizer industry is bound to grow, as it serves in the field for increasing food production. At present there is a shortage of about three to four million tons of fertilizer[3].In organic fertilizer the nutrient supply is more balanced, which helps to keep plants healthy, supplies food and encourages the growth of beneficial microorganisms and earthworms. They help to suppress certain plant diseases, soil-borne diseases and parasites which spread through various means such as insects, soil, etc[4]. Green manuring is an efficacious contributor to soil in building up organic matter. Availability of green manure nitrogen largely depends on quantity and quality of green manure, the time it is turned under and soil properties[5]. During the first year of turning, it was expected about 40-45% of organic nitrogen in green manures to become available for plant nutrition[6].The use of non-traditional organic materials such as weeds is the largely overlooked resource availability for soil fertility remediation.

I. RELATED WORKS

The decayed tissues of unwanted plants to provide nutrients for crops is a crude but effective way of exploiting weeds and is a simpler technique than any of the other alternatives[7,18]. Algae and aquatic weeds are possible sources of organic manure; they are usually as rich in nutrients, richer than many green manure. However, their high water content (92% on the average) has been the major deterrent to the commercial use of aquatic weeds[8,16].In ancient days; the cattle based agriculture was widely practiced. Panchagavya, the five products of cow is used as a foliar application to boost yield of crop plants and to restrict the incidence of common diseases[9].Few farmers in the Southern parts of India have modified the formulations of Panchagavya[10], then used them to increase the biological efficiency[11] of the crops, the quality of fruits & vegetables[12-14]and also to support the growth of beneficial microorganisms[15,20]. It contains growth regulatory substances like IAA, GA, cytokinin, and some essential plant nutrients, effective microorganisms like lactic acid bacterium, yeast and Actinomycetes[16-18]. It also contains biofertilizers like *Acetobactor, Azosprillum* and *Phosphobacterium* and plant protection substances[19]. The biofertilizer potential of Panchagavya prepared in the traditional way and a modified preparation amended with seaweed extract has been evaluated for the biofertilizer potential[17].

III. MATERIALS AND METHODS

A. Plant material collection and extract preparation[21,17]**:** Fresh sample of aquatic weed *Eichhornia crassipes* (Fresh samples collected from lake) was collected at Erode district. Root, leaf and shoot portions were taken and shade dried and extracted using aqueous extraction.**Panchagavya preparation**[23]: Panchagavya was prepared by mixing cow dung(fresh-2.5kg), cow's urine (1.5L), cow ghee (500ml)and kept in the room temperature for 3 days and then cow milk (1L) and curd (1L) were added and mixed well. The panchagavya stock solution was ready after 21 days.

B. Weed Based Panchagavya[14]**:** prepared in the 1:1 ratio of weed extract with panchagavya stock solution.**Quantitative Analysis:** Protein estimation was done by Lowry *et al.,* (1951) methodology. The method A.O.A.C. (1990) will be used for the estimationof crude fibreand the ash content.**Moisture content:** 2g of fresh weed sample was weighed and placed in cruciable and ignited in an oven at 105°C.**Swelling capacity (SWC):** SWC of weed samples was analysed by the bed volume technique after equilibrating in excess solvent[13].

C. Design and layout of the Experiment[24]**:** The effect of panchagavya preparation and weed extract on germination and development of *Arachis hypogeal* (groundnut) seedling were studied by following the below design, T_0 : Control, T_1 : Seeds were soaked in weed based panchagavya 5% for 5h and sown, T_2 : Seeds were soaked in weed based panchagavya 10% for 5h and sown, T_3 : Seeds were soaked in weed based panchagavya 15% for 5h and sown, T_4 : Seeds were soaked in weed based panchagavya 20% for 5h and sown, T_5 : Seeds were soaked in panchagavya 5% for 5h and sown, T_6 : Seeds were soaked in panchagavya 10% for 5h and sown, T_7 : Seeds were soaked in panchagavya 15% for 5h and sown, T_8 : Seeds were soaked in panchagavya 20% for 5h and sown, T_9 : Seeds were soaked in weed 5% for 5h and sown, T_{10} : Seeds were soaked in weed 10% for 5h and sown, T_{11} : Seeds were soaked in weed 15% for 5h and sown and T_{12} : Seeds were soaked in weed 20% for 5h and sown.

D. Microbial Analysis and Enzyme study: Spread plate technique was used for microbial analysis. Enzymes such as protease, amylase and lipase activity were studied and their zone of inhibition was noted.

E. Growth Parameters[5]**:**

(i) Germination Percentage: In agriculture, the germination rate describes how many seeds of a particular plant species or variety are likely to germinate. The germination percentage was estimated by the below given formula:

GP = No of Seeds Germinated/ Total no of Seeds ×100

(ii) Root and Shoot Length Measurement: The shoot length, root length, leaf length and leaf width was measured for a period of 7 days using thread and a centimeter scale.

(iii) Seedling Dry Weight (g/10 Seedlings): The same 10 normal seedlings used for measuring shoot and root length were put in butter paper pockets and kept in an oven maintained at 100 ± 2°C for 24 hrs. To cool the seedlings, they were kept in a desiccator after drying. The weight of dried seedlings was recorded and means dry weight was expressed in milligrams.

(iv) Estimation of photosynthetic pigments[3]: Pigments from leaves of those developing seedlings were extracted by treating them with 80% acetone and the amounts of chlorophyll a and chlorophyll b pigments were determined.

Chlorophyll a = 12.21 A663 – 2.81 A646 mg/g of tissue, Chlorophyll b = 20.13 A646 – 5.03 A663 mg/g of tissue Where, A646 and A663 represent the optical density (OD) values at the respective wavelengths.

IV. RESULTS AND DISCUSSION

A. Quantative Analysis: Among the various parameters, moisture level and crude fiber of *Eichhornia crassipes*was found to be 1.71/2g and 0.036g. Benjama *et al*., (2012) reported 5.2% and 3.3% of moisture level and 64% and 60% of crude fiber in seaweeds *Gracilaria fisheri* and *Gracilaria tenuistipitata* respectively. Swelling capacity of *Eichhornia crassipes* was 2.5cm^3/g, which was higher than the seaweed *Ulva lactuca* (0.3cm^3/g), reported by Yaich *et al*., (2011). Ash content was found to be 0.16g in *Eichhornia crassipes* similar results were reported by Jafari, (2010) (Table 1).pH range of Aqueous extract of *Eichhornia crassipes* 6-7, which was similar to water hyacinth manure (7.8pH) reported by Lata and Veenapani (2011). pH range of Panchagavya showed 5.5-6, which was consistent with the previous report (pH 5.8) given by Ali *et al*., (2011). pH of weed based panchagavya was 5-5.5. Mineral composition for weed, panchagavya, and weed based panchagavya are listed in Table 2. Lata and Veenapani (2011) showed 49.79 C Mol kg^{-1}, 6.88 C Mol kg^{-1}, 115.72 C Mol kg^{-1} and 0.86% of calcium, magnesium, iron and nitrogen in water hyacinth manure. Benjama *et al*., (2012) reported

1455.3mg/100g and 1980.3mg/100g of chlorine in seaweeds *Gracilaria fisheri* and *Gracilaria tenuistipitata* respectively. Ali *et al*., (2011) accounted 1.4% of nitrogen content present in panchagavya and Graciela *et al*., (2011) reported 8.72% protein in seaweed *Sargassum fillpendula*. Further experimental studies deals with *Arachis hypogaea* and itwas exposed to *Rhizobium* sp and *Phosphobacter* sp and to different concentrations of 5%, 10%, 15 % and 20% weed based pancahgavya, panchagavya and *Eichhornia crassipes.*

B. Microbial Analysis

Total bacterial count was observed on 7^{th} and 14^{th} day after seeding process. (Table 3-5). Maximum microbial population obtained on 14^{th} day after seeding was 112×10^{-6} (10% weed based panchagvya), 102×10^{-6} (10% panchagavya) and 150×10^{-6} (20% weed) over the control (10^{-5}). Ali *et al*., (2011) studied the effect of organic farming practice in soil health using panchagavya and sanjibani and observed the total microbial count before planting black gram (2.7×10^{7}), after harvesting black gram (6.7×10^{8}) and after harvesting mustard (3.1×10^{8}).

C. Enzyme activity

The enzyme activities were measured by zones (mm) for weed based pancahgavya, panchagavya and *Eichhornia crassipes*, observed on 7^{th} and 14^{th} day after seeding. Figure 1represents the enzyme activity for 7^{th} day, 20% weed based panchagavya showed maximum zones (mm) for all enzymes (protease-13.55±2.19; amylase- 18.55±2.19; lipase- 18.25±1.76) than the control (protease- 12.3±1.83; amylase-14.3±1.83; lipase- 15.051.48) and 5% weed based panchagavya showed minimum enzyme activity (protease-12.5±2.5; amylase-13.5±2.12; lipase-11.65±0.91).

Figure 2, illustrates enzyme activity for 7^{th} day. Maximum activity was observed for 10% panchagavya, (protease- 18.85±1.06; amylase- 19.3±1.83; lipase-20.7±0.98) and minimum for 5% panchagavya (11.75±1.06; amylase- 12.1±1.55; lipase- 11.4±0.84). The enzyme activity was highest for 10% weed (protease-19.75±1.06; amylase-20.75±1.06; lipase- 19.75±0.35) and lowest for 15% weed (protease-13.25±1.76; amylase- 12.7±2.40; lipase- 11.75±2.47) on 7^{th} day as shown in Figure 3.Maximum enzyme activity of 14^{th} day was observed from 15% weed based panchagavya (protease-18.9±1.27; amylase-17.2±1.69; lipase- 23.35±0.91) than control (protease- 19.7±0.42; amylase-14.9±0.14; lipase-16.9±0.14) and minimum for 5% weed based panchagavya (protease-13.9±1.27; amylase-14.3±0.98; lipase-17.85±1.20) as explained in Figure 4. The enzyme activity was maximum at 20% panchagavya (protease-18.4±0.84; amylase-15.2±1.69; lipase-15.4±1.97) and minimum at 5% panchagavya (protease-15.85±1.20; amylase-14.45±2.05; lipase-14.05±1.48) on 14^{th} day as showed in Figure 5. Figure 6 proved the maximum enzyme activity of 10% *Eichhornia crassipes* (protease-11.9±1.27; amylase-17.8±1.20; lipase-19.7±0.42) and minimum for 5% *Eichhornia crassipes* (protease-11.65±0.91; amylase-14.85±1.20; lipase-16.75±0.35) on 14^{th} day.Sangeetha and Thevanthan (2010), depicted that spraying the seedlings with seaweed based panchagvya increased the activities of three antioxidant enzymes namely; superoxide dismutase, glutathione reductase and glutathione peroxidase in the leaves of *Arachis hypogaea, Vigna mungo, Cyamopsis tetragonoloba, Lablab purpureus, Cicer arietinum* and *Oryza sativa* var. ponni.

D. Growth parameters

From Figure 7,8 and 9 germination was more effective in 10% weed based panchagavya (100%), 5% panchagavya (85.34±0.52) and 20% weed (71.14±0.38) than the control (56.79±0.49) and was minimum for 20% weed based panchagavya (42.42±0.60), 15% panchagavya (42.26±0.51) and 5% weed (57.62±0.67).Venkatraman *et al*., (1997) experimented with the prepared extract from seaweed *Sargassum plagiophyllum* and commercial seaweed extract (SM3) and reported that liquid seaweed fertilizer promoted seed germination and enhanced early seedling growth upto a concentration of 0.75% in blackgram and 1.5% in greengram. Sharda *et al*., (2014) reported the highest percentage of germination (88%) using water hyacinth manure on wheat plant.The selected test plant, *Arachis hypoaea* was rendering to different concentrations (5%, 10%, 15% and 20%) of weed based panchagavya, panchagavya and weed. It was noted that when treated with at 5% of weed based panchagavya, the test plants have showed enhanced growth compared to control in terms of the shoot length (12.9±0.14), root length (11.75±0.35), plant height (24.65±0.49), dry weight (0.6±0.14), wet weight (2.58±0.06) and plant weight (3.07±0.04) as explained in Figure 10, 10(a). From Figure 11, 11(a) 10% panchagavya was more effective than control in terms of the shoot length (13.75±0.35), root length (12.55±0.77), plant height (25.2±0.42), dry weight (2.9±0.04), wet

weight (2.42±0.05) and plant weight (2.42±0.05). 20% weed was effective than control and had shoot length (11.4±0.56), root length (9.7±0.42), plant height (21.6±0.56), dry weight (0.61±0.04), wet weight (2.36±0.07), plant weight (2.9±0.56) as illustrated in Figure 12, 12(a).

Sharda *et al*., (2014) experimented on wheat plant using water hyacinth manure and had significant increase in the fresh weight (13.85gm), dry weight (1.24gm), biomass (12.59), root (14.75cm) and shoot length (15.99cm) when compared to control. Majid (1983) have reported the enhancement in yield of rice, corn, sesame, brinjal, onion and gourd, using water hyacinth compost, water hyacinth manure and also used in combination with other aquatic weeds. Ranjitha *et al*., (2015), experimented on brinjal plant using various concentration of panchagavya (25%, 50%, 75%, 100%) and found increased plant growth (25.2cm), leaf length (8.17cm), leaf weight (1.19g) and root length (10.2cm) for 100% treatment.

On fresh weight basis, the addition of various concentration of weed based panchagavya, panchagavya and *Eichhornia crassipes* had significantly enhanced the content of chlorophyll a, chlorophyll b and total chlorophyll. Maximum concentration of chlorophyll a, chlorophyll b and total chlorophyll was observed in 20% weed based panchagavya (12.7±0.24), 20% panchagavya (11.04±0.22) and 20%*Eichhornia crassipes* (7.22±0.16) than the control (5.23±0.07). Minimum concentration was observed in 5% weed based panchagavya (6.74±0.38), 5% panchagavya (5.53±0.28) and 5% *Eichhornia crassipes* (6.3±0.17) (Figure 13, 14, 15). Ranjitha *et al*., (2015) observed the total chlorophyll (154.97 mg/g) in brinjal plant (*Solanum melongena*) using panchagavya.

Table 1. Analysis of physiochemical parameters on *Eichhornia crassipes*

S. No	Parameters	Concentration
1	Moisture	0.855/g
2	Swelling capacity	2.5cm^3/g
3	Ash	0.16/g
4	Crude fiber	0.036/g

Table 2 Analysis of physiochemical parameters on Weed, Panchagavya and Weed Based Panchagavya

S. No	Mineral Concentration	Weed	Panchagavya	Weed based panchagavya
1	pH	6-7	5.5-6	5-5.5
2	Total Hardness (mg/L)	125	300	90
3	Calcium (mg/L)	85	80	30
4	Magnesium (mg/L)	40	280	60
5	Nitrogen (mg/L)	0.38	0.49	0.32
6	Iron (mg/L)	3.2	1.6	0.92
7	Chloride (mg/L)	486.374	238.224	168.742
8	Phosphate (mg/L)	0.05	0.0007	0.00041
9	Protein (mg/L)	2.3	3.2	2

Table 3 Total bacterial count for various concentration of Weed Based Panchagavya

S. No	Pot name	Biofertilizer preparation	Total Bacterial CountCFU/g		
			Day 1	Day 7	Day 14
1	T_0	Ordinary soil sample	TNTC	46×10^{-6}	1×10^{-5}
2	T_1	1500 g Soil + 5% Weed Based Panchagavya + 100ml of Biofertilizer	TNTC	TNTC	111×10^{-6}
3	T_2	1500g Soil + 10% Weed Based Panchagavya + 100ml of Biofertilizer	TNTC	TNTC	112×10^{-6}
4	T_3	1500g Soil + 15% Weed Based Panchagavya + 100ml of Biofertilizer	TNTC	TNTC	43×10^{-6}

5	T_4	1500g Soil + 20% Weed Based Panchagavya + 100ml of biofertilizer	TNTC	TNTC	23×10^{-6}

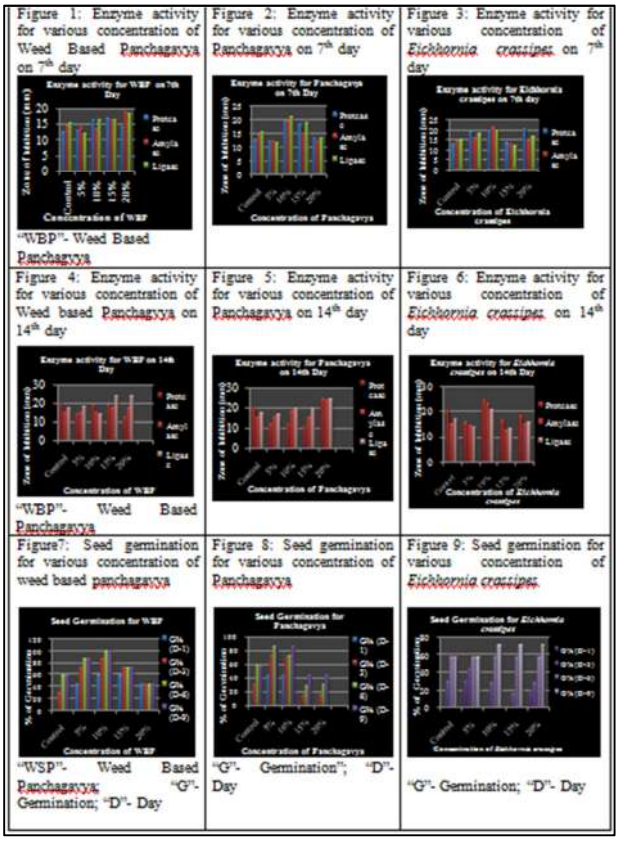
Table 4 Total bacterial count for various concentration of Panchagavya

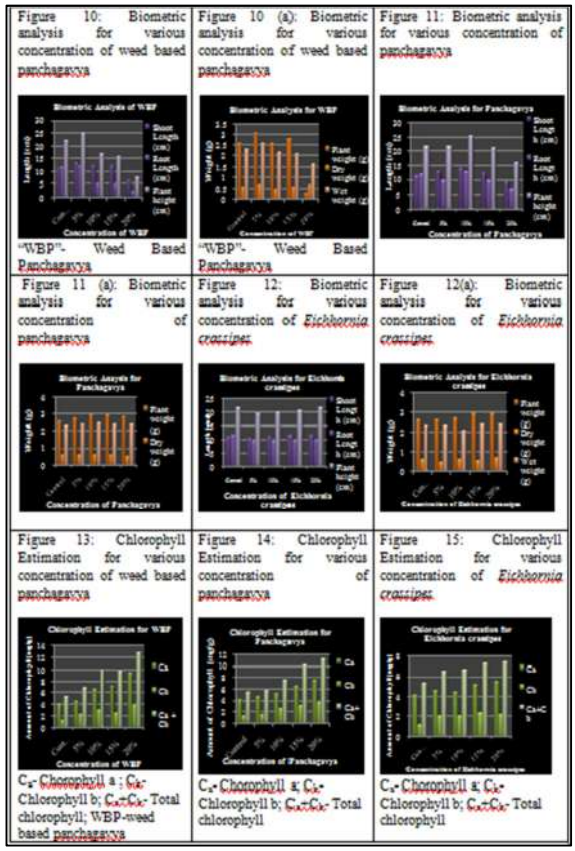
S. No	Pot name	Biofertilizer preparation	Total Bacterial Count CFU/g		
			Day 1	Day 7	Day 14
1	T_0	Ordinary soil sample	TNTC	46×10^{-6}	1×10^{-5}
2	T_5	1500g Soil + 5% Panchagavya+ 100ml of Biofertilizer	TNTC	TNTC	98×10^{-6}
3	T_6	1500g Soil + 10% Panchagavya + 100ml of Biofertilizer	TNTC	TNTC	102×10^{-6}
4	T_7	1500g Soil + 15% Panchagavya + 100ml of Biofertilizer	TNTC	TNTC	41×10^{-6}
5	T_8	1500g Soil + 20% Panchagavya + 100ml of Biofertilizer	TNTC	TNTC	22×10^{-6}

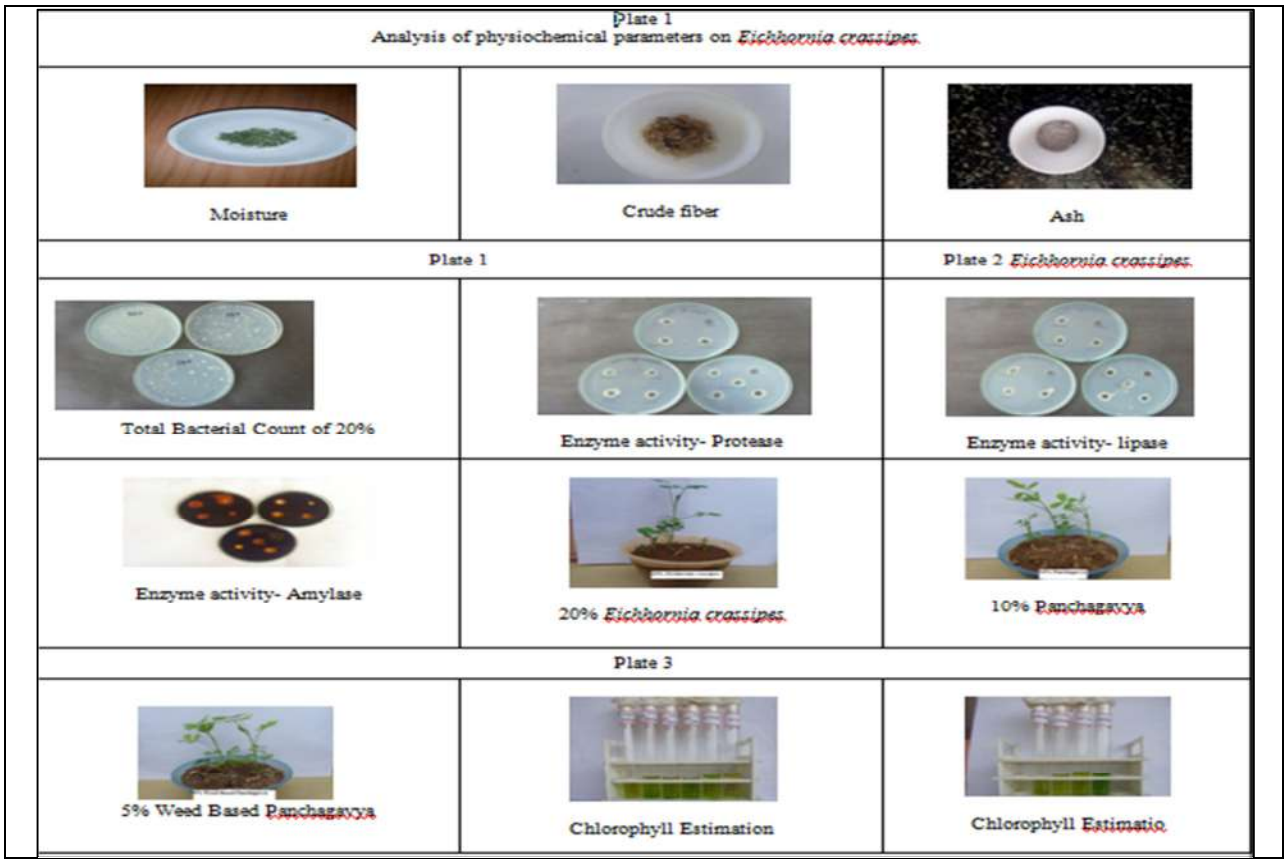
Table 5 Total bacterial count for various concentration of *Eichhornia crassipes*

S. No	Pot name	Biofertilizer preparation	Total Bacterial CountCFU/g		
			Day 1	Day 7	Day 14
1	T_0	Ordinary soil sample	TNTC	46×10^{-6}	1×10^{-5}
2	T_9	1500g Soil + 5% Weed + 100ml of Biofertilizer	TNTC	170×10^{-6}	117×10^{-6}
3	T_{10}	1500g Soil + 10% Weed + 100ml of Biofertilizer	TNTC	185×10^{-6}	112×10^{-6}
4	T_{11}	1500g Soil + 15% Weed+ 100ml of Biofertilizer	TNTC	190×10^{-6}	110×10^{-6}
5	T_{12}	1500g Soil + 20% Weed + 100ml of Biofertilizer	TNTC	170×10^{-6}	150×10^{-6}

"TNTC"- Too Numerous To Count







V. CONCLUSION

Germination was observed up to 9 days. In 10th day 10% weed based panchagavya showed 100% and 87.5% germination compared to control and other fertilizer. Biometric measurement was studied after 15th day of plant growth; it was focused on, root length, shoot length and total plant height. Maximum plant height was recorded on 10% panchagavya. Total dry weight and wet weight increased at the concentration of 5% weed based panchagavya.Soil bacterial growth was measured on 7 and 14th day. Bacterial population was measured using serial dilution method up to 10^{-6}th dilution. Good bacterial growth (CFU/g) level maintained on 20% weed extract fertilizer upto 14th day. Soil bacterial enzymes (amylase, protease, lipase) were analyzed in 7th and 14th day. Enzyme activity was estimated using standard plate assay method. No major variation was observed in enzyme production in all the pot concentration when its compare to control pot. It was indicating the bacteria utilized carbon and nitrogen source from bio- fertilizers like panchagavya, weed extract and weed based pangavya. Chlorophyll content were analysed for all the pot plants. 20% weed based panchagavya produced maximum chlorophyll content compared with other fertilizer mixing pots. Among all the parameters, 10% Weed Based Panchagavya was effective when compared to control and other biofertilizer.

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SP-7

COMPARATIVE STUDY ON ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACTS, HERBAL OILS AND COMMERCIAL ANTIBIOTICS AGAINST *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Water is a major part of our human surveillance. There are various outbreaks of disease occurs through water contamination. Water gets contaminated through improper sanitation and due to pollution too. One among the water disease is caused by *Pseudomonas aeruginosa* (*P.aeruginosa*). It also causes nosocomial infection. The strain has started to cause severe infection which are resistant to nearly all type of powerful antibiotics. Hospital associated *P.aeruginosa* causes infection like UTI, lung infection, wound infection, ear infection and septicemia. The infection becomes life threatening in individuals who have weakened immune response. This work is to bring an alternative method of herbal extract usage for antibiotics to overcome multi drug resistant microbial evolution. A comparative antibacterial activity study on herbal extract (Neem), herbal oil (thyme oil) and commercial antibiotics were tested against *P.aeruginosa*. The result showed that thyme oil gave positive result with high reactivity than tested antibiotic. The herbal extract does not stimulate any side effects compare to antibiotics which cause allergic reactions in certain individuals.

KEYWORDS*: Antibiotics, allergy, Pseudomonas aeruginosa, multi drug resistance, herbal oils, antibacterial activity*

I. INTRODUCTION

Water is essential to life. Availability of safe and good drinking water can result in significant benefits to health. Due to pollution and other factors, this water gets contaminated and serves as a source of infection and also origin of various diseases. Some species that are found in contaminated water are *Vibrio cholerae*, *Salmonella typhi*,*Salmonella Shigella, Vibrio, Campylobacter*, *Aeromonas, Pseudomonas* and *Mycobacterium sp*.

Pseudomonas aeruginosa is a Gram-negative bacterium that continues to be a major cause of opportunistic nosocomial infections, causing around 9-10% of hospital infections[1].*Pseudomonas aeruginosa* belongs to the genus*Pseudomonas(sensu stricto)*[2]. According to the Centers for Disease Control (CDC), the overall incidence of *P.aeruginosa* infections in US hospitals averages about 0.4 % and the bacterium is the fourth most commonly-isolated hospital borne pathogen accounting for 10.1 % of all hospital acquired infections.In hospital, *P.aeruginosa* finds numerous reservoirs such as in disinfectants, respiratory equipment, food, sinks, taps, and mops.

II. RELATED WORKS

The antibiotic of the class tetracycline has been presently used up for the treatment of *Pseudomonas* infection. Tetracyclines have the beneficial effects in the treatment of various diseases where excess nitric oxide has been implicated in the pathophysiology of these disease[3]. Considering about the emerging multidrug resistance strain an alternative approach of herbal extracts (Neem, Thyme oil) effectiveness on *Pseudomonas aeruginosa* against antibiotics (tetracycline) was made in this study. This main objective of this study is[4-6]to isolate and identify the pathogen from drinking water[7-9]to correlate the isolates with stool and sewage water[10,11]comparative study of herbal extracts, herbal oils and commercial antibiotics.

III. MATERIALS AND METHODS

A. Sample collection

The samples are collected from three different sources such as the stool, sewage and municipal drinking water. The samples are collected from the south – east region of Tamil Nadu in the district of Coimbatore.

B. Transport

The specimen was transported as early as possible. Refrigeration of the sample is done in the case of sewage and municipal drinking water. But the stool sample is not refrigerator due to the chance of losing some species like *Shigella.*

C. Methods

a. Serial dilution and spread plate

In order to determine the viable cells present in samples --the stool, sewage and municipal drinking water. The serial dilution of the sample was employed. This method involves decimal dilution of the samples. Once diluted the suspension is transferred into different agar medium which are employed to support the growth of these microorganisms. The diluted suspension is uniformly distributed on agar plates by the spread plate method. The dilutions of 10^{-1}, 10^{-2}, 10^{-4}, 10^{-6} are plated on Nutrient agar, MacConkey agar, Endo agar and SS agar mediums.

b. Isolation and identification of bacterial pathogens from stool, sewage and municipal drinking water samples

The colonies of each representative isolate were then characterized by standard bacteriological methods (morphologically and biochemically), sub cultured and maintained in sterile nutrient agar slants. The isolates were subjected to microscopic analysis by staining techniques and the colony morphology of the isolates was studied by growing the cultures on various agar media. The morphological identification is done on Gram staining. The biochemical characteristics of the isolates were studied by performing indole production, methyl-red, Voges-Proskauer, citrate utilization, catalase production, oxidase production, triple sugar iron agar test, starch hydrolysis, casein hydrolysis, carbohydrate fermentation and urease production tests. The identified bacterial isolates were then tested for its sensitivity and resistivity against herbal oils and commercial antibiotics.

c. Collection of herbs, herbal oil and commercial antibiotics

The herbal oils and commercial antibiotics were selected for the present study was collected from in and around Coimbatore district, Tamil nadu, South – east coast of India. The methanolic extracts of the plants were prepared by mixing 6 g of medicinal plant powder with 100 ml of 80% methanol in an airtight conical flask and kept at room temperature overnight. After 12 hours of extraction, the solution was filtered using cheesecloth and the filtrate was kept at room temperature for evaporation of methanol. The solution was filtered to get the concentrated extract.

d. Antibacterial activity by well diffusion Method:

The comparative study between herbal oils and commercial antibiotics is performed by well diffusion method (8).The nutrient agar medium is prepared and sterilized at 121ºC and poured into sterile petriplates and allowed to solidify. Wells of 6 mm were punctured using a well borer. 0.1% inoculum suspension of the test culture was swabbed uniformly over the surface of the agar. 100 µl of each herbal oil and antibiotic was loaded into the well and the plates were kept for incubation at 37ºC for 24 hours. The result was evaluated in terms of zone of inhibition, measured and recorded in millimeters.

IV. RESULTS

A. Sample collection and Transport

The samples (stool, sewage and municipal drinking water) were collected from the south-east part of Tamil Nadu, Coimbatore. The stool sample was collected in a sterile container and stored in an anaerobic condition. The other two samples were collected in a sterile container. It was transported in 10 hrs at room temperature.

B. Processing

The stock solution is prepared by transferring the sample in 100ml of distilled water.

C. Serial dilution and plating:

The stock solution was serially diluted and platted on to the specific agar plates such as Nutrient agar, MacConkey agar, Endo agar, SS agar respectively by the spread plate method.

D. Colony morphology

Plate 1: No Growth. **Plate 2:** Circular, Smooth, Opaque White Coloured Colonies. **Plate 3:** Circular, Cylindrical, Smooth White, Pink Coloured Colonies. **Plate 4:** No Growth. **Plate 5:** Circular, Cylindrical White Coloured Colonies. **Plate 6:** Circular, Cylindrical, Smooth, Translucent, White, Pink Coloured Colonies. **Plate 7:** Circular, Smooth, Opaque, White, Pink Coloured Colonies. **Plate 8**: No Growth. **Plate 9:** Circular, Smooth, Opaque White Coloured Colonies. **Plate 10:** Circular, Cylindrical, Smooth, Translucent, White, Pink Coloured Colonies. **Plate 11:** Small, Circular Pink Colonies. **Plate 12:** Circular, Smooth, White Pinkish Colonies (represented in fig 3-15).

1. Grams staining

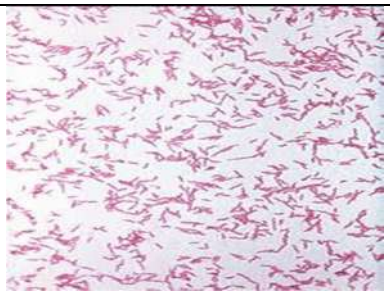

Figure 1: Colony

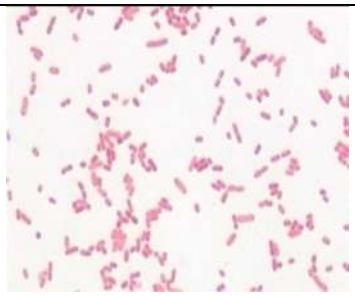

Figure 2: Colony 2

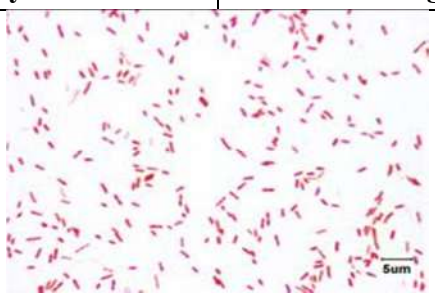

Figure 3: Colony 3

2. Drinking water sample platted on various agar Media:

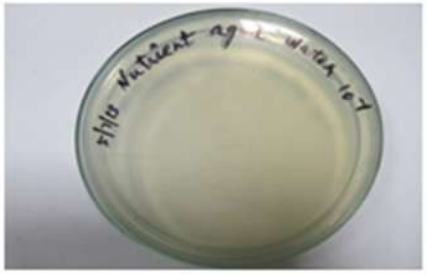

Figure 4: Plate 1(Nutrient agar)

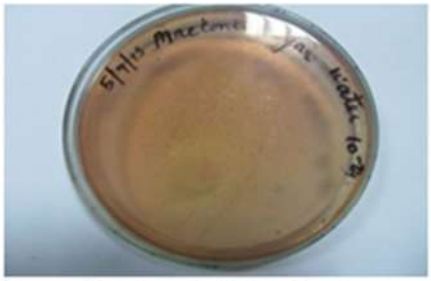

Figure 5: Plate2 (MacConkey agar)

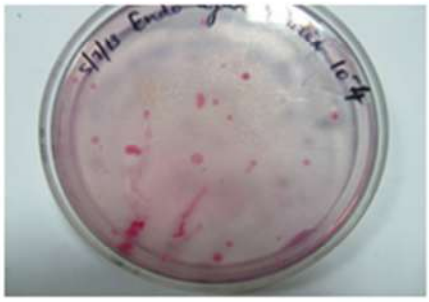

Figure 6: Plate 3 (Endo agar)

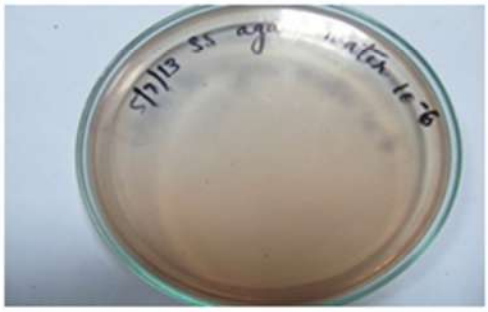

Figure 7: Plate 4 (SS agar)

3. Sewage sample platted on various agar Media:

Figure 8: Plate 5 (Nutrient agar)

Figure 9: Plate 6 (MacConkey agar)

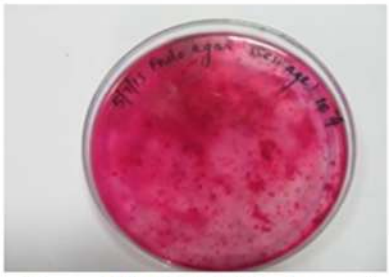

Figure 10: Plate 7 (Endo agar)

Figure 11: Plate 8 (SS agar)

4.Stool sample platted on various agar Media:

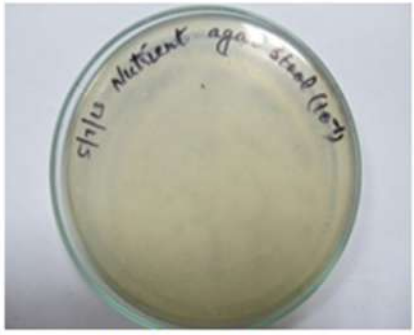

Figure 12: Plate 9 (Nutrient agar)

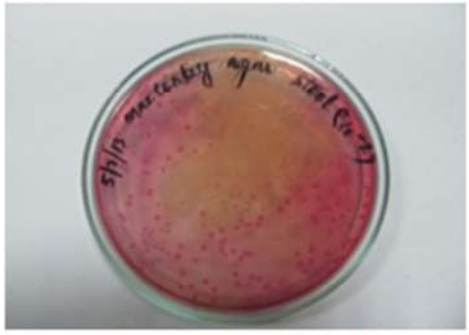

Figure 13: Plate 10 (MacConkey agar)

Figure 14: Plate 11(Endo agar)

Figure 15: Plate 12 (SS agar)

Figure 16:Plate 13: Antibacterial Activity against Pseudomonasaeruginosa

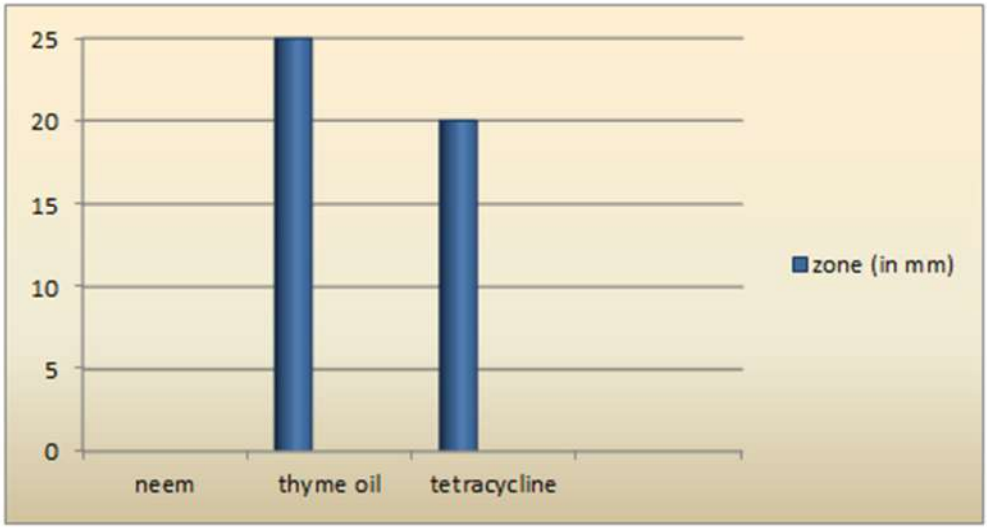

Figure.17: In this figure we can examinate that zone level of thyme oil is 25 mm and antibiotics is 20mm which shows thyme oil is highly reactive against *Pseodomonas aeruginosa*than the tested antibiotics.

*E. **Grams Staining:***

In grams staining the three different colonies were found to gram negative rods (represented in fig 1, 2 & 3).

F. Biochemical test:

The biochemical test result for the isolated organisms are tabulated below

Biochemical test	Colony 1	Colony 2	Colony3
Indole	Negative	Negative	Positive
Methyl red	Positive	Positive	Positive
Voges-proskaver	Negative	Negative	Negative
Citrate	Positive	Positive	Negative
Urease	Negative	Positive	Negative
Triple sugar iron	A/a	Ak/a	A/a
Carbohydrate	Negative	Negative	Positive
Gelatin hydrolysis	Positive	Positive	Negative
Starch hydrolysis	Negative	Negative	Negative
Casein hydrolysis	Negative	Negative	Negative
Catalase	Positive	Positive	Positive
Oxidase	Positive	Negative	Negative

With reference to the standard biochemical test results to the above mentioned results, the results were found to be 1. Colony 1 (Drinking water) - *Pseudomonas aeruginosa. 2.* Colony 2 (Sewage) - *Proteus mirabilis.* 3.Colony 3 (Stool) - *Escherichia coli*

G. Antibacterial activity results:

The antibacterial activity of the herbs and their oils against Pseudomonas was experimented by well diffusion method at which Neem (*Azadirachta indica*) showed no effect but thyme oil had the higher zone of about 25mm (represented in table 1) when compared with the commercially available antibiotic(Tetracycline) with the zone of about 20mm (represented in fig 16).

Organism	Zone of inhibition (in mm)		
	Neem	Thyme oil	Tetracycline
Pseudomonas aeruginosa	0	25	20

V. DISCUSSION

The antibacterial results proved to be high in herbal oils when compared to that of the commercially available antibiotics. Similar studies were also done with Antimicrobial activity of thyme oil against multidrug resistent strains of *Pseudomonas sp.,*(3). Studies explaining the effectiveness of thyme oil has also been carried out concluding its effectiveness against various pathogens (9).The modulation activity of commercial antibiotic was also discussed (10). Hence this paper brings out the alternative use thyme oil to the commercially used antibiotic for the treatement of *Pseudomonas aeruginosa* infection which prevents the side effects and also is available at recent costs.

VI. CONCLUSION

The reasons for arise of the epidemic diseases may be due to improper sanitation, environmental issues etc.Proper sanitation and taking the steps for maintenance of hygiene and proper health has to be followed for maintaining proper health. This paper gives an alternative approach for treating diseases with herbs in order to prevent the side effects of using chemical antibiotics. *P.aeruginosa* is one of the persistent and recurrent opportunistic pathogen responsible for life threatening recurrent infections in patients with CF.(4)(11) Despite advances in antimicrobial therapy, the mortality and morbility associated with *P.aeruginosa* indused UTIs remain significantly high(6) .The comparative antibacterial activity was tested for the herbs, herbal oils and commercial antibiotics against the identified pathogen by the well diffusion method and herbal oils (thyme oil) showed high activity when compared to the commercial antibiotic to the identified pathogen so it can be considered for the treatment of *Pseudomonas aeruginosa* infection.

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SP-8

A NOVEL APPROACH OF PROTEASE ENZYME PRODUCTION USING TANNERY EFFLUENT AS A SUBSTRATE

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ABSTRACT

Leather industry is one of the major industrial sectors of India. Protease enzyme producing microorganism was isolated from tannery effluent and identified on the basis of morphological cultural, and biochemical characterization. Optimization study was done for analyze the various parameters such as pH, bacterial inoculum, carbon source, nitrogen source and agro industrial waste. The optimum growth was found on 8 pH, carbon source- maltose, beef extract as nitrogen source and orange peel in agro industrial waste and the enzyme have the destaining property in this study.

KEYWORDS: ***Protease, Tannery effluent, Optimization, Destaining property***

I. INTRODUCTION

Enzymes have various applications and widely found in many sources like plants, animals and microbes. They are commercially used in the detergent food, pharmaceutical, diagnostics and fine chemical industries. These enzymes mainly function in a narrow range pH, temperature, and ionic strength[1-3].Proteolytic enzymes are of great commercial importance because it contributing to more than 40% of the world's commercially produced enzymes that accomplished the enzymatic unhairing[4-6].

II. RELATED WORKS

Approximately 50% of the enzymes produced are used for industrial process[7-9]. Microbial proteases are the most important hydrolytic enzyme, so it is widely used for physiological and commercial process[5]. Although proteases have been identified from different sources and they have used more for industrial purposes. Tanneryeffluent give good potential bacteria that could produce protease active at dual extremities of alkaline pH and high temperature because they produce alkaline in nature[10-12]. The present study focused on isolation of microorganism from tannery effluent (in and around erode district). The optimization study was performed to analyse on various parameters such as pH, bacterial inoculum, carbon source, nitrogen source and agro industrial waste and enzyme destaining property[13].

III. MATERIALS AND METHODS

a. Sample collection: Tannery sludge and effluent were collected in sterile container from leather processing company in and around Erode district.

b. **Isolation and identification of bacteria from tannery sludge**: Sample was serially diluted and test taken for morphological and biochemical characterization study.

c. Optimization of enzyme production using various parameters: The effect of pH on protease production was assessed in various pH ranging from 7.0, 8.0, 9.0, 10.0. Effect of Bacterial inoculum concentration ranging from 0.5%, 1%, 1.5% and 2%. Effect of various carbon sources such as lactose, glucose and maltose at the level of 0.5%, 1%, and 1.5%, incubated at room temperature for 24 hours, 48 hours and 72 hours. Effects of various nitrogen sources such as beef extract, gelatin and potassium nitrate at the level of 0.5%, 1% and 1.5% and incubated at room temperature for 24 hours, 48 hours and 72 hours. Effects of various agro industrial waste sources such as orange peel, black gram husk and sugarcane baggase at the level of 0.5%, 1% and 1.5%, and incubated at room temperature for 24 hours, 48 hours and 72 hours. The enzyme activity of each parameter checked separately.

d. Standard estimation of Protein: Enzyme activity was assayed using casein as the substrate. The reaction mixture consisted of 20% of casein and 0.15 ml of enzyme solution and stopped by adding TCA then the precipitate was removed by centrifugation, the supernatant was taken and 5 ml of 0.44M Na_2CO_3 and 0.20M Na_2HCO_3 was added and its absorbance was measured at 660nm.

e. Standard estimation of protease assay: Caseinase activity was assessed by the modified procedure of using 2% casein. Casein solution (0.5ml) with an equal volume of suitably diluted enzyme solution was kept in the water bath and 10% TCA was added. The mixture was centrifuged and to the supernatant 5 ml of 0.44M Na_2CO_3 and two-fold diluted Folin Ciocalteau reagent was added. The absorbance was measured at 660 nm in calorimeter.

f. Estimation for protein: Extracellular protein was estimated by Lowry method and absorbance was measured at 660nm in calorimeter.

g. Partial purification: The crude enzyme was first saturated upto 20% with solid ammonium sulfate and then centrifuged, further saturated upto 70% with solid ammonium sulfate and again centrifuged. The pellets obtained were dissolved in minimum volume of 0.1M phosphate buffer; pH 6.2 these solutions were dialyzed against 500 ml of the same buffer at 4°C to remove the excess salt. By using modified Reese's method the proteolysis activity was determined.

h. Destaining of blood: The cloth were stained with blood and allowed to dry for few minutes. Before immersing the cloth in enzyme, it is treated with 2% formaldehyde and washed with water. Then the cloth pieces were immersed with 5 U/ml of partially purified enzyme and incubated at 40-50^{0}C. Distaining was observed at specification time interval. After every time interval, the cloth pieces were rinsed with water and dried. The same procedure was done for the control except incubation with the enzyme solution.

IV. RESULT AND DISCUSSION

Morphological and biochemical test result the organism similar to *Klebsiella sp.*Figure 1 and 2 explained that the maximum total enzyme production (5500 µg/ml) and protease production (240µg/ml) was observed at pH 8.Similar results were reported by Kinjal *et al*., 2016 that his results proved that the alkaline protease was produced in neutral to alkaline pH. The metabolic activity of microorganisms was very sensitive to the change in hydrogen ion concentration of their environment. Protease production was affected if pH level was higher or lower compared to the optimum value. Maximum activity for protease production was obtained by *Bacillus sp*. at pH 8. Figure 3 and 4 revealed that the effect of bacterial inoculum on enzyme production. Maximum enzyme production (Protein) and Protease production was found at 2% of inoculums concentration such as 900µg/ml and 260µg/ml respectively.

Table 1 and 2, show the various Carbon sources on enzyme production, such as Lactose, Glucose and Maltose. 0.5%, 1% and 1.5% of each carbon sources were taken for the study. Out of three different carbon sources, Maltose was found to be the best carbon source, allowing maximum total enzyme production (9500 µg/ml) and protease production (450µg/ml) at the concentration of 1.5% (Figure 5 and 6). Similar results were reported by Tuhina *et al*., 2016 based on both results that we concluded Maltose found to be the best carbon source for maximum protease production. Different Nitrogen sources are taken for the protein and protease production. Gelatin, Beef extract and Potassium nitrate (0.5%, 1% and 1.5%) were chosen for the experiment (Table 1 and 2). Based on enzyme production that we concluded the Beef extract resulted in the highest Total enzyme production 4700 µg/ml and protease production 370µg/ml at 1.5% concentration (Figure 7 and 8), followed by 1.5% concentration of Gelatin Total enzyme production 927.5µg/ml and protease production 340g/ml. Tuhina *et al*., 2106 reported the maximum protease production in presence of Beef extract. That we concluded the best Nitrogen source for protease production was Beef extract for *Klebsiella sp*. Several researchers have also reported that organic Nitrogen sources were found better for enzyme production than inorganic sources[4].Various Agro Industrial wastes are added to the medium (Table 1 and 2). After 72 hours of incubation. Production medium containing 0.5%, 1% and 1.5% of Sugarcane baggase, Orange peel and Black gram husk, compared to all other Agro wastes. Orange peel (1.5%) produced highest enzyme activity presented in Figure 9 and 10. The total enzyme production was found to be increased 13500µg/ml and protease production was 760µg/ml. Various Agro Industrial wastes are added to the production medium. After 18 hours of incubation production of enzyme in Orange peel 310 U/ml, Rice bran 320 U/ml, Wheat husk 610 U/ml, Sugarcane baggase 390 U/ml and for Raw potato starch 400 U/ml. The maximum production of enzyme was seen in Wheat husk (610 U/ml) reported by Ashok raja *et al*., 2012.

For the larger production of protease, the best optimized parameters were chosen for the enzyme production (Table 3 and 4). pH 8, 2% of Bacterial inoculum, 1.5% of Lactose, Maltose and Orange peel added to the medium with the supplementation of Tannery effluent. After 72 hours of incubation the partial purification was carried out by Dialysis method. The dialysed enzyme was treated for blood stain removing purpose. Goat blood was applied to the cloth and washed with water as a control. Compared to water, maximum amount of stain were removed by protease enzyme (Fig 9). The result is far better comparable to the earlier reports of protease from *Pseudomonas aeruginosa*[8].

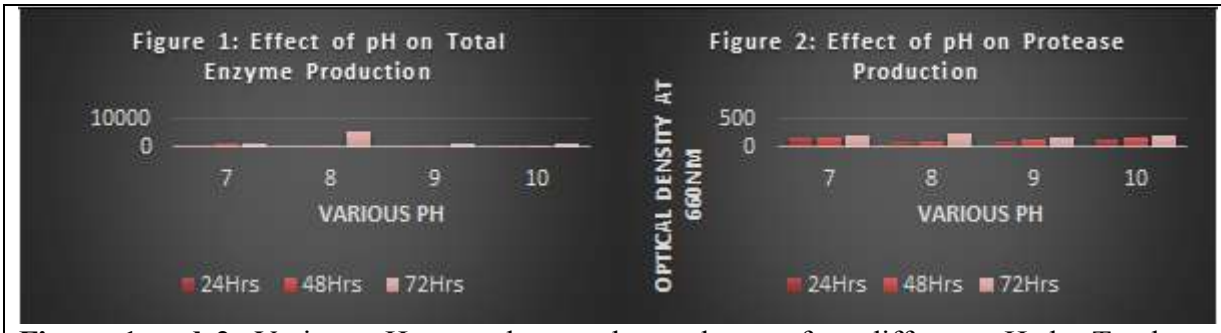

Figure 1 and 2: Various pH was taken to the study, out four different pH the Total enzyme production and Protease production was effective in pH.

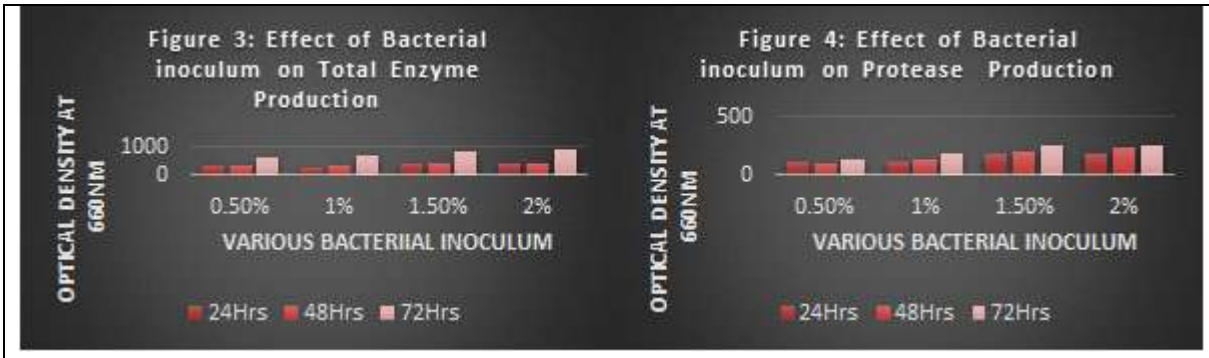

Figure 3 and 4: Different bacterial inoculum concentration was taken for the study, the maximum Total enzyme production and Protease production was found in 2% of bacterial inoculum concentration

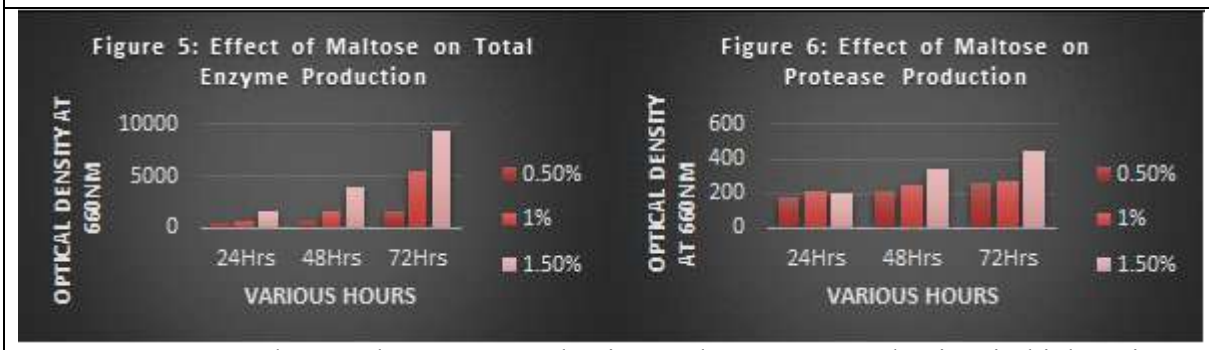

Figure 5 and 6: The Total enzyme production and Protease production is highest in Maltose when compared with Lactose and Glucose

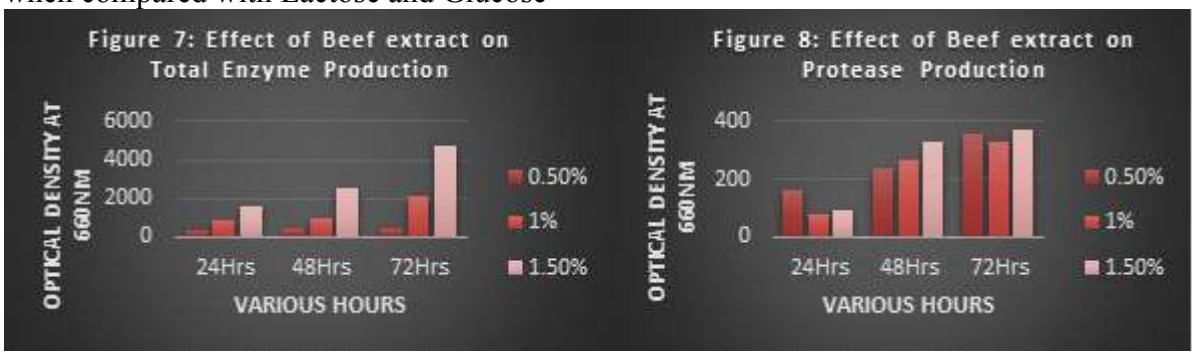

Figure 7 and 8: Beef extract produced maximum Enzyme production and Protease production when compared with Potassium nitrate and Gelatin

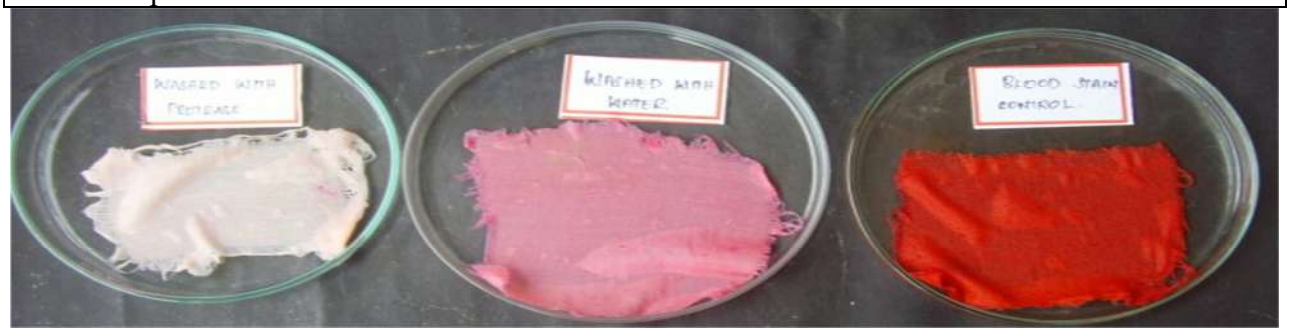

Figure 9: Destaining of cloth

Table 1:Protease assay

S. No	Test	24Hrs			48Hrs			72Hrs		
		0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
1.	Lactose	433	170	433	775	2000	750	850	5500	2675
2.	Glucose	500	642.5	195	642.5	750	395	1150	5000	1725
3.	KNO_3	425	600	625	625	625	925	825	900	927.5
4.	Gelatin	485	335	320	625	500	470	837.5	700	625

5.	Black gram husk	1150	1940	1650	1595	3950	6250	2400	4175	3200
6.	Sugarcane baggase	1000	2000	1750	1700	3500	5375	1850	4175	6250

Table 2: Protease assay

S.No	Test	24Hrs			48Hrs			72Hrs		
		0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
1.	Lactose	130	280	180	220	290	320	240	220	330
2.	Glucose	180	240	250	240	280	300	260	340	310
3.	Gelatin	140	140	120	250	300	260	320	310	340
4.	$KN0_3$	160	140	100	170	170	180	280	310	220
5.	Black gram husk	200	220	400	380	360	340	300	460	440
6.	Sugarcane baggase	230	300	280	420	310	300	240	320	450

IV. CONCLUSION

The isolate was identified as a potent protease producing bacterial species from tannery effluent. The protease activity was optimized using pH, bacterial inoculum, carbon source, nitrogen source and agro industrial waste. The enzyme is used for destaining and commercial purpose.

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We thank to the Management of Sri Krishna Arts and Science College, Coimbatore, Tamil Nadu, India for providing necessary facilities to carry out this work.

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SP-9

BENZENE DEGRADATION BY WILD TYPE OF SPHINGOMONAS URSINCOLA

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ABSTRACT

Benzene is found to be the major component found in crude oil and cigarette smoke. Humans are exposed to benzene regularly which has multiple effects and practically affects all the systems of the body. At high levels benzene in the air can cause cancer, even comma or death. Hence, this research is focused on degradation of benzene by bacteria named *Sphingomonas ursincola.* The major objective is to isolate and incorporate the plasmid of a benzene degrading bacteria into the competent *E. coli* DH5α cell. The samples were collected from benzene contaminated soil and water. The isolates were selected based on screening with different concentrations of benzene like 6% and 8%. Plasmid isolation was done for the highest degrading isolates. Among the two recombinants, only one was able to transform and degrade benzene. The isolated bacterium was exposed to benzene stress and the results show that the bacterium was able to fully degrade benzene in the media after 3 days. The bacterium from sample was identified from various tests which expresses the *Sphingomonas sp.* This bacterium was identified as *Sphingomonas ursincola* by biochemical tests and 16s rRNA sequencing. This helps in the degradation of benzene and hence this organism can be used for degradation of multiple organic contaminants.

KEYWORDS: ***Sphingomonas ursincola, Benzene Biodegradation, Recombinant, E. coli DH5α***

I. INTRODUCTION

Benzene exposure has been a major health issue for many decades. Benzene (C_6H_6) is a volatile compound known for its carcinogenic effects. It is highly inflammable. It is commercially used along with other hydrocarbons like toluene and xylene[1].Benzene is also a component of crude oil, gasoline, and cigarette smoke[2].Exposure of this solvent happens through many ways viz., air, soil and water. Chronic exposure of benzene can lead to reproductive, neurological and endocrinal problems[3]. The exposure of benzene through petrol bunks and garages also has harmful effects. Usually this happens due to leaks from the petrol storage tanks or spills of petrol and diesel around garages. Most of the benzene evaporates into the air, but a small amount gets diffused into the soil. In case of petrol bunks, the groundwater is affected the most[4].Exposure through any of the above ways can affect the health and well being of people who live in these polluted regions. The best solution for this is bioremediation[5-8]. Microorganisms are used to degrade the aromatic hydrocarbons using aerobic and anaerobic pathways. Microbial aerobic degradation is achieved by incorporation of molecular oxygen into the inert hydrocarbon molecule[9-13].These intermediates are further broken down or oxidized via the Krebs cycle. Bacteria can use the compounds as sole source of carbon and energy [6]. This process is used in many bio filters and bioremediation techniques[14,15]. There are more than 200 species of bacteria found in non- contaminated soil that can degrade benzene and these are used for many purposes, but studies say that the bacteria isolated from contaminated water and soil, which are indigenous to those regions, are more capable of dealing with environmental and degrading conditions[6]. Hence, the objective for this study is to collect and enumerate the water and soil samples from contaminated areas and 16s rRNA sequencing is executed to screen benzene from the organism.

II. METHODS AND METHODOLOGY

I. **Sample isolation:** The soil samples were collected near to garages and the water sample was collected near to industrial area (in and around Bangalore). The colonies were enumerated and isolated in selective media-Bushnell Haas broth. The three samples were collected. The first sample was collected from soil around a garage which seemed to be contaminated from old petrol and oil. The second sample

was collected from soil from a different garage which was contaminated from engine oil. The third sample was collected from a lake in a clean glass bottle. The samples were taken to the lab immediately.

II. **Morphological and biochemical test:** Serial dilution for the samples was done within three hours after collection. The aliquots from 10^{-2}, 10^{-3} and 10^{-4} were spread on Nutrient Agar plate for enumeration.Morphological and Biochemical characteristics study were done.

III. **6%&8% Benzene screening:** The cultures were inoculated in B.H. broth with 6% &8% benzene concentration. The broth did not have carbon source other than benzene. They were incubated at 37ºC in shaking incubator.The colonies which degraded benzene the best were selected for plasmid isolation.

IV. **Isolation of plasmid:** The plasmid was isolated with the procedure given[7]. The bacterial colonies which degraded 8% benzene were inoculated in 50ml of L.B broth and were kept for overnight incubation in shaking incubator. The overnight grown cultures were transferred to centrifuge tubes and were kept in ice water for 5 minutes. The pellet was obtained by centrifugation at 6000 RPM for 8 minutes at 4ºC. 150µl of E buffer was added and the pellet was resuspended in it. 300µl of Lysis solution was added and mixed well. The contents were transferred to 2ml eppendorf tubes and kept for incubation in water bath at 60ºC for 60 minutes. Then 900µl of phenol-chloroform (1:1) mixture was added to the tubes and they were mixed well. Centrifugation was done at 6000 RPM for 8 minutes and the supernatant was collected. The supernatant was treated with chloroform-isoamyl alcohol (24:1) and mixed gently. Following centrifugation (1000 RPM for 10 minutes) the supernatant was transferred to new eppendorf tubes. 3M sodium acetate ($1/10^{th}$ volume of supernatant) was added and double the volume of absolute ethanol was added. This was stored overnight around -20ºC. Following this, the pellet was obtained by centrifugation (12000 RPM for 10 minutes) and washed with 70% ethanol. After the final centrifugation the supernatant was discarded and pellet was air dried. It was then suspended in 15µl of T.E. buffer and stored at -20ºC.

V. **Agarose gel electrophoresis:** 0.8% agarose gel was prepared using TAE buffer. The precipitate (20 µl) was mixed with 3.5µl of loading buffer (0.25% Bromophenol blue, 30% glycerol). This was loaded in the wells and gel was run.

VI. **Competent cell preparation:** 100µl of *E.coli* DH5α was inoculated in two tubes of 10 ml of L.B. media, in which one tube contained 8% benzene (The inoculation in broth, with the benzene, was done to show that *E.coli* DH5α cells were not capable of degrading benzene). The tubes were kept for overnight incubation at 37ºC. The grown cultures were transferred to a bottle of sterilized L.B media and were kept for 3 hours incubation at 37ºC. The grown cultures were transferred to centrifuge tubes and centrifuged at 6000 RPM for 8 minutes. After discarding the supernatant, the pellet was washed with 2ml of chilled Calcium chloride (0.1mM). The tube was kept in ice for 30 minutes incubation after which it was subjected to centrifugation and washing with calcium chloride (0.1mM). This was then stored in refrigerator for transformation.

VII. **Transformation:** Three sterilized 1.5ml eppendorf tubes were taken and in each tube 150µl of competent cell cultures were added. The isolated plasmids (4µl) were suspended in two of the eppendorf tubes and the third one was left as it was. They were incubated in ice water for 20 minutes after which they were subjected to heat shock treatment. This was done by placing the tubes in hot water (42ºC) for 100 seconds. Then the tubes were placed in cold water for 100 seconds. 900µl of L.B media was dispensed into the eppendorf tubes and they were incubated for 1 hour. Following centrifugation, the supernatant was discarded till only $1/4^{th}$ remained. The pellet was mixed thoroughly using sterile microtips. The pellet with only the DH5α cells was inoculated in B.H. broth having 8% benzene. The pellet containing the competent cells along with the plasmids were inoculated in two B.H. broth bottles, one with 6% benzene and the other with 8% benzene. These bottles were incubated at 37ºC for 24-48 hours. The protocol for competent cell preparation and transformation was done following the Basic Protocol[8].

III. RESULT

A. **Morphological and biochemical test:**Twelve colonies were altogether isolated from the samples.The Gram Staining result is given in the Table 1.Seven colonies showed growth in 6% benzene (Fig 1).The

biochemical tests for the seven colonies were carried out. The results are given in Table 2 and Fig 2 (a-f)After 24 hrs of incubation of the isolated culture, growth was observed in isolates Fig 3(a & b) in 8%benzene. The plasmid was isolated from the selected two cultures.In agarose gel electrophoresis, the gel was placed under UV and the thin band was observed which showed that the plasmid was present (Fig 4).

B. Transformation: The E. coli DH5 alpha cells were grown in L.B. media, but the growth was not seen in the broth with 8% benzene Fig 5(a). This shows that the competent cells do not have the ability to degrade benzene or any other characteristics that could interfere in the expression of the plasmid. The grown culture was washed twice with chilled 0.1mM Calcium chloride and was used for transformation. The plasmid isolated before was dispensed along with 150µl of the competent cells in eppendorf tubes. These were then subjected to Heat shock treatment for 100 seconds. During this time period, the calcium chloride present helped in expansion of the pores so that the plasmid could enter the competent cell. After this, L.B. media was added in the tubes so that the cells could grow. It was incubated for 1 hour at 37ºC. The pellet was obtained and it was pipetted into three bottles of 50 ml B.H. broth. One bottle contained only the E. coli DH5 alpha cells with 8% benzene concentration (which was selected as control), while the other two contained benzene in concentrations of 6% and 8%. No growth was observed in the bottle containing only DH5 alpha cells with 8% benzene. Transformation occurred only in the bottles having bacterium of sample 3 Fig 5(b) and 5(c). This was seen by the turbid growth and lack of oil bubbles in the media. The surface of the media appeared less shiny, indicating that benzene was degraded and used. The isolate from sample 3 showed better degradation. The bacterium from sample 3 was identified from biochemical tests to belong to the *Spingomonas sp.* This bacterium was sent for 16s rRNA sequencing at Yaazh Xenomics, Madurai, Tamil Nadu. The analysis showed that the organism was homologous with *Sphingomonas ursincola*. The phylogeny tree and BLAST analysis is given in Fig 5(d) and (e).

TABLE1 Gram staining results

SAMPLE 1		SAMPLE 2		SAMPLE 3	
Colony	Morphology	Colony	Morphology	Colony	Morphology
1	+, Rod	1	-, Rod	1	+, Cocci
2	+, Rod	2	+, Cocci	2	+, Cocci
3	+, Cocci	-	-	3	-, ovoid
4	+, Cocci	-	-	-	-
5	+, Cocci	-	-	-	-
6	-, Cocci	-	-	-	-
7	+, Cocci	-	-	-	-

TABLE 2 Biochemical test result

Sl. No.	Colony	Catalase	Oxidase	Indole	MR	VP	Citrate	Nitrate	Urease
S	A		M	P		L	E		1
1	S13	+	+	-	+	-	+	+	+
2	S16	+	+	-	-	+	-	+	-
3	S17	+	+	-	-	+	-	-	-
S	A		M	P		L	E		2
1	S21	+	+	-	+	-	+	-	-
2	S22	+	+	-	+	-	+	-	-
S	A		M	P		L	E		3
1	S31	+	+	-	+	+	+	+	-
2	S33	Weak reaction	+	-	-	+	-	+	-

Abbreviation of S13 – Sample 1, colony 3.

Figure 1. 6% benzene degradation.

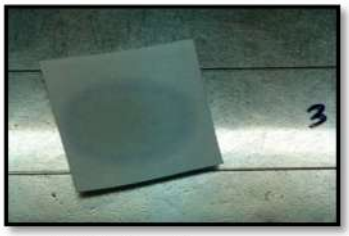

Figure 2(a) Oxidase Test

Figure 2 (b) Catalase Test

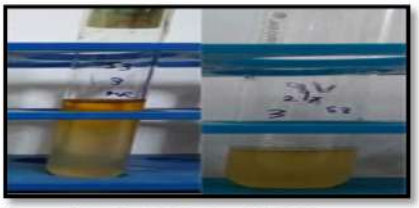

Figure 2 (c) Methyl Red and VP Test

Figure 2 (d) Indole and Citrate Test

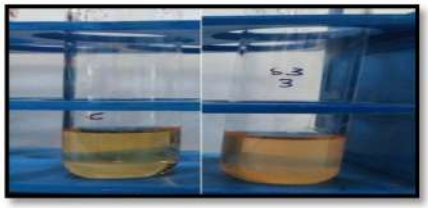

Figure 2 (e) Urease Test

Figure 2 (f) Nitrate Test

Figure 3(a) 8% degradation of first isolate

Figure 3(b) 8% degradation of second isolate

Figure 5(b) Transformation result of first isolate.

Figure 6 Complete degradation of 8% benzene bywild type of *Sphingomonas ursincola.*

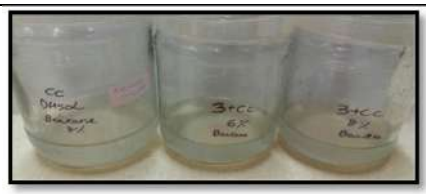

Figure 5(c) Transformation result of second isolate.

Figure 4 Band formation to check the presence of plasmid. A- sample 1. B- sample 3.

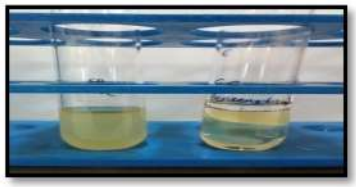

Figure 5(a) Inability of CC to grow in benzene.

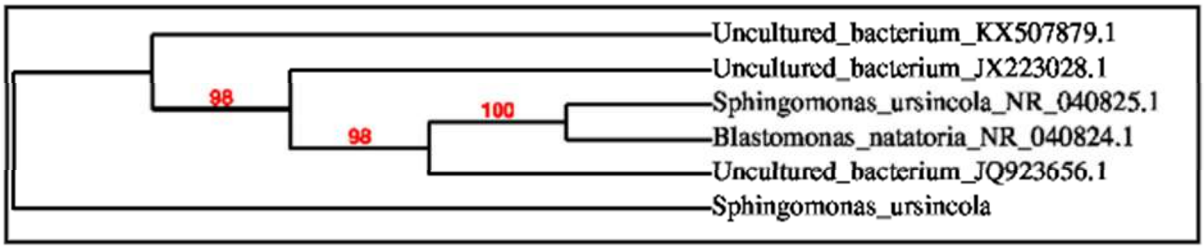

Figure 5(d) Phylogeny Tree

>Contig1

CCAAAGAAAAACAACTATTTTAGAGTTTGGATCATGGCTCAGAACGAACGCTGGCGGCATGCCTAACACATGCAAGTCGAACGAAGGCTTCGGCC
TTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTGGGTTCGGAATAACAGTGAGAAATTACTGCTAATACCGGATGATGACTTCGGTC
CAAAGATTTATCGCCCAAGGATGAGCCCGCGTAAGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCTTTAGCTGGTCTGAGAGG
ATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCA
ATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCAGGGATGATAATGACAGTACCTGGAGAATAAGCTCCGGCTAACTCCGT
GCCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCCATTCAAGTCAGAGGTGAAAG
CCCGGGGCTCAACCCCGGAACTGCCTTTGAAACTAGATGGCTTGAATCTTGGAGAGGCGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGAT
ATTCGGAAGAACACCAGTGGCGAAGGCGACTCGCTGGACAAGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG

GTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCGGGTTCATGGAACTTGGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACG
GTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGC
GTTTGACATGTCTAGTATATTTCCCAGAGATGGGTTATTTCAGTTCGGCTGGCTAGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCTTTAGTTGCCATCATTCAGTTGGGCACTCTAAAGAAACCGCCGGTGATAAGCCGGA
GGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACACGCTGGGCTACACACGTGCTACAATGGCGGTGACAGTGAGCAGCTAGATCGCGAG
ATCATGCTAATCTCAAAAAGCCGTCTCAGTTCGGATTGTTCTCTGCAACTCGAGAGCATGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGC
CGCGGTGAATACGTTCCCAGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGATTCACCCGAAGGCGCTGCGCTAACCCGTAAGGGAGGC
AGGCGACCACGGTGGGTTCAGCGACTGGGGTGAAGTCGTAACAGGGTAAACCGTAAATTTTTTTTACTAATTTTAT

Figure 5(e) BLAST Analysis. The complete sequence analysis showed 97% similarity with *Sphingomonas ursincola*.

IV. DISCUSSION

Benzene pollution is a widely known phenomenon and it poses a threat for anyone exposed to it. Bioremediation is the most efficient solution for this problem. The aim of bioremediation is to overcome the limiting factors that slow down biodegradation rates[5]. This can be done by many techniques. Bacteria which can degrade these contaminants efficiently are often used in these techniques. In this study, the isolated bacterium was exposed to benzene stress and the results show that the bacterium was able to fully degrade benzene in the media after 3 days (Fig. 8). To enhance the metabolic efficiency of microorganisms for specific environmental applications, biomolecular engineering is carried out to construct a microorganism with desirable catabolic pathways using recombinant DNA technology[12]. The plasmid was isolated from *Sphingomonas ursincola* it was transformed into an *Escherichia coli* DH5α cell. When this recombinant was introduced to a media with 6% and 8% benzene as the only carbon source, the degradation was not as efficient as the wild type. Even this can be remedied. One successful method is to incorporate different plasmids into one recombinant cell. The construction of recombinant strains for the mineralization of chloroaromatics by patchwork assembly is reviewed, in which pathways obtained from various bacteria were combined via conjugation into a single recombinant host to provide a complete pathway for mineralization of particular compounds. The same could be done in this case. The pathway of benzene degradation needs to be studied in *Sphingomonas ursincola.* Once this data is found, a recombinant can be created with the genes taken from *Sphingomonas ursincola* as well as genes from another bacterium which degrades benzene using pathways different from *Sphingomonas.* This genus of bacteria has been used before for the degradation of Polycyclic Aromatic Hydrocarbons[9].

V. CONCLUSION

Studies have shown that the organisms of this genus can be used for degradation of aromatic hydrocarbons also. As the rate of degradation happens to be slow for this bacterium, biosurfactants can be used to enhance the degradation. In this study the recombinant showed a slower degrading rate. It was observed that the addition of biosurfactant increased the biodegradation efficiency for slow-degrading consortia [11]. This means that, if a consortium was made using the recombinant organism their rate of degradation could be increased by the addition of Biosurfactants. The organisms of the genus *Sphingomonas* contain glycosphingolipids [13] instead of the common Lipopolysaccharides. Most of the organisms belonging to *Sphingomonas* are non-human pathogenic, which makes it safer to use this for bioremediation. As they contain glycolipids they could be used for biosurfactant production. Organisms of this genus have been used also in the bio-precipitation of uranium using alkaline phosphatase [14]. Ławniczak *et al* [11]put forth steps to consider when designing a biosurfactant mediated process.

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SP-10

OPTIMIZATION OF SURVIVAL AND SPORE FORMATION OF *PAENIBACILLUS POLYMYXA* DURING PRODUCT STORAGE

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ABSTRACT

Paenibacillus polymyxa is an endospore-forming bacterium that is found in environments. It is nonpathogenic and used as bio fertilizer which can promote plant growth in agricultural ecosystem. The quality of bio fertilizer was affected, if both the bacterial growth and room quality control was not maintained properly. Spore cycle preserve the bacteria from starvation, drying, freezing, chemicals, and heat. On favorable conditions, the spores germinate, with each spore again becoming a vegetative cell with the ability to reproduce. The product is stored in spore form, which should not revive in order to protect the product from spoilage. The present study was to maintain the spore for long time without reviving till transit. *Paenibacillus polymyxa* was treated with three different organic acids such as Manganese chloride, Sorbic acid and potassium dihydrogen phosphate at 19, 20 and 21 mm concentration. Sorbic acid at 20 mm concentration gives more spores and it germinates faster than other two treatments. Sorbic acid can give the highest survival rate of bacteria and spore formation during storage of biofertilizer.

KEYWORDS: ***Paenibacillus polymyxa, organic acids, biofertilizer***.

I. INTRODUCTION

Paenibacillus polymyxa is a Gram-positive, rod-shaped bacterium, which is also motile. It achieves motion via peritrichous flagella[1]*aenibacillus polymyxa* is an endospore-forming bacterium that is non-pathogenic and found in environments[2-4]. The soil level and porosity is improved due to organic compounds released from *P. polymyxa*[5-7]. Organic acids can affect the heat resistance of spores[2,8]. This microbe has a role in ecosystem function and potential role in industrial processes[3]. Secondary metabolites of *P. polymyxa* have wide applications in agricultural ecosystems, bio preservation in food and medicine industry, bioflocculation in waste water and mineral processing[9].The problem occurs during storage of bio fertilizer were the growth quality control and room quality control. The present study was focused on *Paenibacillus polymyxa* spore induction test and germination process. The growth of bacteria was compared with three organic acids with different concentrations.

II. MATERIALS AND METHODS

a. **Morphology and Biochemical characteristics:** *Paenibacillus polymyxa,* industrial strain was used for the present study. Morphological and Biochemical test were performed for the selected bacteria.
b. **Determination of growth curve**:Turbidimetric analysis was done todetermine the generation time of *Paenibacillus Polymyxa* and interpreted using graphical analysis.
c. **Spore Induction and germination test**:Organic acids in different concentration (19, 20 & 21 mm) were mixed in nutrient broth (ie) Manganese chloride, sorbic acid, potassium dihydrogen. After sterilization *Paenibacillus polymyxa* was inoculated and reading was taken from 0^{th} hour. The readings are taken using hemocytometer. The count of spore & vegetative cells of treated *Paenibacillus polymyxa* in different organic acid, in different concentration and in different interval of time was interpreted in graphical analysis. In spore germination test, the bacteria were added to nutrient broth containing acids of selective concentration. After incubation for 48 hours the germination test was done by plating technique. The results were noted.

III. RESULTS AND DISCUSSION

A. **Morphology and Biochemical characteristics:** Under staining techniquesselected bacteria was found as gram positive, endospore forming bacteria Plate (1.1&1.2). The biochemical characteristics showed positive results for *P.polymyxa*. (Plate1.3 to1.6)

B. **Determination of Growth curve:** The growth of *P.polymyxa* can see in 7hours and spores started to form at 24 hours and after 48 hours rough colonies of spore have formed (A. **E.** FRANCIS **AND** *JOAN* E). The shows the growth of *p.polymyxa* and optical density is interpreted in (Fig1).

C. **Spore induction test**: The spore induction test has done and it gives the different count in different organic chemical and the readings are tabulated by CFU/µL value and it is plotted in graph.*Cfu=Total no of colonies/Volume of sample× Dilution factor*
The Count of spore and vegetative cell in Manganese chloride, Sorbic acid and potassium-di-hy-phosphate at 19, 20 & 21 mm concentration were represented in graphical analysis (Fig2-10). In three concentrations of Manganese chloride, Sorbic acid and potassium-di-hy-phosphate, there was no considerable growth has shown from 0 to 5, *P.polymyxa* has shown its considerable growth from 18^{th} to 96^{th} hour. The growth of *p.polymyxa* in control has shown in graph Fig11.By counting the vegetative cell and spore in different acids in different concentrations in different time standardized the manganese chloride at 19 mm concentration, sorbic acid at 20 mm concentration and potassium phosphate at 19 mm concentration gave more count of vegetative cell and spore. The two concentrations of respective acids have taken for germination test.

D. **Germination test:** The plating was done on nutrient agar for all three acids in the selected concentration .After 24 hour of incubation the plates were taken out and counted the colonies. Colony forming unit for all three acids after 24^{th} hours incubation showed 1.5×10^4 Cfu/µl in Manganese chloride, 2.5×10^4 Cfu/µl in Sorbic acid and 5×10^3 Cfu/µl in Potassium-di-hy- phosphate. In germination efficiencyof sorbic acid at 20 mm(2.5×10^4 Cfu/µl) gives the more count than other two acids and it germinate fast than other two acids. (Plate 1.7) (Figure 12)

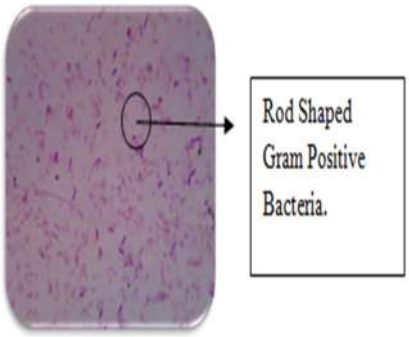


Plate1.1: Grams staining

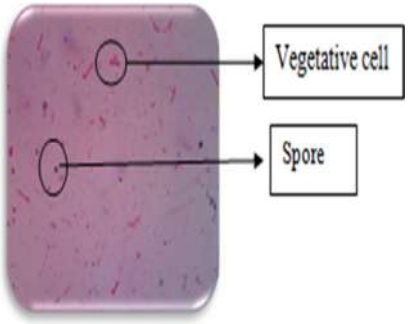


Plate1.2:Endospore staining

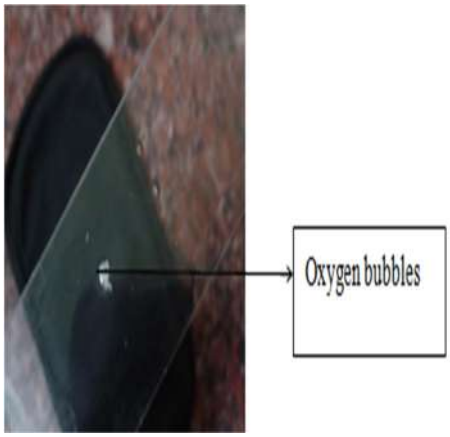


Plate1.3: Catalase

Plate1.4:Starch hydrolysis test

Plate 1.5: casein hydrolysis test

Plate 1.6: Gelatin hydrolysis

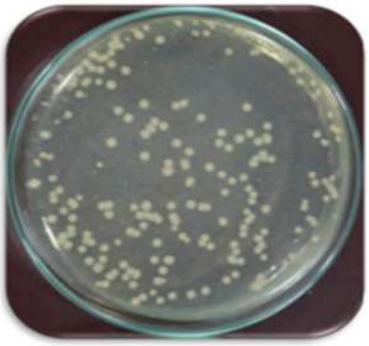


Plate1.7:Formation of colonies in sorbic acid

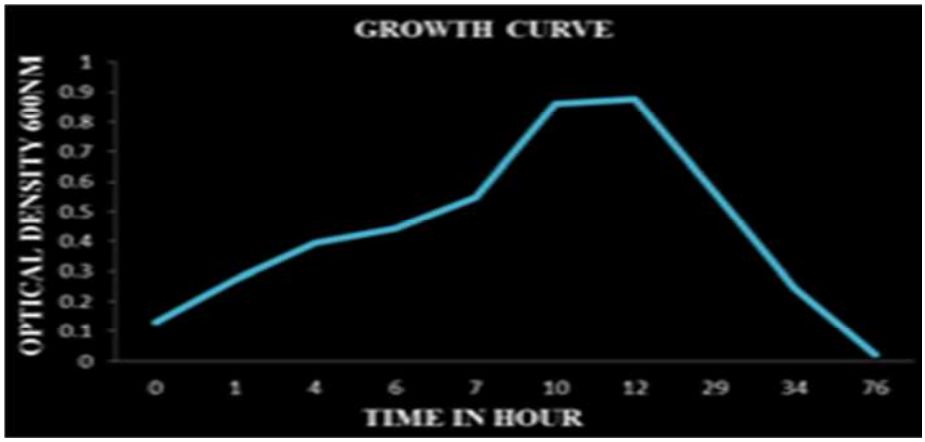


Fig. 1 Growth curve

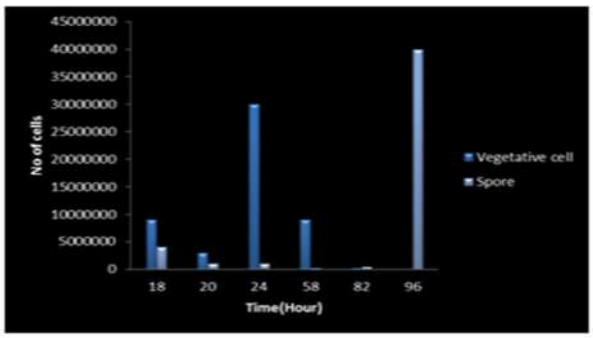


Fig2:Manganese chloride 19mm

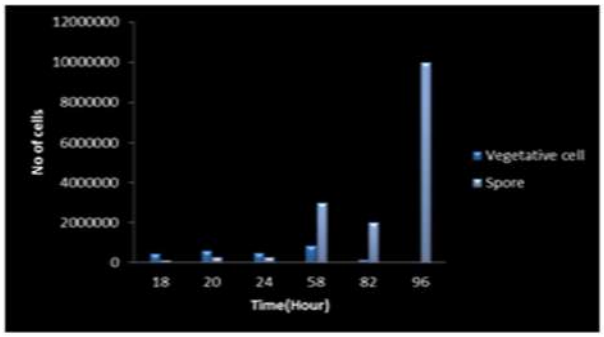


Fig3:Manganese chloride 20mm

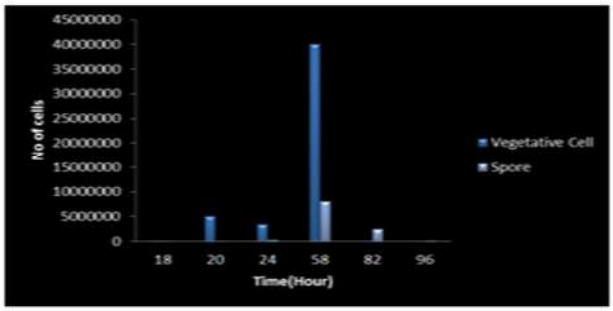


Fig4:Manganese chloride21mm

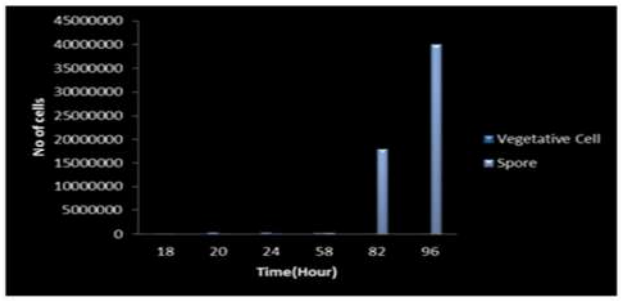


Fig 5:Sorbicacid at 19th milimolar

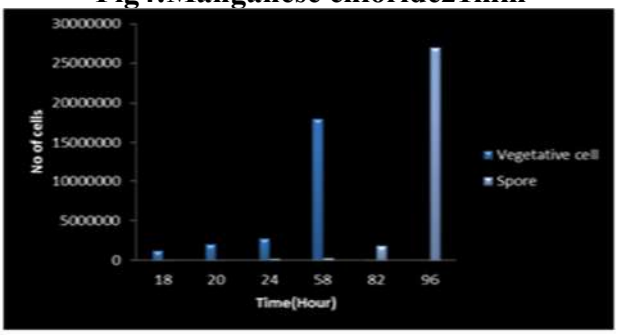


Fig 6:Sorbic acid at 20th milimolar

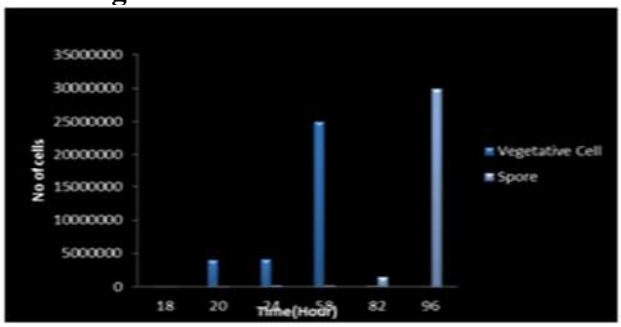
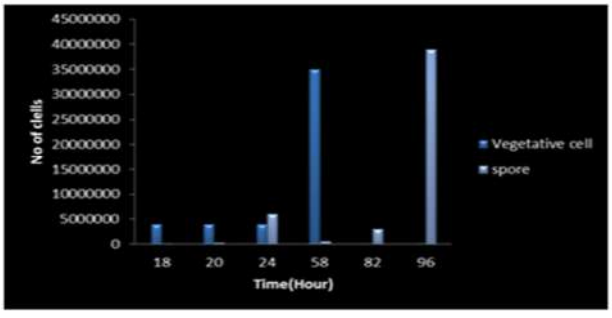


Fig 7:Sorbic acid at 21st milimolar



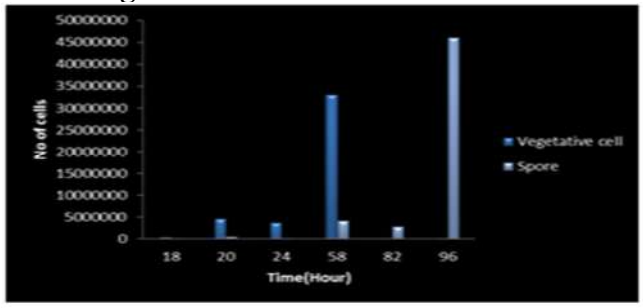


Fig8:Potassium phosphate at 19th milimolar

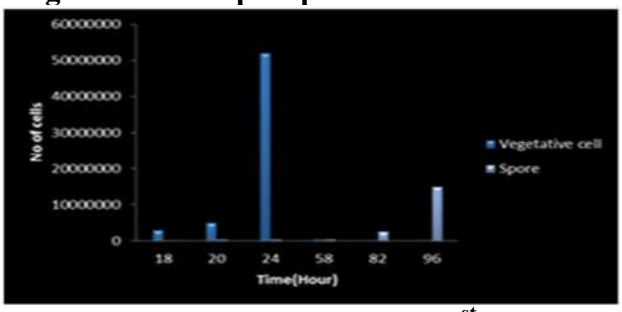


Fig 9:Potassium phosphate at 20th milimolar

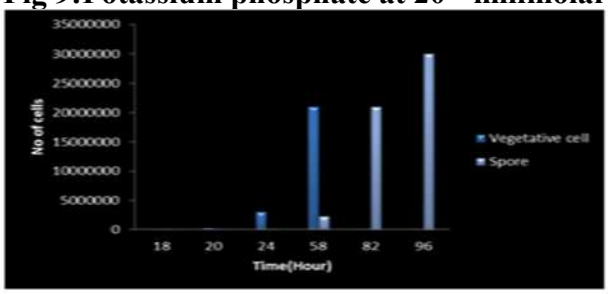


Fig 10:Potassium phosphate at 21st milimolar

Fig 11:Control for spore and vegetative cell count

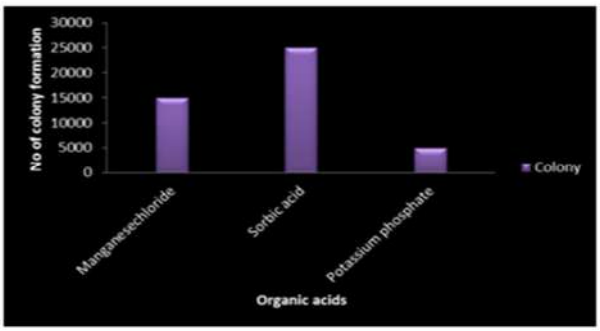


Fig 12: Colony formation in three organic acids

IV. CONCLUSION

In conclusion Sorbic acid at 20 mm concentration gives more spores and it germinates faster than other two organic chemicals. It led to the highest survival rate and spore formation during storage of biofertilizer which can be implemented with further studies for the preparation and preservation of bioorganic fertilizers.

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SP-11

A SURVEY PAPER ON THE DRK GENES IN DROSOPHILA MELANOGASTER

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ABSTRACT

The JAK- signal pathway transmits info from a physical object in the chemical signals to the nucleus that leading to DNA transcription and expression of genes concerned in immunity, proliferation, differentiation, and oncogenesis in insects. Here it known six genes families concerned in the STAT pathway in the silkworm, silkworm moth as well as seven in drosophila, three in arthropod genus Gambia, 6 in Apismellifera,. Comparative analysis showed that Drosophila melanogaster has higher number of STAT pathway-related gene families that are present and it has a unique gene family called which is not present in other insects. There is an interaction between external actions and internal reactions that allows a cell to live. Each receptor like a watch senses stimuli and starts to transfer corps of signals to the castle of the nucleus in order to impress vital responses.

KEYWORDS: ***JAK- signal pathway, DNA, Oncogenesis, Drosophila melanogaster***

I. INTRODUCTION

Over the past five years, comparative studies have affected beyond straightforward characterizations of variations in organic phenomenon levels among and between species to finding out variation in restrictive mechanisms. We still understand comparatively little regarding the precise chain of events that lead to most restrictive variations, but we have taken significant steps towards understanding the relative importance of changes in different mechanisms of gene regulatory evolution.. This pathway has been extensively studied in vertebrates and protostomes and shown to play basic roles in the development and performance of the immune and alternative cells. However, our understanding of the origins of the individual pathway parts and their assembly into a functional pathway has remained restricted.The JAK-STAT signaling pathway transmits information from extracellular chemical signals to the nucleus resulting in DNA transcription and expression of genes involved in immunity, proliferation, differentiation, apoptosisand oncogenesis. The JAK-STAT signal cascade consists of three main components: a cell surface receptor, a Janus kinase(JAK) and two Signal transducer and substance of Transcription (STAT) proteins. Disrupted or dysregulated JAK-STAT functionality can result in immune deficiency syndromes and cancers.

II. METHODS AND MATERIALS

The ***Drosophila melanogasters*** genome and protein sequences were downloaded from the flybase database[1] and utilized for this study. The predicted protein sequences associated with drk genes of *Drosophila melanogaster*, *Anopheles gambiae*, *Apis mellifera*, *Tribolium castaneum*, were downloaded from the NCBI database[2], Ensembl Metazoan database[3], or species-specific databases such as bombyx mori[4], BeeBase for *A. mellifera*[5].The drk gene sequences that collected from the various database of each organism are merged. This set of sequences were blasted using standalone blast against *Drosophila melanogaster*sequence which are downloaded from flybase database[6].Based on the multiple sequence alignment results, neighbor-joining phylogenetic trees[7]forstat-related genes from all surveyed insects wereconstructed using MEGA4.0[7].

A. BLAST

In addition to providing BLAST sequence alignment services on the web, NCBI also makes these sequence alignment utilities available for download through FTP [8].This allows BLAST searches to

be performed on local platforms against databases downloaded from NCBI or created locally. These utilities run through DOS-like command windows and accept input through text-based command line switches. There is no graphic user interface.

B. Clustalw

Clustalw[7] is one of the standard programs implementing one variant of the progressive method in wide use today for multiple sequence alignment. The W denotes a specific version that has been developed from the original Clustal program.

C. MEGA

Molecular Evolutionary Genetics Analysis (MEGA) is computer software for conducting statistical analysis of molecular evolution and for the constructing phylogenetic trees.

III. RESULTS AND DISCUSSION

A. Construction of Phylogenetic Tree

Based on this domain we can search the result andcollected that sequence from the insects and silkworm protien sequences are compained and to make a single file then these sequences are selected for their multiple sequence alignment using the online software called clustalw, which is based on the results to construct a phylogenitic tree in tree format and further its visualiesd by the software called mega.

V. CONCLUSION

In conclusion, the phylogenetic tree provides useful clues about the evolutionary relationshipbetween the surveyed sequences of STAT-related genes in silkworm and other insects.It shows that the common fruit fly, *Dmelanogaster*, is a well studied and highly tractable genetic model organism for understanding the molecular mechanisms of human diseases. Many basic biological, physiological, and neurological properties are conserved between mammals and the*fruitfly*and nearly 75% of human disease-causing genes are believed to have a functional homolog in the fly,by the identification and drk related gene function in other organisms beneficial in human immune related development.

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SP-12

RESEARCH ON YIELD DEVELOPMENT OF BANANA BASED ON MEDICATION AND FERTILIZATION FOR VIRUS INFECTED DISEASE USING DATA MINING ALGORITHM

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ABSTRACT

Banana is essentially called as tropical crop which grows excellently in temperature between 13ºC to 38ºC and 75 to 85%. Mostly in India banana is being cultivated in climate ranging from humid tropical climate to dry mild subtropical climate. Banana trees need a modest climate to develop and produce fruit. Most of the banana trees stop growing when temperatures drop below 53 degrees Fahrenheit. In this paper the yield estimation and preventive measures taken for medication and fertilization of banana is calculated from first stage to final stage using data mining algorithm. Initially plantation of banana is called the first stage where the banana is planted. Then in the stage of manure and fertilizing, bananas get mostly affected from the diseases. By following the preventive measures banana can be controlled from the infection of diseases which increases the yield and production rate of banana.

KEYWORDS: Banana, medication, manure, fertilization, data mining.

I. INTRODUCTION

Banana is not a real type of tree banana does not come under these categories also but it is said as banana palm. There are many leaf connected together called as banana trunks.Below the soil there is a growth of new leaves[1-4]. The banana plant takes minimumof ninemonths time for the cultivation of bananas. After the cultivation time is completed the main plant decades but around main banana there are various suckers grown which are said as baby plant[5-8].Below the soil there are many rhizomes present the rhizomes are called as corms[9]. The rhizomes consist of enormous starting point this points later develops into the suckers. There are two ways to plant a sucker[10]. The sucker can be kept at the origin near main plant or it can be taken and kept in the new place. If there are many suckers presented near the main plant the suckers should be taken and planted in different place[11]. Too many suckers at the same place cause damage which affects the growth of the banana plant. Banana can be grown in all climates the main and major source for growing banana is water and soil. Soil is important in banana plantation it acts as a catalyst. Proper manure and fertilizer should be added to the soil. If there is heavy temperature then more number of water should be poured into the fields if the temperature is low minimum amount of water should be poured into the fields. If watering the field is done correctly 25% of work is done.

II. PLANTATION OF BANANAN

Many of them said that banana plant can be grown from the seeds but growing banana from seed is not an easy process sometimes a plant arises from seed but the fact is most properly this does not works. Instead of seeds suckers can be planted suckers comes from the root of the banana plant so it will easily develop into a plant there are two type of suckers large suckers and small suckers. Large suckers have leaves at it edges so it will take only short time to fruit whereas small suckers does not have edges on it so it will take long time to fruit. Large suckers are grown near mother banana plants so it should be carefully taken the cutting of larger sucker should be done from the base root of the sucker so it does not affect the growth while planting it in the new place but main drawback in planting suckers is the diseases the suckers get easily affected by the diseases. Pit planting is also done for the cultivation of banana plant the suckers are planted at the centre of the pit the soil is kept completely covered and tightly packed so it holds some grip for the plantthe pits are presented below the ground level. The plant should be carefully pressed while planting it into the soil the

plant should not be planted below the ground level. The irrigation must be done immediately after plantation is over for the best cultivation of the banana drip irrigation should be done drip irrigation improves the usage of water in the fields watering should be done carefully over watering lefts the plan to become decade.

III. MANURING AND FERTILIZING BANANA

The more amount of nutrients added in the banana the more the yield is obtained nutrients are added manually to the soil. Nitrogen, phosphorous and potash are the important nutrients required for the banana to avoid lack of nutrients proper fertilizers should be added in the soil to obtain good growth and yield. The intercrops damages the root systems of banana easily so to avoid damages in root systems intercropping should be avoided so the root does not gets damaged or gets decayed.

IV. BANANA PLANT WEEDING

The method of taking unwanted plants from the soil is called as the weeding by taking away the unwanted plants gives space for the growth of the plant. To improve the yield of banana micro nutrient foliar spray is used it is made my mixing zinc and copper to the water. The weight and development of the banana can be increased by removing the male bud from the plant it should be removed carefully with the edges so the male bud does not grows again. Bunch spray is used to avoid changes in fruit skin. To save the leaves from the effects of the sunlight bunch covering is used it avoids sunlight and the fruit quality is increased highly because of bunch covering it should not be practiced in the rainy season because it leads to the fruit decade in rainy season bunching should be done carefully any changes in bunching may affect the entire growth of banana. Due to heavy rain the banana tree gets damaged and the balance of the plant is lost without the balance stops the production rate to avoid this propping is done. In propping sticks are placed in triangle shape which avoids the lack of balance in the banana it also helps the banana to grow uniformly.

Table.1 PRECAUTIONARY MEASURES

DISEASE NAME	SYMPTOMS & DAMAGES	PREVENTIVE MEASURES
Panama Wilt	margins to mid-rib of lower leaf becomes yellow, At the base leaves gets broken and starts hanging	Diseased plant and surrounded soil should be removed immediately. free planting material should be injected with 2% Aretan soil drench
Mycosphaerella leaf spot	Leaves noted with spots with greyish center and yellow halo appearances. pinkish colour fruits with irregular shape	Fungicide should be applied at the start of the infection. intercropping should be done, infected leaves should be destroyed.
Anthracnose (fig1.2)	Black spots seen on fruits. Pink colour layer on fruits	To minimizing the disease in cold storage cooling with 14°C should be done.8 times in a month Bavistin 1% and Chlorothalonil 0.2% should be sprayed.
Bacterial wilt	Leaves moves upward with yellow colour, centre portions remains uncoloured.	To find manually and destroy infected plant, formaldehyde is used for farm cleaning.
Bacterial soft rot(fig1.1)	Young plant get affected. infected plant comes out of the soil easily but root remains in the soil.	Once infected plant is detected it should be destroyed immediately.

Banana Bract Mosaic Virus	Young leaves spotted with yellow bands. Affected leaves look abnormal. Leaves get affected in bunches.	Infected plant parts should be destroyed. To control this disease intercropping should be done with cucumber.
Banana Streak Virus	Yellow appearance on the leaves which slowly destroys the leaves. In older leves black streaks are noted. The plant which is severely infected should be destroyed.	The plant with starting stage of infection should be cleared with help of pesticides.

Fig 1.1 Banana Tree (Raise) Fig1.2 Banana Tree (Bent)

V. HARVESTING THE BANANA PLANT

At the maturity stage the banana should be harvested to get more yields. After refining process the fruit can reach the consumption stage. Maturity indices involve the shape, length, colour, growth and the development of the fruit which also contains market holds of the banana. Once the maturity and harvesting is completed the bunch should be removed this process is called as the removal of bunch. It takes minimum of 100ndays for the removal of the bunch light should not fall on the bunch for that the bunch should be kept in dark area or dark room which improves the softness of the bunch and increases the weight of the bunch after this the post harvesting method comes to the execution. In post harvesting, bunches are carried in Lorries to avoid decades fruits should be washed with fresh water or with the sodium so that the bunch remains fresh and neat.

VI. THE YIELD OF BANANA

The yield gets ready for cultivation between 11 to 12 months after the plantation. The first and second crops get ready within 8 to 9 months for cultivation within 30 months it is possible to harvest 3 crops if crop managed well the yield maybe high. With the help of fertigation and drip irrigation banana yield can be obtained high under tissue cultivation technique. The appropriate yield production of banana is 20 ton per acre sometimes the yield may change it may be high.

VII. CONCLUSION

The research on yield development of banana based on medication and fertilization for virus infected disease using data mining algorithm is the real time problem faced by the farmers.The overall study lifecycle of banana from starting plantation stage to the cultivation stage is mentioned.The banana plant takes minimum nine monthstime for the cultivation of bananas there are various problems faced by the farmer during this phase. This paper gives clear idea and precautionary measures that should be taken for the growth of banana

from starting stage to the final stage and also provides various techniques for medication and fertilization to protect banana from virus infected diseases. By following this precautionary measures and techniques the yield of banana can be increased.

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SP-13

SMART AGRICULTURE FOR DETECTING SOIL MOISTURE RATE IN WIRELESS SENSOR NETWORKS USING VPVB MODEL

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ABSTRACT

Wireless sensor networks refers to a group of spatially dispersed and dedicated sensors for monitoring and recording the physical conditions of the environment and organizing the collected data at a central location. The complete solution to increase the yield of crops can never be achieved only by monitoring the environmental factors. To overcome these problems, energy efficiency must be implemented in agriculture. The system consists of sensor networks like, soil moisture, temperature and rainfall. With the use of battery, we will make the sensor to get activated. The soil moisture, temperature and rainfall are not necessary to be used for all the time. When these sensors are used in agriculture we can save time and energy. For every 15 minutes the soil moisture will be tested before the crop is cultivated and the results will be sent to the main server. In those remaining times, these sensors will be in SLEEP/SHUT DOWN STATE. This method helps us to save energy and time.Proposed a new model called Variable Path with Variable Bandwidth (VPVB) to compute a path of highest bandwidth in each time slot and also proposed a Dijkstra's algorithms, searching the shortest path from one source to one destination in HPN. The system developed with the main intension of achieving Energy Efficiency.

KEYWORDS: ***Bandwidth Scheduling, HPN (High Performance Networks), Energy Efficiency, Network protocol.***

I. INTRODUCTION

The traffic to estimate the consumption of energy by every network that considers the topography can be done with the help of an end-to-end energy cost model[1]. The bandwidth link in such networks is typically shared by multiple users through advance reservation and it is mainly for transmitting the signals, resulting in alternate bandwidth availability in future time. Most existing bandwidth scheduling algorithms only concern traditional objectives for minimizing data transfer end time.

II. ENERGY EFFICIENCY IN WIRELESS NETWORK

With the quick increment of wireless networking within the world, the energy potency of wireless networking protocols becomes a firm of the many wireless networking stakeholders[2]. They need passion on the energy potency in wireless networking protocols for many logics like design drawback, inexperienced technology policy, price and last user satisfaction[3]. There square measure several capacities for saving the energy in wireless network protocols. Some measure concentrating on saving the energy in many modes like effective/sleep modes. Some measure involved regarding reducing interference and receiving higher signal-noise quantitative relation with a similar transmission radio power[4]. Some measure exercised regarding increasing the speed consistent with the applying and atmosphere to save lots of the time operating underneath active modes[5].

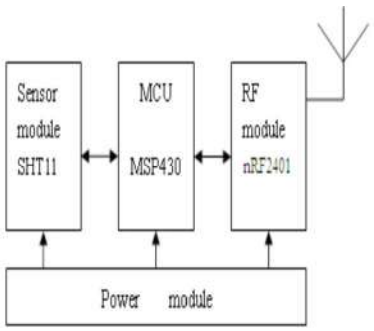

FIGURE 1 MODEL OF ENERGY EFFICIENCY IN WIRELESS NETWORK

1.2 GREEN NETWORKING

Green networking is a hugephraseregardingmovements used to improve networking or make it extraefficient[6]. This term elaborates and covers procedures that lowerstrengthintake, as well asmethods for protecting bandwidth or every othersystemto be able tosooner or laterdecreaseenergy use and secondly, fee[7,8]. The error of inexperienced networking has a number ofessentialapplications, mainly as energywill becomeextrahigh-pricedand thosegrow to beextra approachable of the unfavorableresults of powerutilizationon the environments[9].

1.3 Router

By calculating the suitable way for the information to attain its destination and then moving it, we can use a router that travels information between two or more packet-switched computer networks by analyzing a given data packet's destination IP address.

II. RELATED WORKS

A. SHUT DOWN/SLEEP MODE

"No Agriculture means No Food", because Agriculture is the main source to provide food for our living. Technology should be mainly implemented in agriculture. By doing this paper, we can help farmers to save their energy and time.In the existing work, many people have concentrated only in saving the time but they failed to save energy efficiency. We have bought a solution to this problem by concentrating in energy efficiency. Each time when the soil is tested the result will be immediately send to the server and the soil will be tested for every 15 minutes before the cultivation[7].So in the remaining time, the server and the soil moisture sensor will be in SHUT DOWN/SLEEP mode. By doing this we can save energy and whenever the soil moisture sensor is needed we can use them at certain times. Temperature sensor is as same as soil moisture sensor and it tests the temperature of the field only at the day time. Because at night times the temperature will be cool and there is no need to check the field[2]. Farmers can start the server and the sensors. These sensors will automatically do their work and it reduces the work of farmers. The transmission of huge information in varied kind applications across superior networks (HPN) consumes a major quantity of energy on a daily basis. Information measure programming is a lot of necessary in superior networks as a result to reduce energy consumption within the network and to avoid wasting the energy within the world network environments[10]. Every dedicated channel in these networks generally consists of one or a lot of physical links that are shared by multiple applications in each time and information measure before reservation.The look of economical information measure programming algorithms is vital to maximizing the employment of dedicated network resources and meeting various end-to-end transport performances needs[1]. The topology of a fanatical network is pictured by a graph, wherever every link maintains a listing of residual bandwidths fixed as divided constant functions of your time. We tend to formulate and investigate 5 information measure programming issues to reduce the information transfer finish time below completely different path and information measure constraints:

(i) Variable path with variable information measure (VPVB), that computes the widest (highest bandwidth) path in any time slot;

(ii) Mounted path with variable information measure (FPVB), that computes a set path with variable bandwidths across completely different time slots;

(iii) Variable path with mounted information measure (VPFB), that computes one path in any time slot with identical bandwidth;

(iv)Fixed path with mounted information measure (FPFB), that computes a set path with a continuing bandwidth; and

(v) Multiple mounted ways with mounted bandwidths (MFPFB) that computes multiple occurring mounte ways with constant bandwidths. Most ordinarily existing information measure programming algorithms contemplate solely information transfer time step-down, and particularly restricted efforts are dedicated t energy potency in HPN[5]. From the experimental results, Energy potency of our planned formula reaches 96.8% that is best than the present system.

III METHODOLOGY

Most commonly existing information measure programming formula solely contemplates knowledge transfer time diminution, and particularly restricted efforts are dedicated to energy potency. Existing system papered sleeping mode technique whenever the network equipment's after they don't seem to be used it goes sleep mode [3]. This raises many problems: property loss, long re-synchronization time. Once more constant wake-up price is needed to transition back to the active state. Polynomial time approximation in previous

study that leads knapsack problem which implies wherever there's a requirement for associate optimum object or finite answer wherever associate thoroughgoing search isn't potential[10].This model ineffectual to avoid wasting energy in network instrumentation effectively.

A. PROPOSED SYSTEM

This paper entirely discusses the analysis methodology and also the absolute steps involved in analysis work. The system effectively proposes new model approach referred to as Variable Path with Variable information measure (VPVB) that computes highest information measure path in any time slot[11]. Together with our papered analysis effectively discuss the way to implement VPVB model a lot of expeditiously with the assistance of a Dijkstra's algorithms[12]. Proposed, Dijkstra's formula has been bestowed to looking the shortest path from one supply to 1 destination simple and effective manner.

B. CONTRIBUTION OF THE PROPOSED WORK

A replacement model approach referred to as Variable Path with Variable information measure (VPVB) that computes highest information measure path in anytime slot. A Dijkstra's algorithms method has been applied to looking the shortest path effective manner[12]. VPVB has been applied that guarantees the world diminution of the information transfer finish time. Our paper model effectively monitors and selects their massive information measure for effective knowledge transfer that leads minimize knowledge transfer time. VPVB model improves and achieves Energy potency.

IV. CONCLUSION

The goal of this paper is to minimize energy consumption in the network and to save the energy in the real life network environments. In the existing work, only Time will be concentrated. In this new work we can save both TIME and ENERGY. This will definitely help farmers to reduce their work .The Variable Path with Variable Bandwidth (VPVB) model computes highest bandwidth path in each time slot. For searching the shortest path from one source to one destination,the Dijkstra's algorithmis used effectively .The experimental results are evaluated by using the simulation environmental. This experimental work will minimize the energy efficiency, compared to the previous works. Finally, it can increase network performance.

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SP-14

RESEARCH ON PROFIT ESTIMATION OF JASMINE FARMERS IN LEAN SEASON BASED ON ITS MARKET PRICE AND PRODUCTION USING K-MEANS CLUSTERING ALGORITHM

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ABSTRACT

Tamil Nadu places 2nd rank in marketing of jasmine in India and the production of jasmine in some places in India is 14,194 as approximately. In market the vendors are busy in selling jasmine in weeding, festivals both in lean and peak months. In these months jasmine plays double rate of selling in festive and weeding months, profit is high for farmer in Lean and low price in Peak season. This research is for the farmers who cultivate jasmine to have the better knowledge about the market values in the lean months and to cultivate the jasmine more in lean, so that they get better profit. The jasmine price is collected in varies market and calculated using the K-Means Clustering Algorithm.

KEYWORDS: ***Jasmine, Market price, Peak season, Lean season, K-Means***

I. INTRODUCTION

In South side of Indiajasmine is the oldest profitable crop. Jasmine there be 50 more types of crop in India, but few crops been generally used. It is the successful production into all period. But production of jasmine also profit of crop used for the proper fertilizer along with maintenance. To be chosen properly Temperature, soil, climate for high profit. During peak time jasmine has high production since of Summer Season, but low profit as well during lean time low crop growing because heavy rain period, however high profit. Crop should be planted during the month June on the way to November. The flower takes place later than six month of plant in the month of March - April along with it started blooming in February to April. Then flower decrease in the month of August it is the Lean period[1]. Proper way isto maintain the production of flower. Get high price in lean month be correct month, if farmers choose the month and cultivate they get double profit[2-4].

II. WORKPLAN FOR CULTIVATION OF JASMINE

STEP 1**:** Analysis of soil and temperature - **10 days**
STEP 2:Plantation of jasmine plant - **20 days**
STEP 3**:** Adding fertilizer and manure for growth of jasmine - **5 days**
STEP 4**:** Adding additional fertilizers for defected jasmine plant - **5 days**
STEP 5**:** After planting flower takes place in six month - **183 days**

II. DISTRICT PRODUCTION OF JASMINE

According to over countries India and Tamil Nadu is high cultivation of jasmine, 77,257 tones entire profit inside addition to annual income, 9,370 hectares is the profitable markets for plants many regions in Coimbatore, Tirchy, KanyaKumari also Chennai. Daily taken flowers are of 100 tones on profit of Rs.35 lakhs. During last month amount starting Rs 300 per kg[5-7], also high profit is Rs 600 during December month(Fig. 1, 2)

Fig. 1 Purification of plant in stage A,B and C

III. ANALYSIS OF LEAN SEASON MARKET PRICE

The markets of Madurai, Dharmapuri, Salem, Sathyamangalam, Pollachi, Tirunelveli, Tirupur has the same market price which ranges from Rs.200 - Rs.500 and Coimbatore, Dindigul has the same market price which ranges from Rs. 300 – Rs. 600 and Chennai (Tables 1-4) has the high rate of jasmine flower because it is imported so it is of high price in the month of December is Lean Season-1.[6]

Table 1 Market Price

MONTH	NAME OF THE MARKET	PRICE LIST
DECEMBER	Madurai	Rs.200-500
	Sathyamangalam	Rs.200-500
	Pollachi	Rs.200-500
	Dindigul	Rs.300-600
	Coimbatore	Rs.300-650
	Chennai	Rs.700-840

Fig. 2 Jasmine in bulk

The month is divided into three types as Peak, Lean-1, Lean-2.March on the way to June is Peak, Oct - Feb is of Lean-1 and July, Aug, Sept be Lean-2 time[2].In Peak time production of flowers be high as well as good but profitable flowers be low because high range during month May in Low price and in March it is high price as from Rs.19.67 to Rs.63.14.In Lean-1month the growing of jasmine be low further price is high since low range jasmine and time of October jasmine amount is Low as well as December profit be high as from Rs.45 - Rs.108.62. In Lean-2 cultivation is no yield or less but profitable amount of buds is high during three months July, August, Sept since Rs.200 to Rs.250. Flower market in Madurai be famous few tones of jasmine exported to some country[3].

Table 2 Yield of Jasmine (2015-2019)

YEAR	MONTH	FLOWER YIELD(Kgs)
2015	Jan 14 - Nov 14	554.7
2016	Jan 15 - Nov 15	514.3
2017	Jan 16 – Nov 16	510.4
2018	Jan 17 - Nov 17	614.2
2019	Jan 18 – Nov 18	628.6

From above table flower crop growing during 2015-2019 time January - Nov taken , flowers cultivation per (Kgs) is approximate calculated.2015 the jasmine yield is 554.7 kg, 2016 the yield is 514.3 is decrease comparing with the previous year , 2017 the production is 510.4 is too decrease comparing among last two years because of low rainfall, 2018 be 614.2 is increase from last year, 2019 the cultivation be 628.6 also raises every day because of good rainfall as well as temp is appropriate in support of farmers good profit[7].

Table 3 Monthly Market Price of Jasmine

MONTH	MARKET PRICE LIST
January	Rs. 800 - 1000
February	Rs. 1000 – 1100

March	Rs. 100 – 150
April	Rs. 80 – 120
May	Rs. 50 – 100
June	Rs. 90 – 110
July	Rs. 150 – 200
August	Rs. 200 -250
September	Rs. 450 – 600
October	Rs. 100 – 220
November	Rs. 700 – 800
December	Rs. 700 – 840

The monthly market rate jasmine be splitted into three seasons, market values, amount is added as season wise. Peak season month from March - June amount be high as well as low, high and low rate Rs.100 to Rs. 50.Lean season-1 month is from October to February the price is from low to high, Rs.100 to Rs. 1100 and Lean season-2 months are July, August, September the rate from low as high Rs.90 - Rs.600 amount of approximately calculated[4].

Table 4 Seasonal Tonnes of Jasmine in Market

AREA	TONNES	PEAK SEASON COST	LEAN SEASON COST
Coimbatore	20 to 25 tonnes	Rs 15 to Rs 20 Per Kilogram	Rs 250 to Rs 300 Per Kilogram
Sathyamangalam	30 tonnes	Rs 10 to Rs 15 per kilogram	Rs 200 to Rs 250 per kilogram
Pollachi	5 to 10 tonnes	Rs 10 to Rs 20 per kilogram	Rs 300 to Rs 350 per kilogram
Madurai	11-15 tonnes	Rs 15 to Rs 20 per kilogram	Rs 300 to Rs 450 per kilogram

The seasonal cost jasmine flower marketplace is taken. A small number of flower marketplaces are taken with their seasonal cost and tones used in favour of sales is taken as approximately.Position is chosen based on the above table[9].Sathyamangalam market has the high tonnes as 30 and profit during lean period from Rs. 200-250, Coimbatore is second position in the marketplace tonnes amount of 20-25 tonnes, profit during lean time Rs.250-300, Madurai be third position since tonnes values from 11-15 tonnes, profit during lean period Rs.300-450,Pollachi is fourth position since in marketplace it has low tonnes of 5 to 10 tonnes and the profit during lean period of Rs.300 to 350 per kg[7].

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SP-15

ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED ZnO NANOPARTICLES FROM VARIOUS MEDICINAL PLANTS

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ABSTRACT

This article describes a simple combination reaction between Zinc Nitrate and leaf extract of *Ocimum basilicum, Anisochilus Carnosus, Curcuma neilgherrensis Wight* and *Aloe Vera* plants to prepare ZnO nanoparticles.XRD analysis demonstrates that the prepared ZnO NPs exhibit hexagonal wurtzite structure and are crystalline in nature. The antibacterial activities of the prepared ZnO samples are analyzed against human pathogens i.e. Gram-positive bacteria (*Basicillus subtilis, Staphylococcus aureus)* and Gram-negative bacteria (*Escherichia coli, Salmonella enterica typhi, Pseudomonas aeruginosa)* were showed excellent results.

KEYWORDS*: Zinc Oxide, XRD, Antibacterial Activity*

I. INTRODUCTION

Nanotechnology is a field of science which deals with fabrication, manipulation and makes use of materials ranging in nanometers. Nanoparticles have expressed important progress, advance to broad ranging application in the field of sensors, bio-medical, antimicrobials, agricultural, catalysts, optical fibers, electronics, bio-labeling etc., Arrays of conventional methods are utilized in production of nanoparticles. But these conventional methods are bound with different limits such as cost, production of hazardous, toxic chemicals etc., which has raised the researchers to broaden safe and eco-friendly alternative approach amongst which biological systems have been focused and developed as a preferred green principle process for production of nanoparticles. Employing of plants in the nanoparticle synthesis was rapidly growing interest as a significant source towards reliable and environmentally benign method of metallic nanoparticles synthesis and its characterization. The current analysis emphasizes synthesizing nanoparticles from different plant resources. Plants are well-known to have various remedial compounds which are being exploited ever since from ancient time as a traditional medicine.Medicinal plants and their products play a vital role in the human wellness in several ways. Nanotechnology involves the employ of materials having nano-scale dimensions in the range of 1nm to 100nm. Working with nano-materials has made to have a better understanding of biology. Different chemical methods are preferred for green synthesis of nanoparticles such as hydrothermal synthesis, co-precipitation method and vapour transport etc.[1-4]. The green synthesis method is rapidly increasing due to usage of less toxic chemicals, eco-friendly nature and one step synthesis of nanoparticles[4].

II. RELATED WORKS

In the modern day's many researchers are diverting themselves from using synthetic methods. They are trying to turn towards biological systems; mostly plants are selected for nanoparticle synthesis as it is cost effective and can be easy for large scale production. The metal nanoparticles synthesize using leaf extracts of the plant are the most effectual process of synthesis at a very reasonable cost. Bio-reduction of metallic ions occurs during the nanoparticle synthesis. The nanoparticles synthesized from plant extract are getting more important attention due to the increasing demand to develop clean, nontoxic chemicals, environmentally non-malign solvents[5-7]. The diversification of products will increase radically when genes of different origins are used. There is no need to concentrate on new compounds only, there are many

exciting natural products, of which the application (e.g. as a drug or fine chemical) is hampered by its availability.The antibacterial activities of the all the prepared ZnO samples havebeenstudied.

III. MATERIALS AND METHODS

A. Preparation of the leaf extract

Ocimum basilicum, Anisochilus carnosus,Curcuma neilgherrensis and *Aloe vera* were used in the study for the green synthesis of ZnO nanoparticles. It was identified and authenticated by Botanical Survey of India (BSI), Southern circles, Govt of India. The well-known fresh leaves of the plants were collected randomly from the region of Yercaud and Kolli hills, Eastern Ghats, Tamil Nadu.

Methanol was used for extraction of plant sample. 30g of air dried and powdered plant materials were extracted with 300ml of solvents by using Soxhlet apparatus for 10hrs at a temperature not exceeding the boiling of the solvents. The obtained extracts were filtered using What-man filter paper No.1 and the filtrates were then evaporated in hot air oven at 100°C. The dried extracts were stored in air tight bottles for further use. Preliminary phytochemical analysis of plant material was done by standard protocols as referred by Brain and Turner, 1975 and Evans, 1996 respectively.

Fig.1: Soxhlet apparatus used in the present work

B. Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of *Ocimum basilicum,Anisochilus carnosus*, *Curcuma neilgherrensis and Aloe Vera* was done for the methanol extract and shown in Table 1. The qualitative phytochemical analysis of the *O.basilicum* leaf methanol extract revealed the presence of alkaloids, flavonoids, Terpenoids, phenols and carbohydrates. Steroids, anthroquinone, saponins, tannin, oils and resins were found to be absent in the extract[7-10] reported that the preliminary phytochemical screening of hydroalcoholic extract of *O. basilicum* indicated the presence of flavonoids and phenols[11]reported the presence of triterpenoids, flavonoids, glycosides, carbohydrate, polyphenols and tannins in the ethanol extract of *O. basilicum* leaf[8-10]. Presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids were isolated in the stem bark of *O. basilicum*[13].

The qualitative phytochemical analysis of the leaf methanol extract of *A.carnosus* was done to test for presence of various phytochemicals. The extract was found to have alkaloids, phenols and carbohydrates. Flavonoids, steroids, Terpenoids, anthroquinone, saponins, tannins, oils and resins were absent in *Anisochilus carnosus* extract[14].reported that in the *Anisochilus carnosus* ethanol leaf extract alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, phenols, triterpenoids, and anthraquinones, oils, fats and amino acids were absent[11,12].

In *Aloe vera* leaf methanol extract, the phytochemicals like alkaloids, flavonoids, steroids, anthroquinone, phenols, tannin and carbohydrates were present. Terpenoids, saponins, oils and resins were absent in *Aloe vera* extract. Where as in *C. neilgherrensis* phytochemical constituents like alkaloids, flavonoids, steroids, phenols, tannin and carbohydrates were present and absence of Terpenoids, Anthroquinone, saponins, oils and resins was noted. [13-26]

Table 1: Phytochemical Analysis for different plant extracts

Phyto chemicals	Observations	Extracts			
		Anisochilus carnosus	***Ocimum basilicum***	***Aloe vera***	***Curcuma neilgherrensis***
Alkaloids Mayer's test Wagner's test	Cream color Reddish brown solution/ precipitate	+ +	+ +	+ +	+ +
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown/Orange color precipitate	- -	+ +	+ +	+ +
Steroids Liebermann-Burchard test	Violet to blue or Green color formation	-	-	+	+
Terpenoids Salkowski test	Reddish brown precipitate	-	+	-	-
Anthro quinone Borntrager's test	Pink color	-	-	+	-
Phenols Ferric chloride test Lead acetate test	Deep blue to Black color formation White precipitate	+ +	+ +	+ +	+ +
Saponin	Stable persistant	-	-	-	-
Tannin	Brownish green/Blue black	-	-	+	+
Carbo hydrates	Yellow/ brownish/blue / green color	+	+	+	+
Oils & Resins	Filter paper method	-	-	-	-

+ represent presence, - represent absence

C. Green synthesis of ZnO Nanoparticles

For the synthesis of ZnO powder, 50ml of plant leaves extract was taken and boiled at 60°–80°C by using a stirrer-heater. Then, 5grams of zinc nitrate was added to the solution as the temperature reaches 60°C. This mixture was then boiled until it is converted to a deep certain coloured suspension. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 100°C for 1hour, and then it was grained using a mortar and pestle. A light white colored powder was obtained and this powder was carefully collected and sent for different characterizations.

D. Preparation of Pure ZnO Nanoparticles

Zinc Nitrate was first dissolved in de-ionized water and this mixture was stirred at room temperature for 30 minutes to get a homogenous and transparent solution. Then ethanol was added as a complexing agent on this $Zn(NO_3)_2$ solution drop by drop and the solution was stirred continuously around 30 minutes. Sodium Hydroxide solution was prepared by adding it with the de-ionized water and this mixture was stirred for 30 minutes. In the prepared $Zn(NO_3)_2$ solution, Sodium Hydroxide solution was added drop by drop and this mixwas continuously stirred with the magnet at 70°C for 120 minutes. A white color precipitate was obtained which was separated by centrifugation and washed several times with double distilled water. The precipitate was dried in oven at 100°C for 1hour to get ZnO powder sample. The obtained final products were dried, cooled and stored in an air tight container.

IV. RESULTS AND DISCUSSION

A. Characterization of ZnO NPs

Structural characterization were carried out via XRD, for samples prepared with different leaf extracts viz., *Ocimum basilicum L., Anisochilus carnosus (L), Curcuma neilgherrensis* Wight and *Aloe vera*. The Green Synthesized ZnO samples are named as ZnO A *(Ocimum basilicum)*, ZnO B *(Anisochilus carnosus)*, ZnO C (*Curcuma neilgherrensis)*, and ZnO D *(Aloe vera)* in further discussions.

B. XRD analysis

Fig. 2 shows the intensity is larger in Green synthesized nanoparticles while comparing to the pure ZnO nanoparticles. The detected (h k l) peaks are at 2θ values are corresponding to the lattice planes (100), (002) and (101) respectively. They are in agreement with the standard JCPDS card 036 – 1451 card for hexagonal wurtzite ZnO. The highest intensity was observed in the ZnO nanoparticles synthesized from leaf extract of *Curcuma neilgherrensis*. XRD pattern of the sample ZnO C exhibits all three diffraction peaks (100), (002) and (101) corresponding to the Wurzite ZnO with strong (002) diffraction peak compared with (100) and (101) peaks. The intensity of the (100) and (101) peak is reduced and (002) peak is increased, that leads the growth towards the c-axis. The grain size has been determined using Scherer's semi-empirical formula and the values are shown in table 2.

Table 2: Grain sizes of ZnO nanoparticles with different plant extracts

Samples	2θ(°)	FWHM (°)	Grain size (nm)
ZnO Pure	34.79	0.1968	42.28
ZnO A (*Ocimum basilicum)*	34.10	0.1968	42.20
ZnO B (*Anisochilus carnosus)*	34.39	0.1968	42.38
ZnO C (*Aloe vera)*	34.34	0.1968	42.23
ZnO D (*Curcuma neilgherrensis)*	34.79	0.1968	42.28

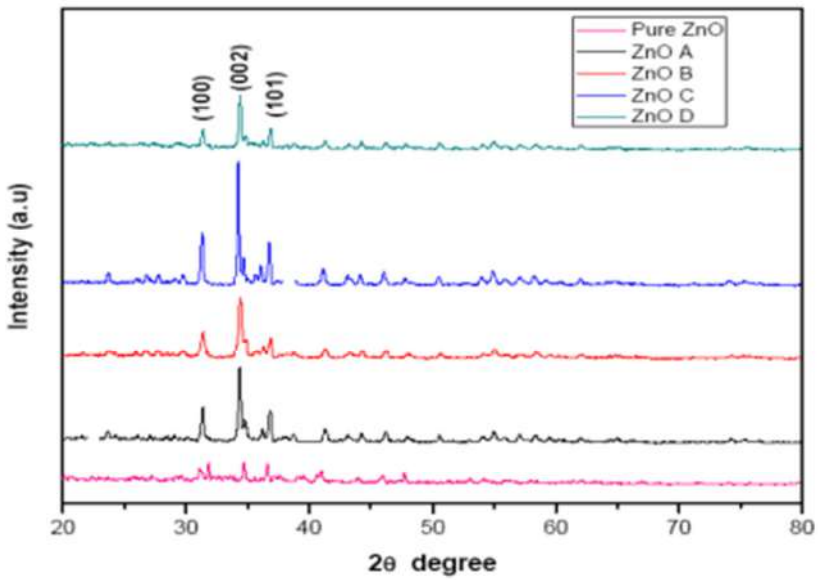


Fig.2: X- ray diffraction patterns of Green synthesized ZnO and Pure ZnO nanoparticles

C. Antibacterial activity

- **Preparation of inoculums**

  Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria (*S.typhi, S.aureus, B.subtilis, E.coli* and *P.aeruginosa*) that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0X 10^6 colony forming units (CFU/ml) for bacteria[13-16].

- **Antibacterial susceptibility test**

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The sterile discs of 6 mm were loaded with 20, 30, 40 and 50µl of test solution (ZnO Nps) [17-21]. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

- **Antibacterial activity of Pure ZnO Nanoparticles**

The antibacterial activity of Pure ZnO Nanoparticles towards various human pathogens tested by disc diffusion method and was represented in fig 3. *In vitro* antimicrobial activity of *ZnO* Nanoparticlesstudy against five pathogenic bacteria on the basis of zone of inhibition revealed that the ZnO nanoparticles is quite effective against both gram positive and gram negative bacteria and is more effective towards gram positive bacteria i.e. *Basicillus subtilis* and *Staphylococcus aureus* than gram negative bacteria. As seen in table 3, ZnO nanoparticles show the lower inhibition in 20, 30 and 40µl concentration. The maximum inhibition zones of 18mm, 19mm, 18mm, 20mm and 19mm in 50µl of concentration were obtained for ZnO nanoparticles against *E. coli, S.typhi, P.aeruginosa, B.subtilis* and *S. aureus* respectively. However, when compared to standard control, lower antibacterial activity of ZnO NPs was observed in all the bacterial pathogens.

Antibacterial properties are very important tool for control of harmful bacterial strains especially in the treatment of various infectious diseases. D. Suresh *et al* observed excellent bactericidal activity was shown by the Nps on *Klebsiella aerogenes, Escherichia coli, Plasmodium desmolyticum* and *Staphylococcus aureus*. Synthesis of multifunctional ZnO Nps using naturally occurring plant products has been advocated as a possible environment friendly alternative to chemical methods. [22,23]

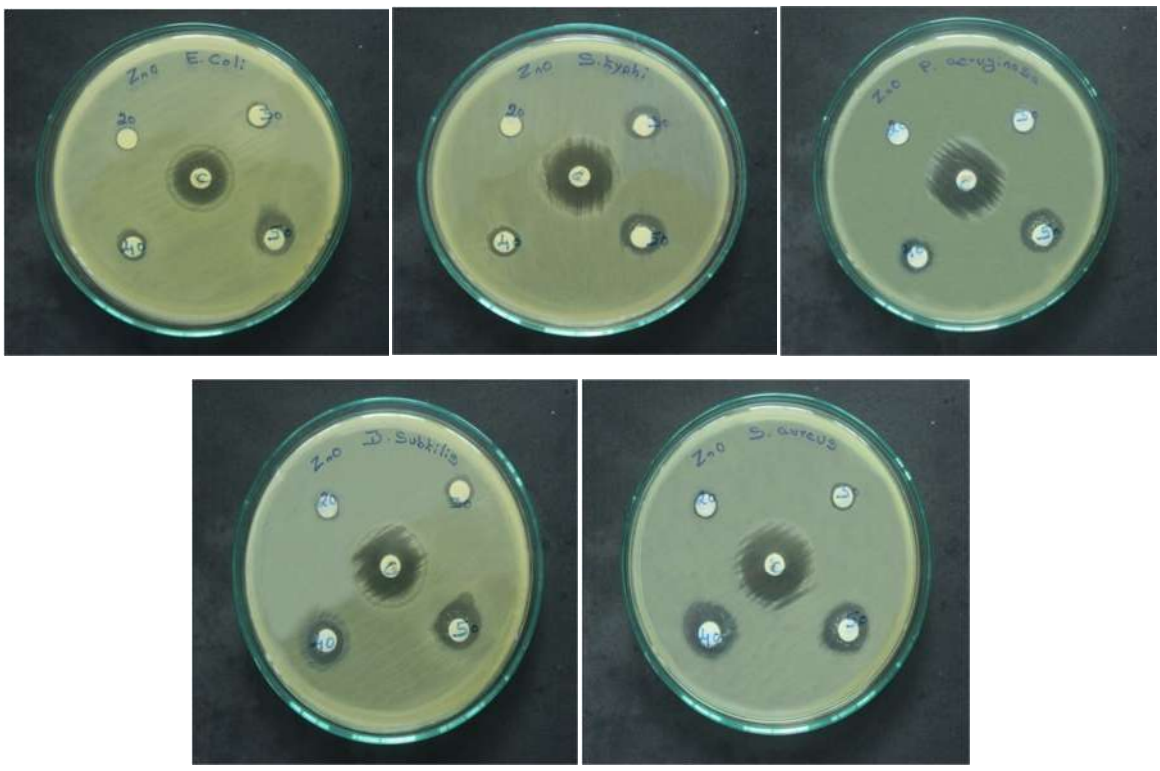


Fig.3: Antibacterial activity of pure ZnO nanoparticles

Table 3: Inhibition Zone of pure ZnO Nanoparticles

S.No	Organisms	Control	Zone Of Inhibition (mm)			
			20µl	30 µl	40 µl	50 µl
1	*E.coli*	24mm	7mm	11mm	13mm	18mm
2	*S.typhi*	25mm	8mm	14mm	16mm	19mm
3	*P.aeruginosa*	26mm	7mm	9mm	13mm	18mm
4	*B.subtilis*	27mm	7mm	12mm	16mm	20mm
5	*S.aureus*	28mm	8mm	11mm	16mm	19mm

- **Antibacterial activity of ZnO nanoparticles synthesized from *Ocimum basilicum***

The antibacterial activity of ZnO nanoparticles synthesized from *Ocimum basilicum* towards various gram positive and gram negative bacteria were tested by disc diffusion method and were represented in the Fig.4. The antibacterial activity results of synthesized ZnO nanoparticles from methanol extract of *O. basilicum* showed good effect against *S.typhi, S.aureus, B.subtilis, E.coli* and *P.aeruginosa*. As seen in table 4, the maximum zone of inhibition was observed for *E. coli* (16 mm) and minimum zone of inhibition observed for *S.aureus* (14 mm), *P.aeruginosa* (10mm), *S.typhi* and *B.subtilis* (13 mm) in 50 µl concentration. The *in vitro* antibacterial activity revealed that the synthesized methanol extract had significant activity against all the microorganisms tested. The extract showed higher activity in high concentration (50 µl) but no activity in lower concentration (20 and 30 µl) and except against *S.typhi* and *B.subtilis* in 30 µl. However, when compared to standard control, *Ocimum basilicum*leaf extract assisted ZnO nanoparticles exhibit lower antibacterial activity against all the bacterial pathogens. Khoshkholgh *et al.,* 2012 reported that the antibacterial activities of the ethanol and methanol extracts of *O. basilicum* showed that the inhibition zone for *Klebsiella pneumonia* was less as compared to *Staphylococcus aureus, Staphylococcus epidermitidis, Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Shigelladysenteriae, Klebsiella pneumonia, Bacillus cereus, Bacillus subtilis, Proteus vulgaris, Serratiamarcescens*. The methanol extracts were reported to be more effective than the ethanol extracts against all the organisms[24].In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss, etc. It is also known to have antifungal properties (Manjunatha, 2006). Saponins have been implicated as bioactive antibacterial agents of plants (Arts and Hollman, 2005; Scalbert *et al*., 2005). Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and antibacterial properties. Plant derived natural products such as flavonoids, terpenoids and steroids etc., have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [25,26]. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory (Abubakar, 2009).

Table 4: Inhibition Zone of ZnOnanoparticles synthesized fromOcimum basilicum

S.No	Organisms	Control	Zone Of Inhibition (mm)			
			20µl	30 µl	40 µl	50 µl
1	*E.coli*	23mm	00mm	00mm	11mm	16mm
2	*S.typhi*	22mm	00mm	07mm	11mm	13mm
3	*P.aeruginosa*	24mm	00mm	00mm	09mm	10mm
4	*B.subtilis*	22mm	00mm	07mm	10mm	13mm
5	*S.aureus*	22mm	00mm	00mm	09mm	14mm

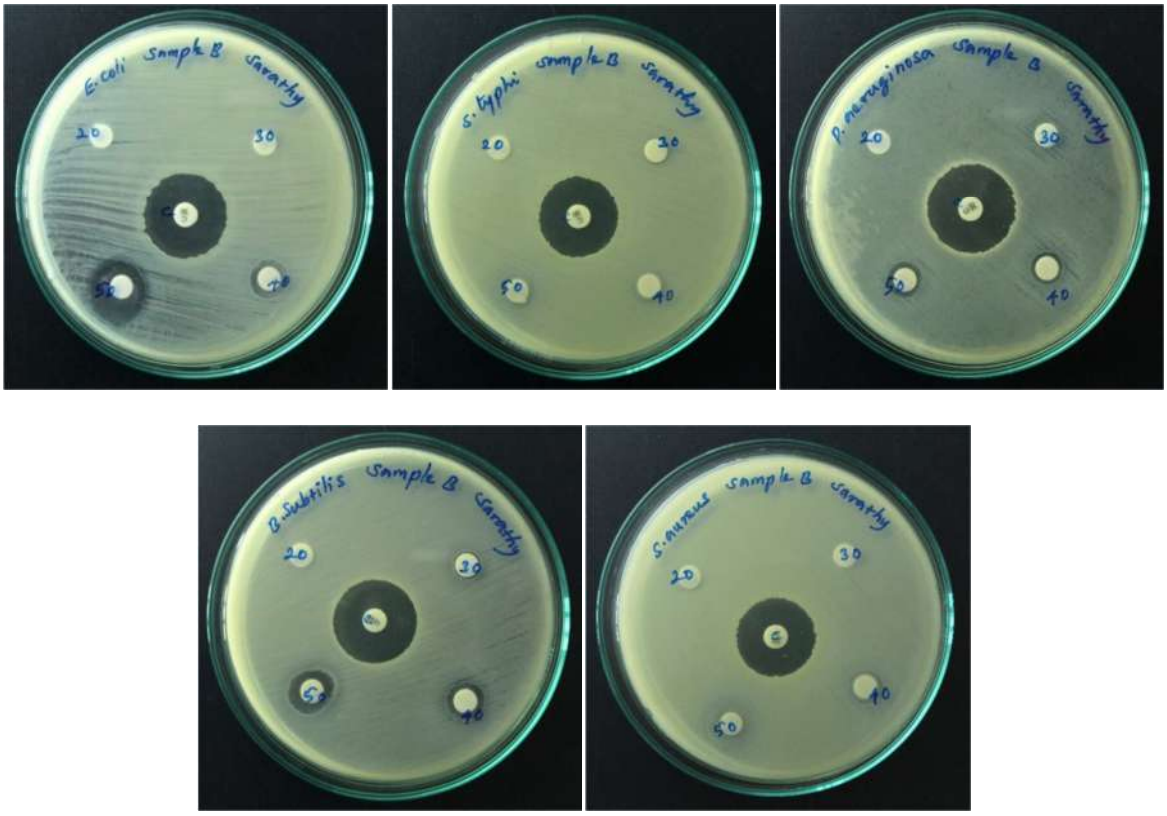
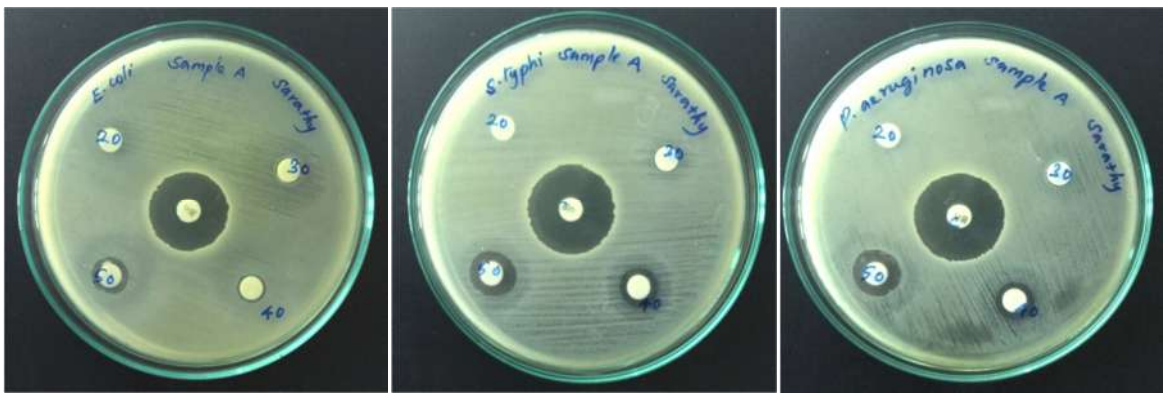


Fig.4: Antibacterial activity of ZnOnanoparticles synthesized from Ocimum basilicum

- **Antibacterial activity of ZnO nanoparticles synthesized from *Anisochilus carnosus***

The antibacterial activity of ZnO nanoparticles synthesized from *Anisochilus carnosus* against various human pathogenswere tested by disc diffusion method was shown in fig 5. As seen in table 5, there was no activity against any organisms at concentration of 20µl. Only *S.aureus, E.coli* and *P.aeruginosa* were inhibited (7mm) at concentration 30µl. At concentration 40µl and 50µl, highest inhibition was found against *P.aeruginosa* and *B.subtilis* followed by *S.typhi.* The maximum inhibition zones of 10mm, 11mm, 13mm, 13mm and 10mm in 50µl of concentration were obtained from the *Anisochilus carnosus*leaf extract assisted ZnO nanoparticles against *E. coli, S.typhi, P.aeruginosa, B.subtilis* and *S. aureus* respectively. When compared to standard control, lower inhibition was observed against all the bacterial pathogens.

Anbuvannan *et al.,* 2015 reported in their study that the antibacterial activity of the *Anisochilus carnosus* synthesized ZnO nanoparticles against *S. paratyphi, V.cholerae, S. aureus,* and *E. coli* showed inhibition zones of 6mm, 10mm, 7mm and 9mm respectively. When compared to control, *Anisochilus carnosus* synthesized ZnO NPs showed a smaller zone of inhibition.



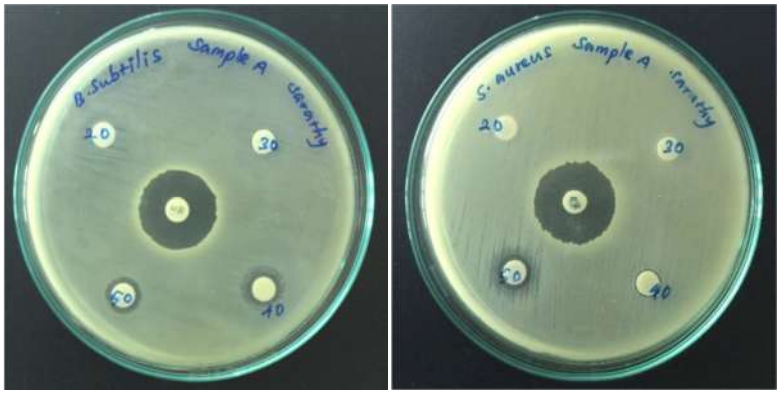


Fig.5: Antibacterial activity of ZnO nanoparticles synthesized from Anisochilus carnosus

Table 5: Inhibition Zone of ZnO nanoparticles synthesized from Anisochilus carnosus

S.No	Organisms	Control	Zone Of Inhibition (mm)			
			20µl	30 µl	40 µl	50 µl
1	*E.coli*	22mm	00mm	07mm	08mm	10mm
2	*S.typhi*	22mm	00mm	00mm	09mm	11mm
3	*P.aeruginosa*	24mm	00mm	07mm	11mm	13mm
4	*B.subtilis*	21mm	00mm	00mm	10mm	13mm
5	*S.aureus*	23mm	00mm	07mm	08mm	10mm

- **Antibacterial activity of ZnO nanoparticles synthesized from *Curcuma neilgherrensis***

The antibacterial activity of ZnO nanoparticles synthesized from *Curcuma neilgherrensis* against various human pathogenswere tested by disc diffusion method was shown in fig 6. As seen in table 6, ZnO nanoparticles show the maximum inhibition in 20, 30, 40 and 50µl concentration. The maximum inhibition zones of 27mm, 23mm, 28mm, 26mm and 24mm in 50µl of concentration were obtained for *Curcuma neilgherrensis*leaf extract assisted ZnO nanoparticles against *E. coli, S.typhi, P.aeruginosa, B.subtilis* and *S. aureus* respectively. However, when compared to standard control, enhanced antibacterial activity of ZnO NPs was observed in all the bacterial pathogens.*In vitro* antibacterial activity of methanolic extract of *Curcuma neilgherrensis*was studied against five pathogenic bacteria. On the basis of zone of inhibition revealed that the extract is quite effective against both gram positive and gram negative bacteria and is more effective towards gram negative bacteria i.e. *P.aeruginosa* and *E.coli*.

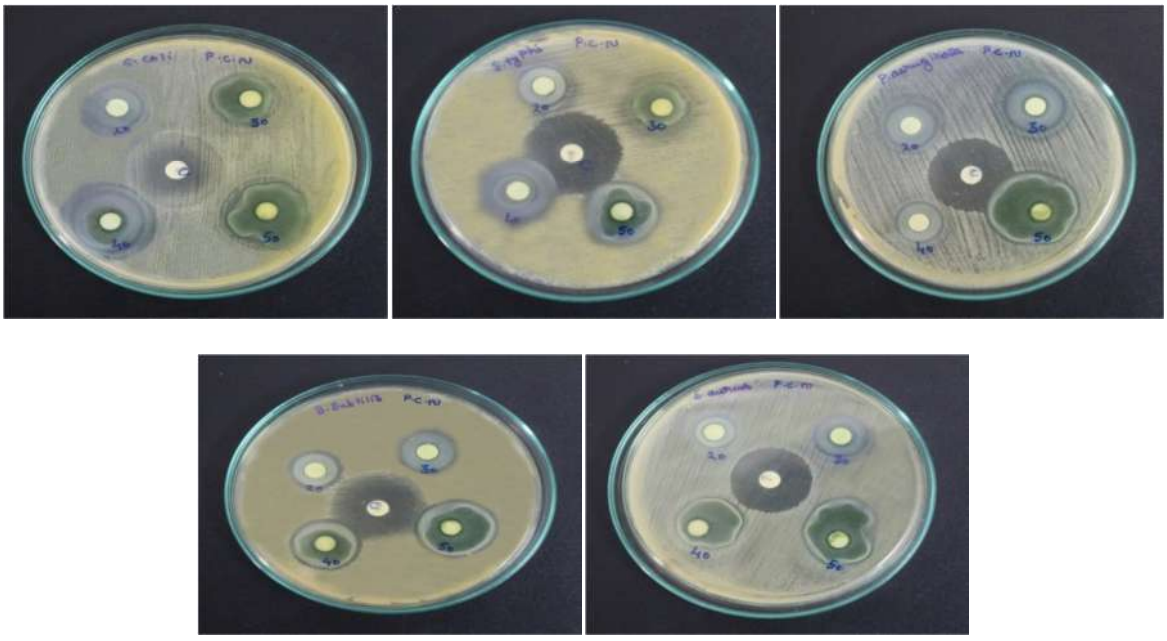


Fig.6: Antibacterial activity of ZnOnanoparticles synthesized fromCurcuma neilgherrensis

Table 6: Inhibition Zone of ZnOnanoparticles synthesized from*Curcuma neilgherrensis*

S.No	Organisms	Control	Zone Of Inhibition (mm)			
			20µl	30 µl	40 µl	50 µl
1	*E.coli*	23mm	20mm	21mm	25mm	27mm
2	*S.typhi*	26mm	14mm	16mm	21mm	23mm
3	*P.aeruginosa*	26mm	15mm	18mm	19mm	28mm
4	*B.subtilis*	24mm	16mm	17mm	21mm	26mm
5	*S.aureus*	23mm	12mm	16mm	20mm	24mm

- **Antibacterial activity of ZnO nanoparticles synthesized from Aloe *vera***

The antibacterial activity of ZnO nanoparticles synthesized from *Aloe vera* against various human pathogenswere tested by disc diffusion method was shown in fig 7. As seen in table 7, there was no activity against *B.subtilis* at concentration of 20 µl. Better activity was seen against *S.aureus* and *S.typhi* than the other organisms. The least inhibition activity was found in *B.subtilis*. The *Aloe vera* extract at the concentration of 50 µl showed 15 mm diameter zone of inhibition against *E. coli*. This was followed by 21mm, 15mm, 20mm and 21 mm zone of inhibition against *S.typhi , B. subtilis, P.aeruginosa* and *S. Aureus.* When compared to standard control, lower inhibition was observed against all the bacterial pathogens.

De Boer *et al.,* 2005 highlighted that the results of the study showed that the methanolic extract was more effective than aqueous extract. This may be due to the better solubility of the active components in organic solvents.

The antibacterial activity of both green synthesized ZnO nanoparticles were studied against the Gram-negative and the Gram-positive bacteria. The maximum inhibition zones of 28mm, 27mm, 26mm, 24mm and 23mm were obtained for ZnO nanoparticles synthesized using *Curcuma neilgherrensis* Wight against all the five organisms i.e. *P.aeruginosa, S.typhi, E.coli, S.aureus* and *B.subtilis* respectively.

Table 7: Inhibition Zone of ZnO nanoparticles synthesized fromAloe vera

S.No	Organisms	Control	Zone Of Inhibition (mm)			
			20µl	30 µl	40 µl	50 µl
1	*E.coli*	30mm	10mm	12mm	14mm	15mm
2	*S.typhi*	31mm	09mm	15mm	18mm	21mm
3	*P.aeruginosa*	27mm	12mm	13mm	16mm	20mm
4	*B.subtilis*	29mm	00mm	09mm	12mm	15mm
5	*S.aureus*	34mm	10mm	16mm	18mm	21mm

Green method possesses important advantages such as lesser or almost zero contamination in the environment because they are derived from the natural plant leaf extract. The synthesized samples have prominent activities against gram positive than the gram negative bacterial strains.

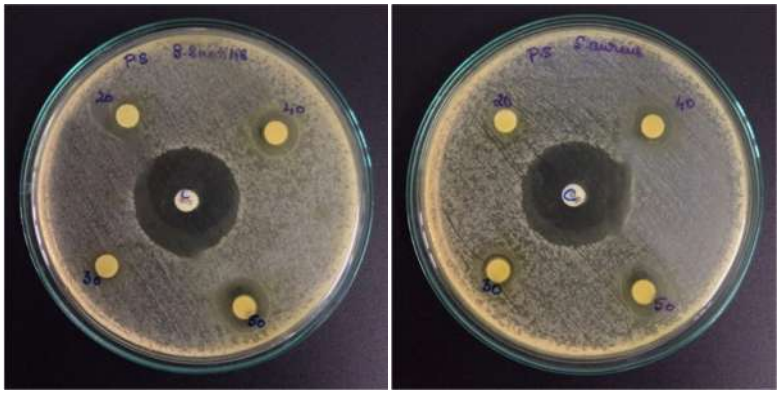


Fig.7: Antibacterial activity of ZnO nanoparticles prepared from Aloe vera

Table 8: Comparison of inhibition zone on 50µl concentration of prepared ZnO samples

S.No	Samples	*E.coli*	*S.typhi*	*P.aeruginosa*	*B.subtilis*	*S.aureus*
1	Pure ZnO	18 mm	19 mm	18 mm	20 mm	19 mm
2	ZnO A	16 mm	13 mm	10 mm	13 mm	14 mm
3	ZnO B	10 mm	11 mm	13 mm	13 mm	10 mm
4	ZnO C	27 mm	23 mm	28 mm	26 mm	24 mm
5	ZnO D	15 mm	21 mm	20 mm	15 mm	21 mm

A comparative analysis of antibacterial activity of ZnO nanoparticles against *E.coli* reported by various researchers is shown in table 8. These results indicate that the Green synthesized ZnO nanoparticles show the better antibacterial activity. Thus the results of the present work are encouraging with green synthesis and it can be taken for further research.

V. CONCLUSION

In this present study, Zinc Oxide nanoparticles were synthesized from the four different (*Ocimum basilicum* L., *Anisochilus carnosus* (L), *Curcuma neilgherrensis Wight* and *Aloe vera)* plant extracts using green combustion method. The prepared ZnO samples were characterized using X-ray Diffraction technique (XRD). Research on ZnO-NPs as antibacterial agent has an interdisciplinary linking physicists, biologists, chemists, and medicine, hence it is the wide spread of their applications. One of these essential applications is in food industry, as an antibacterial agent in food packaging and towards food borne pathogen. The antibacterial influence of ZnO-NPs against food borne pathogens stimulates proficient applications in food packaging, and can be introduced in food nanotechnology.

The ZnO nanoparticles synthesized from the *Curcuma neilgherrensis* leaf extract showed better antibacterial activity against human pathogens due to the presence of both physical and chemical absorber compounds. The chemical absorber compounds include alkaloids, flavonoids, terpenoids, steroids, phenols and tannins. Zinc Oxide acts as a physical blocker compound. Chemical absorbers absorb high-intensity UV rays while physical blockers reflect or scatter them. Hence this can be exploited as a safe and effective ingredient in sunscreen lotions.It is anticipated that this work may be able to enhance further research into novel methodological characterization and clinical correlations in this topic. Meanwhile, solutions would be suggested to consequences of health-related problems by addressing this complex through research and scientific reports.

Acknowledgement

With deep regards and profound respect, I take this opportunity to express my deep sense of gratitude and indebtedness to my supervisor and guide, Dr. M. Saroja M.Sc., M.Phil., Ph.D. Associate Professor, Department of Electronics, Erode Arts and Science College, (Autonomous), Erode-9, for her continuous support and guidance. The help and co-operation from Alagappa University Karaikudi, South Indian Textile Research Association (SITRA), Coimbatore in characterizing the samples are gratefully acknowledged.

Conflict of interest:

The author does not have any conflict of interest.

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SP-16

MICROBIAL DEGRADATION OF AZO DYES USING *Bacillus subtilis*

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ABSTRACT

Environmental pollution causes a major challenge of civilization today where the textile dyes constitute a major source of pollution. Dyes in the textile industry are difficult to remove by conventional waste water treatment methods since they are stable to light and oxidizing agents and are resistant to aerobic digestion. Presence of carcinogens has also been reported in combined waste water of dyeing and printing units. Azo dye like methylene blue and methyl red are used in textile industries can be degraded easily using bacteria like *Bacillus subtilis*. Various concentrations of methylene blue and methyl red were taken as 10, 20, 30, 40, 50 ppm. *Bacillus subtilis* degradation property was found in methyl red compare to methylene blue (10ppm), followed by plasmid was isolated and taken for PCR amplification. In agarose gel electrophoresis the plasmid was found at 630kda- toluene gene. Degraded methyl red was sprayed on green gram (plant) and its growth was observed for three weeks along with control. The test plant was found giving high growth and was estimated with its chlorophyll content.

KEYWORDS: ***oxidizing agents, aerobic digestion, azo dyes, microbial degradation.***

I. INTRODUCTION

Rapidly of industrialization and urbanization around the world has lead to the recognition of environmental relationship between pollution and public health. Most of these dyes are potentially toxic to aquatic life and some are even carcinogenic and mutagenic to humans[1]]. In order to prevent contamination of soil and surface and ground water it is necessary in treating -contaminated wastewater discharged from the textile and other dye - stuff industries[2].Different dyes used in textile industry casually have a synthetic origin and complex aromatic molecular structures, which make them more stable and more difficult to be biodegrade. There are over 10,000 commercial available dyes with a production of over 7×10^5 tons per year[3]. Textile azo dyes are sometimes found difficult to degrade completely and the conventional physico-chemical treatment process are not always suitable enough for their complete degradation and conversion to CO_2[4]. Therefore, focusing on the microbial biodegradation of dyes as a better alternative source of microorganisms, including bacteria, fungi and algae can degrade or absorb a wide range of dyes[5].

II. RELATED WORKS

In contrast, remediation of dying industry effluent by using microorganisms has proved to be the best solution. Since *Bacillus,Pseudomonas, Enterobacter*, *Halobacter* and *Aeromonas* have been reported to exhibit tremendous capability[6]. The biological mode of treatment of dye bath effluents offers unique advantages over the standard modes of treatment. The basic step in the decolourization and degradation of azo dyes is break down of azo bonds, leading to removal of color. Azo dyes known to undergo reductive cleavage where's the result lent aromatic amines are metabolized aerobic condition[7]. The objective of this study was to isolate methyl red and methylene blue degrading bacteria and implementation of degraded water for plant growth.

III. MATERIALS AND METHODS

A. Microbial source & Dye Degradation

Bacillus subtilis was used in the present study to find its degradation property of Azo dye. The Culture medium used in degradation studies were sucrose (0.2 gm), yeast (0.01 gm), KH_2PO_4 (0.012gm) and sodium chloride (0.012gm). Methylene blue and methyl red with different concentration viz., 50, 40, 30, 20, 10 ppm was prepared with Culture medium and inoculated with *Bacillus subtilis*. The culture was incubated in

shaker incubator at 37 °C for 48 hours. The degradation study was calculated using spectrophotometer at 450- 700 and 400- 600 nm respectively.

B. Spectrophotometric analysis

The grown culture in methyl red and methylene blue was incubated in Luria Bertani broth and incubated at orbital shaker. Further it was centrifuged to collect plasmid from isolated colonies.

C. Isolation of plasmid DNA

Lysis technique: 1.5 ml of overnight culture was taken in an Eppendorf tube and cells were collected by centrifugation for 7 minutes at 12,000 rpm. The pellet was thoroughly suspended in 100µl of solution A (25mM Tris HCL, 50mM Glucose, 10mM EDTA) and the solution kept for room temperature for 10 minutes. Then 200µl of solution B (0.2 N Sodium Hydroxide and 10% SDS) was added, mixed gently by inverting the tubes for few times. To that 150µl ice cold solution C (3M Sodium acetate) was added and vortex for a few seconds. The tubes were kept on ice for 5 min. The mixture was then centrifuged at 12,000 rpm for 10 minutes to pellet the plasmid DNA. The clear supernatant was (approximately 400µl) taken in fresh Eppendorf tubes. To those two volumes of ice cold isopropanol (800µl) was added in each tube and kept at -20 °C for 30 minutes. The tubes were again spun at 10000 rpm for 10 minutes and collected the pellet. To that 100µl solution D and 0.5 ml of ethanol was added, mixed well and centrifuged at 5000 rpm for 5 minutes. The pellet was again washed with 70 % ethanol and spun at low rpm. It was finally then dissolved in 20µl TE buffer and stored at - 20 °C.

Extracted plasmid DNA was separated by horizontal electrophoresis in 0.8 % agarose slab gels in Tris-Acetate EDTA (TAE) buffer at room temperature. The 6X gel loading dye was added to the plasmid DNA which in turn was loaded on the gel 15 µl and the electrophoresis unit was run at 50V with an appropriate DNA marker till the dye migrated to a sufficient distance in the gel and visualized under UV trans illuminator.

D. Polymerase chain reaction

The isolated plasmid was amplified by polymerase chain reaction.The plasmid DNA was amplified with required conditions and specific primers using Applied Biosystems PCR (USA) programmed for polymerase chain reaction. It was performed in 50 µl of reaction mixture with CNB- F and CNB- R primers. Amplification was performed in thermo cycler programmed for 35 cycles with required reaction mixtures. PCR products were electrophoresed on 1% agarose gel, stained with Ethidium bromide and visualized under UV-trans illuminator

Primers	Sequences	Base pair size
CNB- F	5' GTGGATGATGCCTCTACAT 3'	19
CNB- R	5' ACCGTATTTTGGGAAGTACA 3'	20

E. Effect of degraded water on green gram

The whole plants of control and treated green gram (with the degraded sample) was taken and measured its length- stem, root and leave. Effect of degraded water on green gram was studied in spectrophotometric analysis. Control and treated plant (for 12 days) was added separately in 95% petroleum ether, 96% methanol and 96% chloroform and homogenized with mortar and pestle. The homogenized was centrifuged at 2500 rpm for one minute. The supernatant was separated and the absorbances were read at 400-700 nm on Elico UV-260 spectrophotometer.

IV. RESULTS

A. Microbial source and degradation study

The organism *Bacillus subtilis* was sub-cultured in nutrient broth and nutrient agar plate. After incubation period the sample was used for further studies. [Figures1, 2]

B. Spectrophotometric analysis

From the readings it is found that both dyes were degraded by *B.subtilis*. In methyl red 10 ppm, 20 ppm and 30 ppm dyes were completely degraded and in methylene blue 10 ppm, 20 ppm concentration of dyes were degraded but not the remaining. So the later was taken for further studies (Figure- 3, 4). The percentage of

degradation was calculated by,

% =Control- Degraded / Control x 100 (Table-1) [Figures 9-15]

C. **Plasmid isolation**

Dye degrading culture was selected for further studies due to its highest percentage of degrading characteristics. Plasmid DNA was isolated from Methylene blue &Methyl red degrading*B.subtilis* colonies using alkaline lysis method. The isolated plasmids were resolved in 0.8% agarose gel and visualised under UV transilluminator. [Figure-5]

D. **Gene amplification by PCR**

In agarose gel electrophoresis the amplified plasmid showed band at 630kda- toluene gene (Figure 6).

E. **Measurement of green gram in control and treated**

Whole plant of green gram of control and treated was measured. The length and size of root, stem and leaves of control and test was tabulated. It is found that test or treated plant has grown well in Methyl red degraded water. It was recorded that Chlorophyll a showed the maximum absorbance at 662 nm, chlorophyll b at 646 nm and total carotene at 470 nm and the amount of these pigments was calculated according to the formulas of Lichtentaler and Well burn in 1985[Figures 16-18].

Table 1: Percentage of degradation of methylene blue and methyl red.

S. No	Concentration at ppm	Methyl red (%)	Methylene blue (%)
1	10	97.06	48.52
2	20	80.47	27.01
3	30	68.39	22.05
4	40	66.81	8.33
5	50	50.3	3.43

Table 2: **Measurement of green gram in control and treated**

Parts of plant	Measurement of length- Test (cm)	Measurement of length- Control (cm)
Root	8.2	6
Stem	18.4	14.5
Leaves	11	8

Table 3: Chlorophyll A and B

Solvent	Control – Absorbance	Treated- Absorbance	Name of the chlorophyll
Methanol	0.418	0.723	Chlorophyll a
Chloroform	0.125	1.427	Chlorophyll b
Petroleum Ether	0.108	0.738	Chlorophyll a

Figure 1:*Bacillus subtilis*

Figure 2: Nutrient agar plate

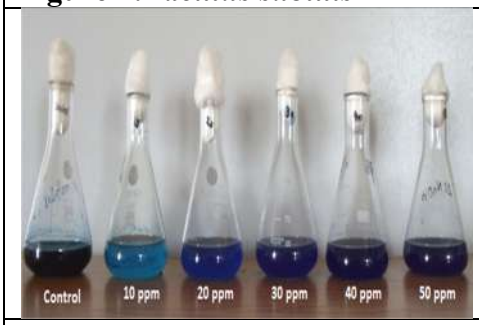

Figure 3. Degradation of methylene blue

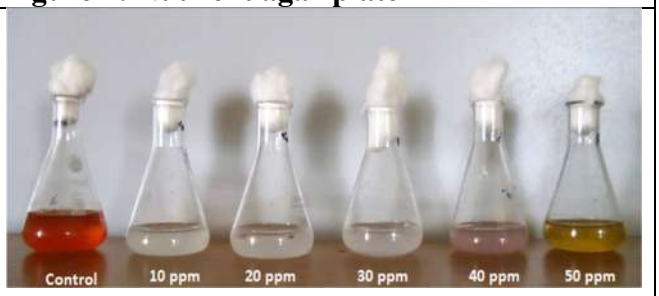

Figure 4. Degradation of methyl red

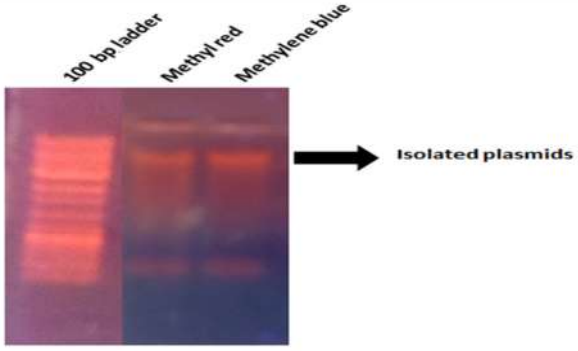

Figure 5: plasmid isolation

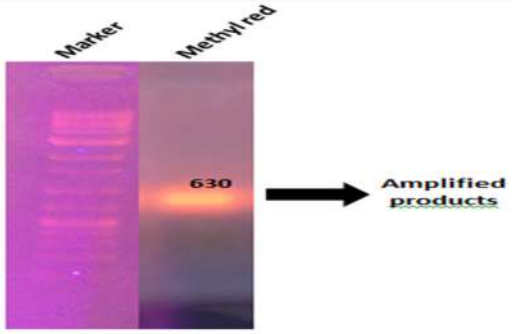

Figure 6: PCR amplified product of methyl red

Figure7: Whole plant of green gram

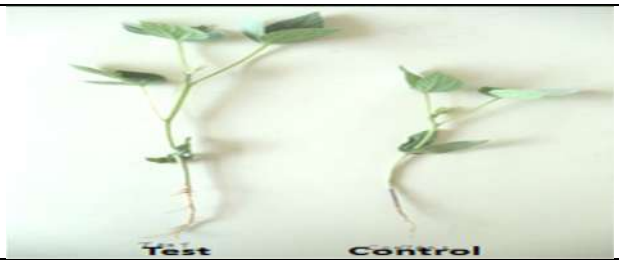

Figure 8. Test and control of green gram

GRAPH

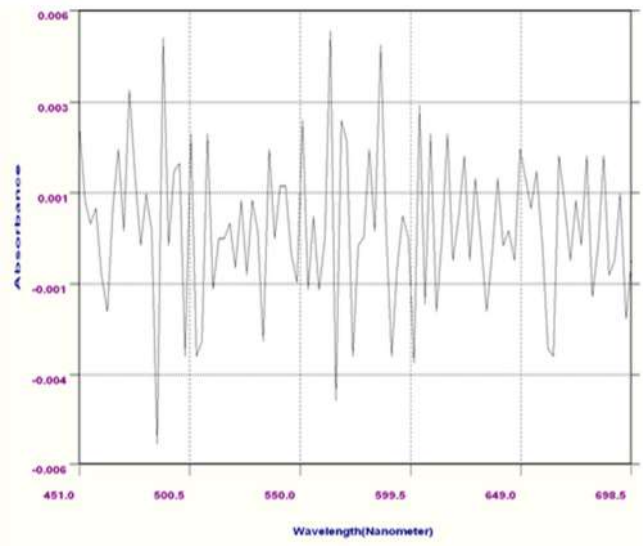

Figure 9:Spectrophotometric analysis of methyl red at 10 ppm

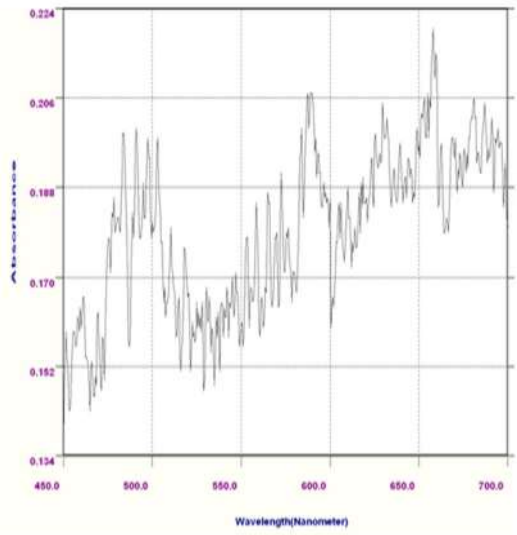

Figure 10:Spectrophotometric analysis of methyl red at 20 ppm

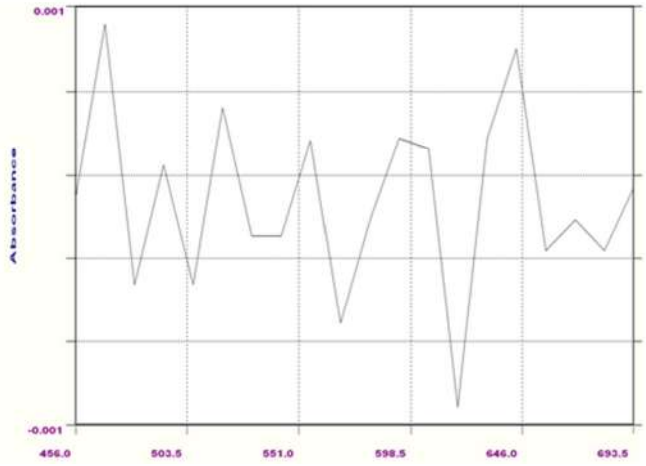

Figure 11:Spectrophotometric analysis of methyl red at 30 ppm

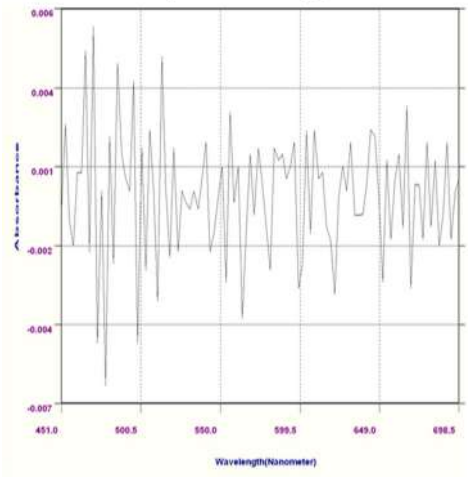

Figure 12:Spectrophotometric analysis of methyl red at 50 ppm

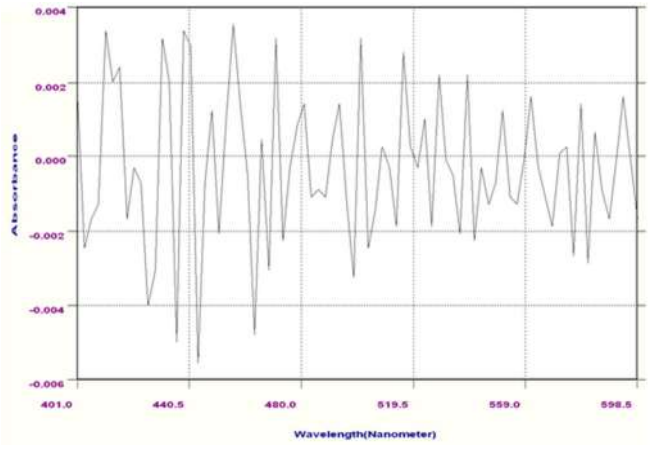

Figure 13: Spectrophotometric analysis of methylene blue at 20 ppm

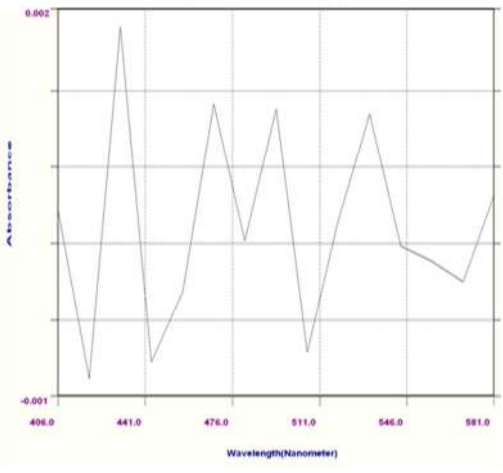

Figure 14: Spectrophotometric analysis of methylene blue at 30 ppm

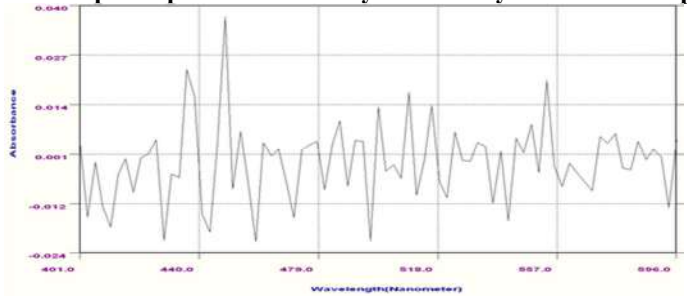

Figure 15: Spectrophotometric analysis of methylene blue at 50 ppm

- **Effect of degraded water on green gram**

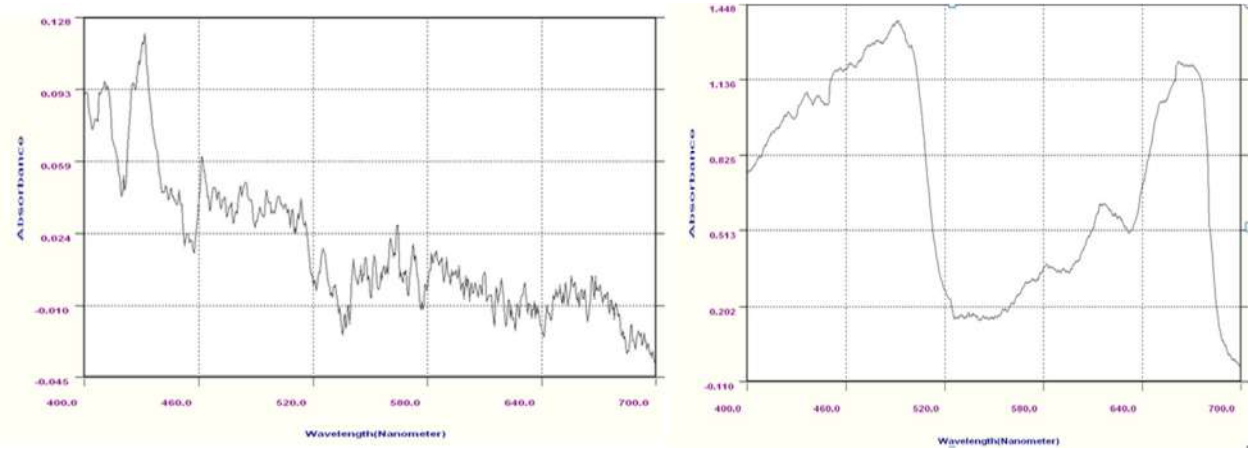

Figure 16: a Control- green gram chloroform **b. Treated- green gram chloroform**

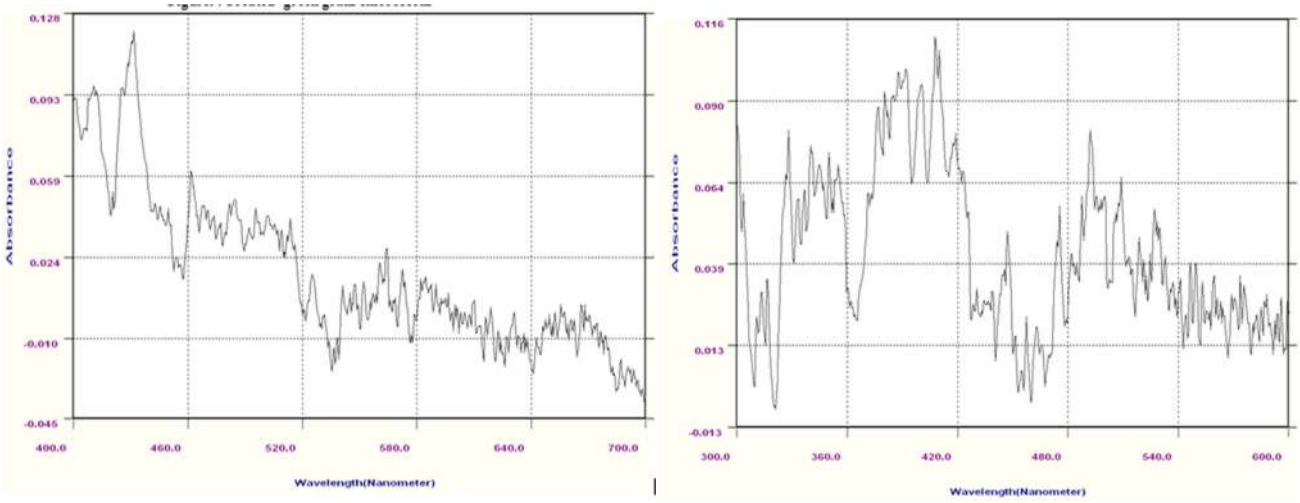

Figure17: a. Control- green gram petroleum ether **b. Treated- green gram petroleum ether**

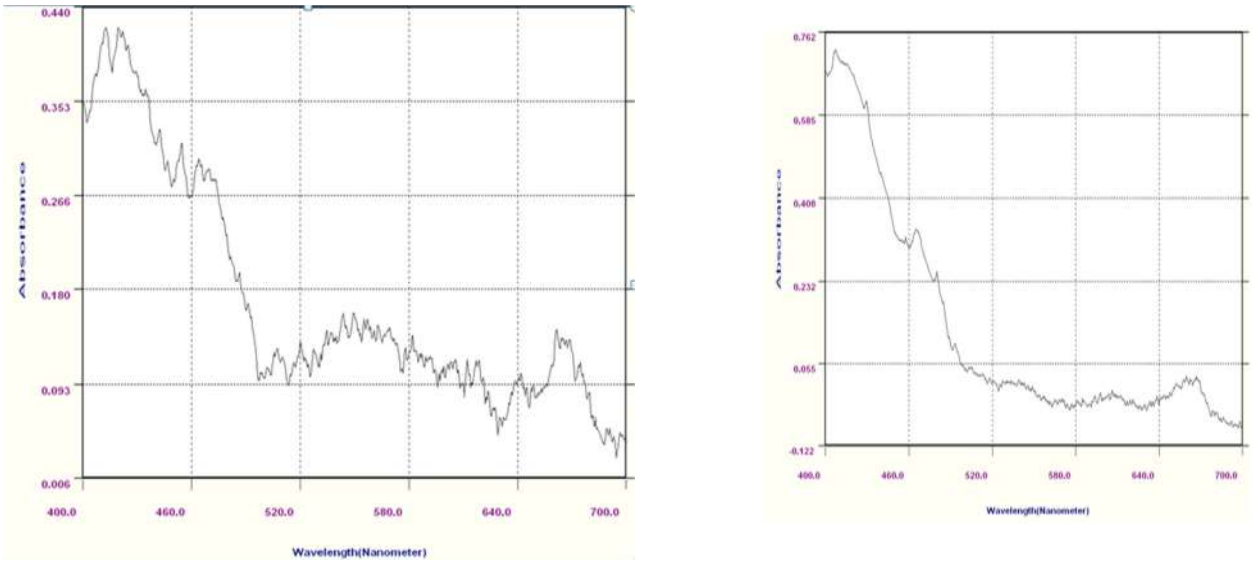

Figure18: a.control -green gram methanol **b. Treated- green gram methanol**

V. DISCUSSION

The growth of the world population, the development of various industries and the use of fertilizers and pesticides in modern agriculture have overloaded not only in the water resources but also the atmosphere and the soil with pollutants. In the few decades, the handling of wastewater appeared to be one of the most important environmental issues. Throughout India, constant attention has been given to the treatment of industrial effluent from textile and dye manufacturing industries. Several researches have demonstrated the

possibility of utilizing for bio treatment of textile water.

Various physical and chemical methods employ in degrading but they are cost expensive and which also creates secondary level of land pollution. Several studies have revealed that pH, temperature, various carbon and nitrogen sources have a significant influence on dye removal efficiency especially in crystal violet by *Bacillus subtilis* ETL- 2211. Similarly Shah *et al.,*[7], demonstrated microbial degradation of textile dye (Remazol Black B) by *Bacillus* species[8-9]. Since there have been several methods like using silver nanoparticles of bacteria, fungi and algae of degrading dye, microbiological degradation using *B.subtilis* is quite easy and more degradable. It is reported that *Pseudomonas aeruginosa*, *Brevibacillus choshinensis* effectively decolorize methylene blue, malachite green, saffranine and crystal violet up to 100 ppm concentration[10].

In the present study, methyl red is degraded more than methylene blue so it could be identified that this bacterial strain has more effective in degrading the compounds present in methyl red. Further degradation studies to be improved in various methods using carbon and nitrogen sources. Similarly, methylene blue has not degraded to the estimated amount so repeated aliquots of microorganism in various concentrations to be added and estimated. Treated dye was found to improve plant growth and total chlorophyll content as compared to control.

VI CONCLUSION

The results of this findings and literature suggest a great possibility for bacteria to be used to decolorize substances from dye wastewaters. This preliminary work can be continued in future for its effective implementation on plant growth.

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We thank the management of Sri Krishna arts and science college, Coimbatore, Tamil Nadu, India for the guidance to carry out this work. I also thank Centre for Bioscience and Nano science Research, Coimbatore, for providing necessary facilities throughout the work.

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SP-17

ANTI-INFLAMMATORY INTESTINAL ACTIVITY OF CLITORIA TERNATEA BY TNBS COLITIS MODEL

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ABSTRACT

Despite recent advances, the inflammatory bowel disease (IBD) remains incompletely understood. IBD is a group of chronic relapsing and remitting disorders of the gastrointestinal tract manifesting as Crown's disease (CD) or ulcerative colitis (UC). A variety of animal models have been utilized in an effort to provide further insights and develop more therapeutic options. In this paper, the effect of *Clitoria ternatea* was studied for the treatment of Ulcerative Colitis in rats. UC was induced by Trinitrobenzene Sulphonic acid (TNBS) through intrarectal administration and the extract of *Clitoria ternatea* 250mg/kg and 500 mg/kg was administered orally. Five groups of rat model were taken to study the effect of *Clitoria ternatea* with control Dexamethasone. There was a decrease in body weight due to decrease in feed intake in response to TNBS induction but after treatment there was normal intake and increase in body weight in groups administered with *C.ternatea.* Myeloperoxidase activity **(**MPO) of TNBS rats was 696.74 units/gm. *Clitoria ternatea* significantly reduced the MPO level to 507.6 and 365.16 mg units/gm and Dexamethasone inhibited the activity to 83.04% in present study.

KEYWORDS*: Clitoria ternatea*, Trinitrobenzene Sulphonic acid, Ulcerative Colitis.

I. INTRODUCTION

Human intestine contains more than 100 trillion "microbiota," involves in complex processes with host mucosal and fundamental physiological processes such as digestion, energy homeostasis, and gut-associated lymphoid tissues antigens & metabolic end-products of gut microbiota[1-7]. However, this homeostatic relationship is perturbed in inflammatory bowel diseases (IBD). Inflammation is a large protective response to stimuli that involves immune cells, blood vessels, and molecular mediators to reject the first cause of cell injury, clear necrotic cells, and tissues[4]. *Clitoria ternatea* (butterfly pea, Tamil: Sangupushpam) belongs to the Fabaceae family. They are used in ayurvedic medicine because of its multipotent bioactive molecules such as alkaloids, tannins, flavonoids, glycosides and phenolic compounds[5]. It cures swelling and prevents pus formation. It is also used in treating digestive problems such as emesis, dyspepsia, constipation jaundice and piles.

II. MATERIALS AND METHODS

A. Sample collection and extraction

Clitoria ternatea samples were collected from in and around Chennai. The sample was extracted (methodology of Indian Pharmacopedia) using a Soxhlet apparatus, filtered and evaporated using a rotatory evaporator (Super fit-ROTAVAP, India). The dried extract was stored at 20°C.

B. Phytochemical Tests

(a) **Carbohydrates Test:** To 2ml of plant extract, 1ml Molisch's reagent and few drops of concentrated sulphuric acid were added. The presence of purple or reddish color indicates the presence of carbohydrates.
(b) **Tanninse Test:** To 1ml of plant extract, 2ml of 5% ferric chloride was added. The formation of dark blue or greenish black indicates the presence of tannins.
(c) **Saponins Test:** To 2ml of plant extract, 2ml of distilled water was added and kept in a graduated cylinder for 15minutes. 1cm layer of foam indicates the presence of saponins.
(d) **Flavonoids Test:** To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. The presence of yellow color indicates the presence of flavonoids.

(e) **Alkaloids Test:** To 2ml of plant extract, 2mlof concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. The presence of green color /white precipitate indicates the presence of alkaloids.
(f) **Quinines Test:** To 1ml of extract, 1ml of concentrated sulphuric acid was added. The formation of red color indicates presence of quinones.
(g) **Glycosides Test:** To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. The formation of pink color indicates presence of glycosides.
(h) **Cardiac glycosides Test:** To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was made to 1 ml of concentrated sulphuric acid. The formation of brown ring at the interface shows the presence of cardiac glycosides.
(i) **Terpenoids Test:** To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added. The formation of red brown color at the interface indicates presence of terpenoids.
(j) **Phenols Test:** To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. The formation of blue or green color indicates presence of phenols.
(k) **Coumarins Test:** To 1 ml of extract, 1ml of 10% NaOH was added. The formation of yellow color indicates presence of coumarins.
(l) **Steroids and phytosteroids Test:** To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring shows the presence of phytosteroids.
(m) **Phlobatannins Test:** To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.
(n) **Anthraquinones Test:** To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones

C. Animal maintenance

Rats weighing 100-140g were housed 4/cage and kept in the animal house for one week for proper acclimatization and controlled conditions of illumination (12 h light/12 h darkness) and temperature ranging 20-25°C. A standard diet has been followed. The studies were conducted in compliance with guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA no. 971/bc/06/CPCSEA), Government of India and approved by the Institute of Animal Ethics commission.

D. Induction of Colitis

After one-week, the rats were divided into five groups composed of 5 rats each. IBD (Inflammatory Bowel Disease) is induced by the intrarectal administration of 2.75 mg of TNBS (6) in 50% ethanol while the rat is under anaesthesia and held upside down for 90 seconds. Dosing proceeded after 24 hours of IBD induction and dosing was continued daily for seven days through oral administration. After 7 days the regimen was completed.

Group 1 : rats were given fresh water and pellets *ad libitum* (control)
Group 2 : TNBS induces colitis
Group3, 4 : TNBS induces colitis and dosed with 250mg/kg & 500mg/kg of *Clitoria ternatea*administered p.o
Group 5 : TNBS induces colitis and dosed with dexamethasone 0.1mg/kg (Standard control)

All rats were killed and colons were harvested. Length and weights (Post flush) was recorded.

E. Measurement of Colon Length, Weight of Colon Tissue

After treatment, there was an increase in body weight comparatively. At the end of 7 days, all rats were killed and large intestines without the caecum were removed after washing in ice-cold phosphate-buffered saline (PBS), they were placed on filter papers to measure their length, after which they were opened to remove the content and weighed. Colonic mucosa samples was scraped, further it was added to 0.5mL of ice-cold PBS and homogenized on ice for 15s. The homogenates thus obtained were centrifuged at 1900 g for 15 min and stored at -80^{o} C till estimation of MPO was done.

F. Myeloperoxidase assay

50 mg colon tissue was mixed in 1ml phosphate buffer (containing the 0.5% hexadecyltrimrthyl ammonium bromide). Three cycles of freezing (-30 degree Celsius), thawing (37 ^{0}Celsius) and sonification was done.

0.1 ml was mixed with 50mM phosphate buffer (containing the o-dianisine dihydrochloride). Absorbance was taken at 470 nm and MPO activity was calculated,

$$\text{MPO} = \frac{X}{\text{wt of the piece of tissue (50mg)taken}}$$

$$\text{Whereas X} = \frac{10 \times \text{change in absorbance}}{\text{wt of theVolume of supernatant taken in the final reaction piece of tissue taken}}$$

$$\text{TNF}\alpha = \frac{X}{\text{wt of the piece of tissue (50mg)taken}}$$

III. RESULTS AND DISCUSSION

A. Phytochemical analysis

Extract of *Clitoria ternatea* were made for the test of various chemical analysis of alkaloids, flavonoids. Both positive (+) & negative (-) results were observed and tabulated.

Table: 1 phytochemical analysis

S.No.	Phytochemical test	Results	S.No.	Phytochemical test	Results
1	Carbohydrate	+	8	Flavanoid	+
2	Terpenoid	+	9	Phenol	+
3	Steroid	+	10	Phlabatannins	_
4	Tannin	+	11	Quinone	_
5	Saponin	+	12	Anthraquinones	_
6	Coumarins	+	13	Cardiac glycoside	+
7	Alkaloid test	+			

B. Evaluation of colitis

Disease Activity Index (DAI) was determined by the method described by (8) at the time of killing the rats.

Percentage of Initial body weight = (weight on day x / weight on day 0) x 100%

Table: 2 Clinical score

Score	Weight loss	*Stool* Consistency*	Occult / gross bleeding
0	None	Normal	Normal
1	1-5%		
2	5-10%	Loose stool	Hemoccult
3	11-15%		
4	>15%	Diarrhoea	Gross bleeding

C. Measurement of body weight and feed intake [Figues 1-3]

The body weight and the feed intake was taken daily during the treatment days. There was a decrease in the body weight due to the decrease in the feed intake response to TNBS induction but when treatment was started, there was normal intake of feed leading normal increase in body weight in groups of *clitoria ternatea*

D. Myeloperoxidase assay [Fig.4]

The inhibition of the control group (group 1) was 0.2µg units/gm of tissue. Activity in the TNBS treated animals (group 2) was 696.74µg units/gm in wet colon. In group 3 & 4 - C*litoria ternatea* showed inhibition levels to 27.15% (507.6 µg units/gm) and 47.59% (365.16 µg units/gm). Dexamethasone inhibited the MPO activity to 83.04% during the study.

The work indicates, the TNBS model is made for the formation of the lesions and cures the human Ulcerative Colitis. The recent research has proved the results that inflammation was reduced by those treatments they include, Anti-ulcerative colitis activity of *Calotropis procera* Linn Amani A.et al. (2018)[1],

Evaluation of anti-ulcer and ulcerative colitis of Sonchusoleraceus EsraaA et al.,(2018)[3], Preventive and curative effect of *Pistacia lentiscu*s oil in experimental colitis Mustafa S et al.,(2018)[9]. *C.ternatea* has showed effective result in the study.

IV. CONCLUSION

Among these, *C.ternatea* has been proven and fulfils the criteria at the initial stage of study and can serve as a useful model in the formation of future novel drugs for therapy of UC. This model works in a better way comparing with all other methods and provided efficient results.

ACKNOWLEDGMENT

We thank the management of Sri Krishna arts and science college, Coimbatore, Tamil Nadu, India for the guidance to carry out this work. I also thank Kavin Bioresearch laboratory, Chennai for providing necessary facilities throughout the work.

ANALYTICAL FIGURE

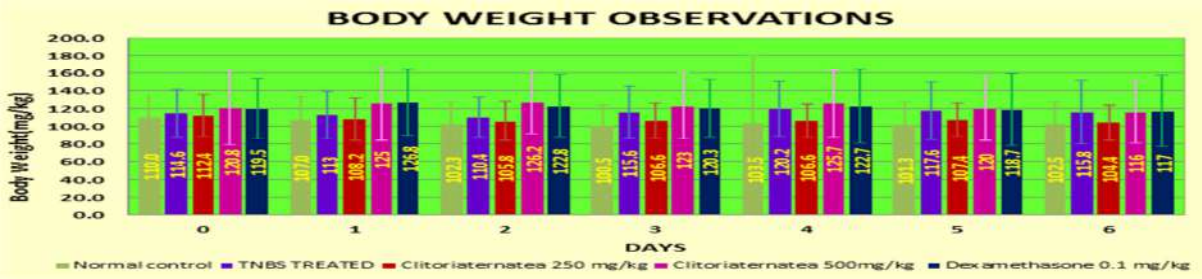

Figure1: Body weight in grams.

The 5 groups of rats with the positive, negative and test were taken.Body weight in the 7 consecutive days during the treatment and the weight of the different groups were analysed under the graphical representation.

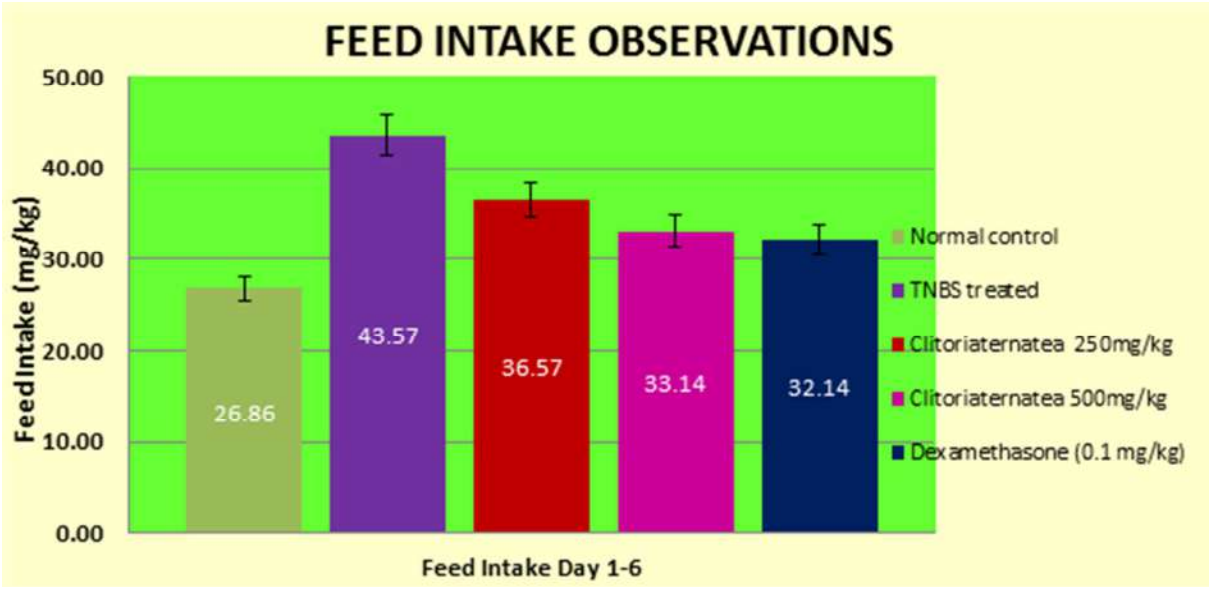

Figure 2 Food intakes in grams.

The feed intake during the process of the treatment and the differences of the feed intake in the initial level and the intake during inflammation of the colitis was observed.

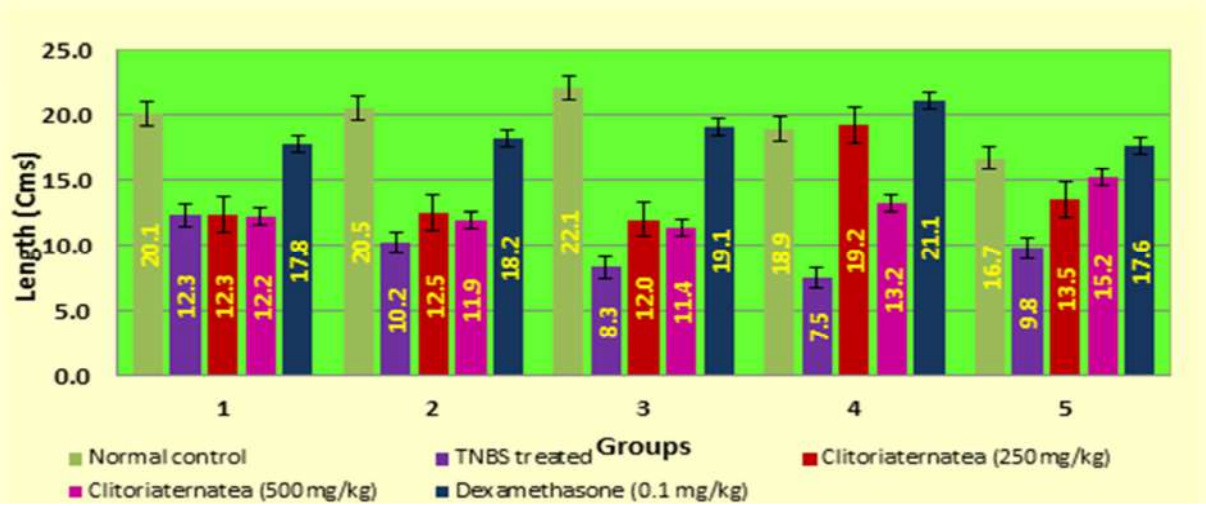

Figure 3 Colon length.

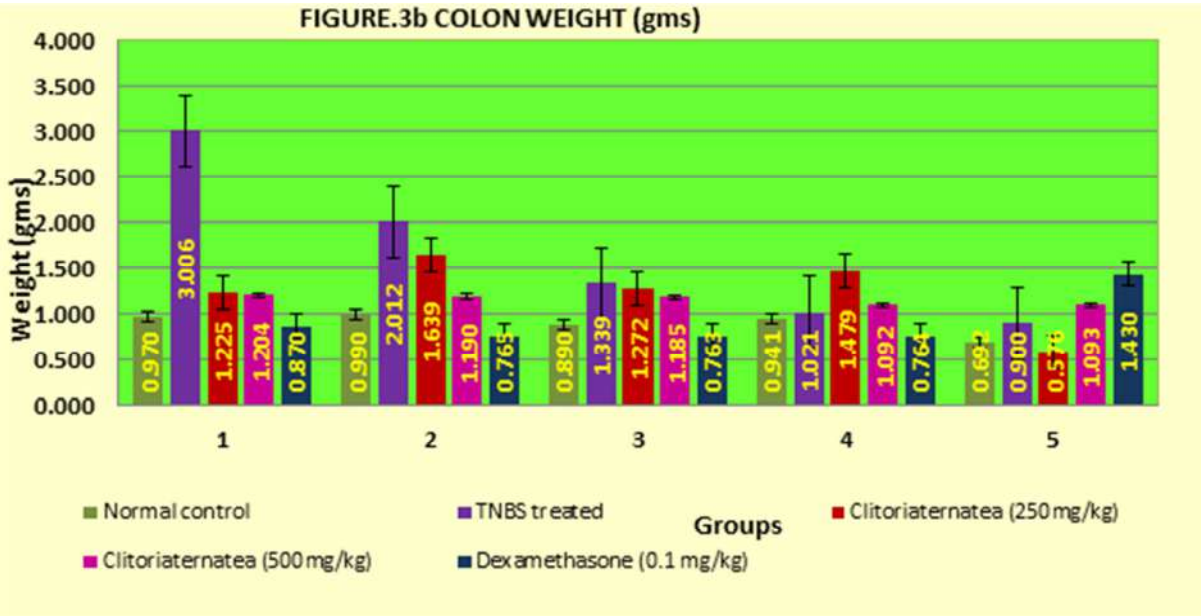

Figure 3b colon weight

The weight of the colon after and before the treatment was analyzed for the identification of the effect of the sample used.

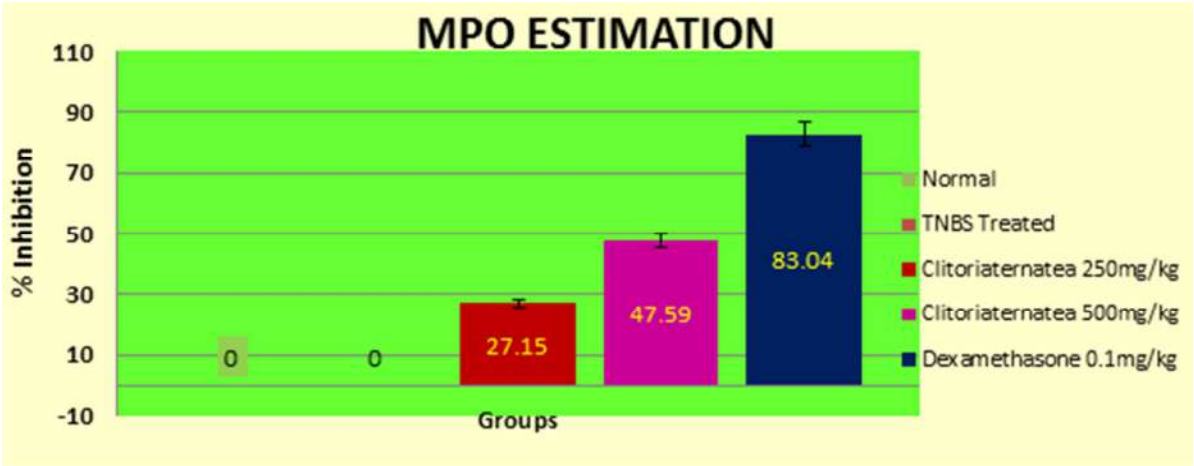

Figure 4.The inhibition of the used sample was identified by the MPO Estimation

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SP-18

ENERGY PROFICIENT AND SECURED ROUTING ARCHITECTUREUSING WSN FOR HABITAT MONITORING

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***Abstract*:**Technology can help the animals and plants for their identification, monitoring and studying their behavior pattern. The proposed architecture can be a reliable routing strategy which can be implemented for the efficient Habitat monitoring in Wildlife. A Wireless Sensor Network (WSN) consists of tiny sensor nodes to sense the environment, where it is actually deployed. The collection information is forwarded periodically to the Base Station (BS). Low cost, high reliability and easy maintenance are the key features to design a routing protocol. The proposed protocol Efficient Route Recovery with Secured and Energy Proficient Routing (ERRSEPR) focuses in minimal energy consumption on each node is carried out using minimal energy, power amplification and efficient routing technique to improve lifetime of the network. It also detects blackhole attack on the network with finding the optimal routing and rerouting using fault tolerant. The optimal routing is obtained using the combination of Artificial Bee Colony (ABC) and Particle Swarm Optimization (PSO). The proposed ERRSEPR has unique behavior in searching optimal path, balancing the network load and network topology maintenance. The proposed ERRSEPR protocol is applied to Ad-hoc On-demand Distance Vector (AODV) routing protocol and the experiment is tested using network simulator 3. The extensive simulation experimental results show that the proposed protocol outperforms than the other several routing protocols.

KEYWORDS: ***Amplification, Adhoc, Optimization, Swarm, Topology, Wildlife***

I. INTRODUCTION

Wireless Sensor Networks (WSN) have an emerging key technology includes environmental sensing, military applications, medical applications and so on. Improving the potentiality of applications for WSN, extremely efficient and easy accessibility routing protocols are needed[1-2]]. For prolonging the lifetime of the WSN, it focuses on the issues like data processing, routing, energy optimization and so on. Among other issues, routing plays a vital role in prolonging the lifetime of the network. WSN is composed of sensors that sense the environment where it is actually deployed and collects the information from the environment and forwards it to the Base Station (BS)[3,4]. Sensor nodes are comprised of tiny sensing, processing unit, RAM, limited battery, transceiver unit to communicate and so on. Some of the optional units of sensors are location finding, power generator and mobilizer to perform a specific task[5-7]. However, due to the dynamic topology and minimal centralized security, the network is vulnerable to the kind of attacks like blackhole attack, node capture, node cloning and Denial of Service (DoS)[8]. In case of equal sequence number in RREP, the RREP is selected based on smallest hop count[9]. In blackhole attack[10], the malicious node forwards a fake RREP to the source to pretend the malicious node has highest sequence number. Therefore, the source decides the malicious node's RREP as a best route and forwards the data to the malicious node.

II. RELATED WORKS

The malicious node receives the original data and forwards the fake message to the BS. In past approaches, trust route is obtained through fake messages. In some cases, the energy efficiency is also considered and improved through the trust route. In some approaches, the energy efficient parameters were added together with the trust to maintain better network lifetime[11,12]. Another issue of WSN is security. WSN is tremendously improving its applications in the recent years[13]. Habitat and environmental monitoring[14] represent a class of sensor network applications with enormous potential benefits for scientific communities and society as a whole. This paper develops a specific habitat monitoring application that is largely

representative of the domain. It presents a collection of guidelines that serve as a basis for a general sensor network architecture for many such applications.

III. METHODOLOGY

The proposed protocol ERRSPER focuses on the major constraints of WSN. They are,

- Trust based Routing
- Energy Proficient Routing
- Fault tolerant routing

IV. RESULTS AND DISCUSSION

A. AODV Protocol

The proposed protocol follows multi-hop flat routing topology. In this topology, all nodes are assigned with equal energy, functionality and roles. To identify and defend the blackhole attack in WSN, the proposed ERRSEPR protocol uses AODV routing protocol [15] [16]. AODV is a reactive distance vector routing protocol. The malicious node which is attacked by blackhole attack selects the RREQ from source node and forwards a fake RREP with high sequence number. Therefore, the source decides the malicious node's RREP is the best route and forwards the data to the malicious node. The malicious node may not send the data to BS or it forwards a fake data to BS. Thus, it leads a malfunctioning of the network and the malicious node also drains the energy of the node resulting to network inefficiency. Therefore, the ERRSPER protocol defines a trust value to identify the malicious node that caused by blackhole attack and defends the network from such attacks. The calculation of proposed trust is presented in next section.

- ***Calculation of proposed trust for ERRSEPR***

Every node performs trust calculation to avoid blackhole attack. When the node N_{src} performs a route detection for node N_{neigh} at t_i time. The trust of node N_{src} to $N_{neigh}$is $\left\{\bigwedge_{N_{neigh}}^{N_{src}}(t_1);\right.$ otherwis$\left\{\bigvee_{N_{neigh}}^{N_{src}}(t_1)\right.$e;

Consider the node N_{src} has n interactions with N_{neigh} during the time t, the detection is as follows,

$$\left\{\bigwedge_{N_{neigh}}^{N_{src}}(t_1)\middle|\bigvee_{N_{neigh}}^{N_{src}}(t_1),\bigwedge_{N_{neigh}}^{N_{src}}(t_2)\middle|\bigvee_{N_{neigh}}^{N_{src}}(t_2),\ldots.\right.$$
$$\left.\bigwedge_{N_{neigh}}^{N_{src}}(t_w)\middle|\bigvee_{N_{neigh}}^{N_{src}}(t_w)\right\}$$

$\bigwedge_{N_{neigh}}^{N_{src}}(t_i)\middle|\bigvee_{N_{neigh}}^{N_{src}}(t_i)$refers the trust value of N_{src} to N_{neigh} at (t_i), when the data is dropped then $\bigvee_{N_{neigh}}^{N_{src}}(t_i) < 0$;

otherwise $\bigwedge_{N_{neigh}}^{N_{src}}(t_i) > 0$.

The proposed ERRSEPR algorithm for trust evaluation (*Trust Evaluation Algorithm)* is defined as follows.

1: Initialize the Network.
2: N number of nodes where: N = 1,2,3 ., n
3: Source Node N_{src} Initialize Route Discovery
4: N_{src} sends RREQ Packets to N_{neigh} Neighbors.
5:N_{neigh}receives the RREQ Packets and forwards the RREP Packets to N_{src}
6:N_{src}checks all N_{neigh} RREP Packets and choose highest sequence number RREP from received RREP and inform the selection to specified N_{neigh}
7:IF more than one N_{neigh} holds highest sequence number then
    Checks for N_{neigh} hop counts
  Select less hop count N_{neigh} and inform the selection to specified N_{neigh}
8: IF N_{neigh} is sink then
    Data routing process completed

Else
Repeat step 3 to step 8 until completing the data process.
9: End process

B. Energy Proficient Routing for ERRSPER

The proposed Energy Proficient routing for ERRSEPR protocol focuses in improving the lifetime of the network through performing proficient data communication. It also focuses in utilizing the minimal energy for communication and uses the battery resources efficiently[17].

The effective data transmission technique is initialized in the time of network formation itself. The proposed protocol follows,

(i) Hop count mechanism – RREQ is forwarded by source node to its neighbors and neighbors replies RREP with hop count to the source node.

(ii) Follows shortest path data communication which is processed through hop count mechanism.

(iii) In proposed ERRSEPR, the distance between the source node and destination node are also received with RREP.

(iv) In deciding route,

a. When distance seems high and hop count is less, then the proposed protocol doses not choose the path as shortest.
b. When distance seems low and hop count is high, the proposed protocol checks other RREP for best route.
c. When a distance is low and hops count is less than the other RREP then sources choose the RREP path as shortest path for communication.
d. If not found, then it chooses the option b as a best path for communication

(v) Finally, the residual energy level of the sensor node also plays a vital role in choosing the proficient path for WSN communications.

(vi) After deciding the communication path,

ERRSEPR amplifies the battery level of the sensor node available in communication path to improve the proficiency of the network. The proposed algorithm for energy proficient routing on ERRSEPR is as follows,

1 For each neighbor node
Node A forwards RREQ
Node A receives RREP with hop count, distance and residual energy from its neighbors.
2 Checks each neighbor's RREP for less hop count to sink, less distance between node and sink node and finally checks the route whether it maintains high residual energy than the other routes.
3 If node A attains the best RREP (From Instance node B) Then
Power amplification changed to HIGH for node A.
Data transmission starts to node B
4 If node B is sink
Successfully received
Communication ends
Power amplification changed to LOW for node A.
5 Else, GOTO step 1
6 End Process

ERRSEPR protocol proposed the equations Eq.3, Eq.4, Eq. 5 and Eq.6 for hop count, distance between node and sink node, residual energy and power amplification respectively.

$$Hop_{count} = \sum_{i=1}^{n-1} 2^{2i-1} \qquad (Eq.3)$$

Where, Hop_{count} denotes Hop Count, n denotes number of nodes and i denotes iterations.

$$Dist = \frac{Dist_{BS}}{Dist_{farthest}} (Eq.4)$$

Where, $Dist$ denotes distance, BSdenotes sink node and $farthest$ denotes farthest node.

$$Resi_{Energy} = \left[\frac{EGY_{current}}{EGY_{maximum}}\right] (Eq.5)$$

Where,$Resi_{Energy}$ denotes residual energy, $EGY_{current}$ denotes current energy and $EGY_{maximum}$ denotes maximum energy.

$$Pwr_{amplify} = \begin{Bmatrix} High_{pwr}\left(\frac{EGY_{current}}{EGY_{maximum}}\right) n = transmittingnode \\ Low_{pwr}\left(\frac{EGY_{current}}{EGY_{maximum}}\right) n = normalnode \end{Bmatrix} (Eq.6)$$

Where, $Pwr_{amplify}$ denotes power amplification, $High_{pwr}$ denotes high power, Low_{pwr} denotes low power amplification.

By combining the equations Eq.3, Eq.4 and Eq.5 the Eq. 7 is proposed for energy proficient routing for ERRSEPR protocol.

$$Energy_{Proficiency} = \left\{\sum_{i=1}^{n-1} 2^{2i-1} + \frac{Dist_{BS}}{Dist_{farthest}} + \left[\frac{EGY_{current}}{EGY_{maximum}}\right]\right\} (Eq.7)$$

Where, $Energy_{Proficiency}$ denotes energy proficiency. Through the Eq. 7 the best energy proficient model is proposed for ERRSEPR protocol. After accomplishing the energy proficiency, the proposed protocol utilises the Eq. 6 for power amplification to amplify the energy to improve the efficiency and lifetime of the network.

C. PerformanceAnalysis

In order to test the efficiency of ERRSEPR protocol, Network Simulator 3.25[13-15] is used. The parameters specified for the proposed ERRSEPR protocol is given in Table I. In this paper, the AODV-SMS(ABC-PSO)[16-18], ActiveSensor[19], EE_QoS[20], FTDR[21]and FBTC[22]are used for comparing with the proposed protocol. The Figure 1 presents the ERRSEPR protocol running in NS 3.25.

Table 1. Network parameters specified for proposed protocol.

Parameters	Value
Network Space	100 X 100
Number of Nodes	100 (0 – 99)
BS	Node 0
TX Power	-5 dBm
Eelec	50nJ/bit
Initial Energy	1J
Packet length	6400 bits
Simulation Time	1000 sec
MAC type	MAC/802_11
Protocol	AODV
Mobility Model	Random Waypoint Mobility Model
Traffic Model	CBR (Constant Bit Rate)

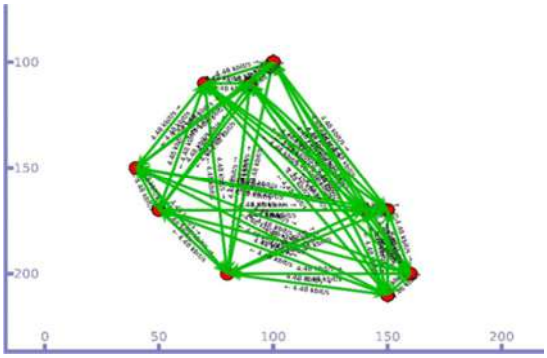

Figure 1: ERRSEPR Protocol

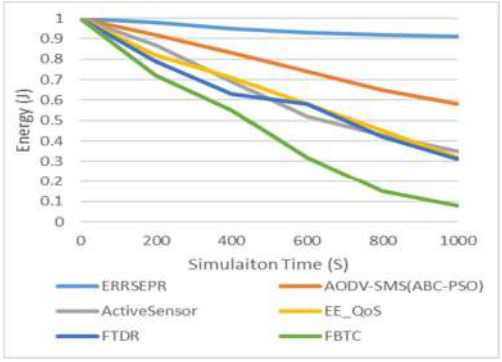

Figure 2: Residual Energy

The parameters used to evaluate the performance of the proposed ERRSEPR protocol are (a) Residual Energy, (b) First Node Dies Ratio, (c) Packet Delivery Ratio, (d) Network Connectivity, (e) DataTransmission Latency

Figure 2 presents residual energy of the proposed ERRSEPR protocol which is compared with AODV-SMS (ABC-PSO), ActiveSensor, EE_QoS, FTDR and FBTC[20]. The proposed ERRSEPR protocol achieves better residual energy than the existing schemes. The maximum energy dissipation is 0.09 J for the proposed protocol that shows it achieves a better energy efficient model than the existing approaches.

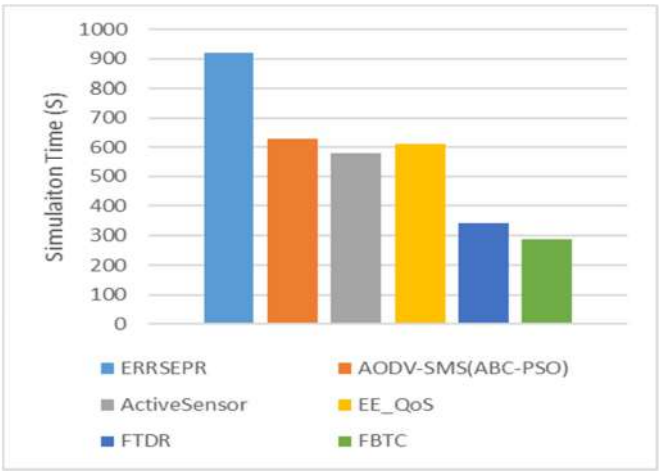

Figure 3 First nodes Dies Ratio

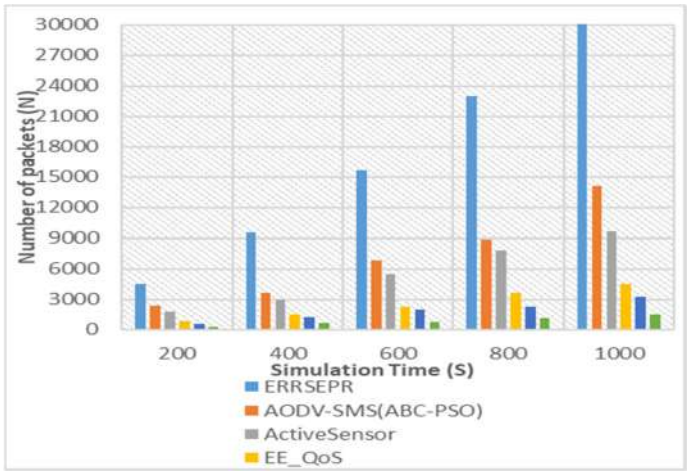

Figure 4 Packet Delivery Ratios

The Figure 3 presents the first node dies ratio between the proposed ERRSEPR and other existing approaches. The proposed ERRSEPR protocol achieves better simulation time i.e. 920 seconds for first node dies in the network as well as the existing approaches attains lesser time for first node dies. The Figure 4 shows packet delivery ratio between the proposed ERRSEPR and other existing works.

The proposed ERRSEPR attains higher packet delivery ratio than the other schemes. The proposed ERRSEPR achieves around 30000 messages whereas the other approaches achieve 15000 and lesser. The Figure 5 shows the network connectivity where the proposed EERSEPR achieves higher network connectivity than the other approaches. The proposed ERRSEPR maintains 99 numbers of alive nodes after the completion of simulation whereas the other models maintain only a few numbers of alive nodes.

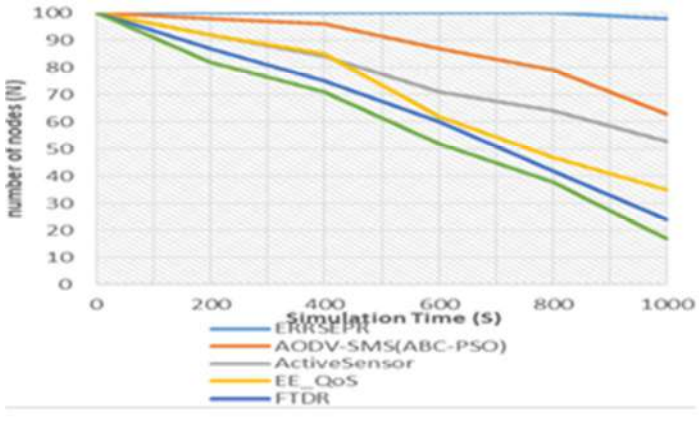

Figure 5 Network Connectivity

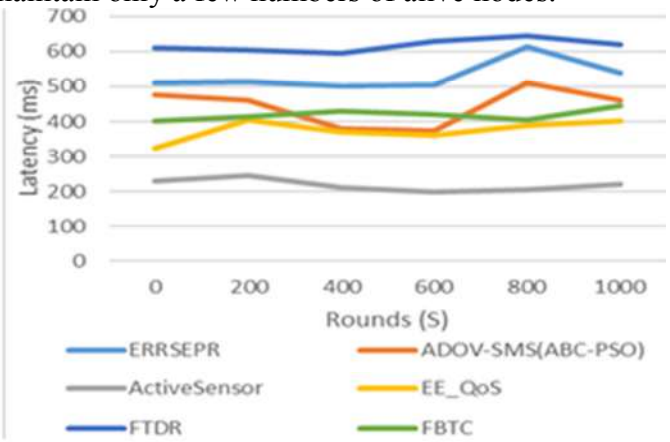

Figure 6 Data transmission Latency

The Figure 6 shows the data transmission latency between the proposed ERRSEPR protocol and other previous works. The proposed ERRSEPR protocol maintains better latency than the other schemes. Thus, the proposed ERRSEPR protocol attains a better result than the other existing works in all simulated parameters. Hence, it proves that the proposed ERRSEPR improves the lifetime of the network through route recovery, secured and energy proficient methods.

V. CONCLUSION

This paper presented the need for wireless sensor networks for habitat monitoring, the networkarchitecture for realizing the application, and the sensor network application implementation. Through route recovery model with secured energy proficient energy optimization model of WSN and load balancing mechanism of the network, this paper analyses the route recovery process between the previous and establishes a secured model with energy proficient approach. The route recovery is obtained using the combination of ABC and PSO and it also helps to recover the route in the network. Further, high successful routing probability with security and network scalability with nodal trust is attained. Through nodal trust the suspicious node is avoided 100% to eavesdrop the message or to forward fake messages. The energy proficient parameters such as residual energy, distance between the node and BS, hop count and power amplification improves the energy and reaches high-energy efficiency than the other previous results. Through the experimental studies, the proposed ERRSEPR presents a better routing protocol model than the existing works. The results are compared using the following parameters such as Residual Energy, First Node Dies Ratio, Packet Delivery Ratio, Network Connectivity and Data Transmission Latency. This improvement shows the successful routing probability and also it is improved to 80 times than the existing works. Use of technology for Wildlife monitoring is a benefit provided by the advances in the research. The data gathered by Wildlife monitoring can be used for number of purposes viz visualization, analysis, interpretation, prediction, etc. Thus, the proposed algorithm can be used to establish a reliable network for the efficient Wild life Habitat monitoring.

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SP-19

AN OUTLOOK ON DRUGGABLE TARGETS TO COMBAT PANCREATIC DUCTALADENOCARCINOMA

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is due to uncontrolled proliferations of exocrine cells in the ducts of pancreas and it is a 'silent cancer' as the PDAC does not signal for any apparent symptoms until its stage IV. The PDAC alone accounts about 95% of pancreatic cancers and 99% of people with stage IV PDAC have been reported to survive less than 5 years. In this review article, comprehensive inhibition of certain proteins playing essential roles in the Ras-mediated signal transduction pathway and autophagy has been hypothesized as an attractive strategy to combat the PDAC. Rationale for the hypothesis and possible druggable targets in the above said biological process has been discussed in detail.

KEYWORDS: ***Autophagy, KRAS, ERK and PDAC***.

I. INTRODUCTION

Pancreas is a large heterocrine gland of human body and plays significant roles in digestion of foods and as well in homeostasis of blood sugar levels. The exocrine cells (duct/acinar cells) of the gland secrete digestive enzymes such as trypsin, alpha-amylase and phospholipases and the endocrine cells (Islets of Langerhans) of the gland produces peptide hormones such as insulin and glucagon[1-4]. The ductal cells form an intricate network of small tubes called 'ducts' for passage of the digestive enzymes to the main pancreatic duct and further merge with the common bile duct (carrying bile from the liver) and eventually drain its fluid into the duodenum at the ampulla of Vater to break down proteins, fats and carbohydrates in the digestion processes[5]. The acinar cells in the pancreas have the intrinsic ability and plasticity to undergo the process of trans differentiation to a progenitor-like cell type with characteristics of duct and this process is termed as acinar-to-ductal metaplasia[6]. This process occurs during the pancreatitis and may represent an initial step towards the development of pancreatic ductal adenocarcinoma[7].

The majority (about 95%) of pancreatic cancer involves the initiation of exocrine pancreas in the ducts of the pancreas, when the exocrine cells start to develop out of control (uncontrolled proliferation). The cellular development avoids apoptosis and leads to the development of adenocarcinoma in the ducts of pancreas and hence the disease is named as Pancreatic Ductal AdenoCarcinoma (PDAC)[8-10]. On the other hand, the endocrine pancreas is composed of small islands of specialized cells called as the islets of Langerhans that are meant for synthesizing peptide hormones[8]. The endocrine cells produce and release hormones such as glucagon and insulin directly into the bloodstream to regulate the glucose concentration in blood[9]. Tumor developed on the endocrine cells is generally known as Pancreatic Neuroendocrine tumor (PNET), which are further grouped into 'functioning types' (insulinomas and gastrinomas) and 'non-functioning types'.

PDAC is the most lethal of all common cancers on the basis of mortality-to-incidence ratio reported in the literature[11]. PDAC is a resistant malignancy driven by an "undruggable" oncoprotein called as KRAS and the PDAC carry a mutant KRAS gene in most cases[12-17]. In PDAC, the indolent tumor shows a rapid progress from diagnosis to death because in most cases PDAC goes undetected until it becomes symptomatic[12]. The Complete surgical resection remains the only potential treatment for cure but only 10–20% of pancreatic cancers are respectable at the time of diagnosis and even the survival rate for PDAC after surgery remains low (15–20%) due to local recurrence[13]. The incidence rate of pancreatic cancer is rising, while prognosis yet remains poor in the case[14]. As a result, pancreatic cancer is estimated to become the third leading cause of death from cancer in the European Union after colorectal and lung cancers[15,16].

II. CURRENT TREATMENT OF PANCREATIC CANCER

Pancreatic cancer is a major cause of cancer-associated mortality due to poor prognosis[18]. At the time of diagnosis for pancreatic cancer, about 15% of patients have a respectable disease (stage I or II), 35% of patients are in a locally advanced stage of pancreatic cancer (stage III), and 50% of patients are in metastatic disease (stage IV)[19]. The application of Palliative gemcitabine has been the standard treatment for pancreatic cancer for many years with a modest of a survival benefit of about 3 months[20]. At present the first-line therapy in pancreatic cancer includes (i) FOLFIRINOX (made up of: folinic acid, 5-fluorouracil, irinotecan and oxaliplatin) and (ii) nab-paclitaxel along with gemcitabine[21]. The combinations of (i) gemcitabine along with cisplatin and (ii) temsirolimus along with bevacizumab are used as second-line treatment but in all cases the survival remains poor[22]. Thus, pancreatic cancer remains as one of the most lethal malignancies and therefore there is a requirement for new therapeutic approaches and development of novel drugs[23]. Unfortunately, the rate of failure of drugs in phase III clinical trials of the cancer, especially for PDAC, is very high (87%) due to the lack of robustness in the preclinical studies underpinning the clinical trials[24].

III. KRAS AND ERK SIGNALING IN PANCREATIC CANCER

Activating mutations in *KRAS* is the most common occurrence (90–95%) in the PDAC[25]. The other mutated genes such as *CDKN2A*, *ARID1A*, *ROBO2*, *PREX2*, *BRCA2, TP53*, *SMAD4* and *MLL3 are also reported in the PDAC.KRAS* encodes a small GTPase that is activated through the binding of GTP and the translocation to the plasma membrane[26].The majority of mutations in *KRAS* occur at codons 12, 13 and 61 to constitutive activation because the protein becomes insensitive to GTPase-activating proteins (GAPs) to induce the hydrolysis of GTP to GDP and turn the RAS protein into its inactive form[27,28].The driver role of mutations in the activation of KRAS in PDAC has been established by the experimental studies conducted on genetically engineered mouse[29]. The activation of KRAS signals through a series of downstream pathways like (i) RAF→MEK→ERK and (ii) phosphoinositide-3-kinase (PI3K)→AKT→mTOR. These signals in downstream pathways show an extensive cross-talk[30]. Although PI3K was considered as a RAS effector, the evidences from recent studies suggest that, PI3K can act as upstream signal to stimulate RAS→ERK signaling and the loss of KRAS expression led to PI3K-dependent ERK signaling[31-33]. The sensitivity to PI3K inhibitors, show an alternative bypass mechanism through canonical (AKT signaling) and non-canonical (ERK signaling) mode of action in PI3K signaling[34-36]. The mechanism of PI3K stimulation in the activation of wild-type RAS to activate ERK remains unclear. Apart from PI3K signaling, the activation of KRAS on cancer cells extends to the surrounding microenvironment and also leads to recruitment of neutrophils[37-39].

IV. ERK INHIBITORS

Targeting an oncogene-driven signaling pathway is a clinically validated approach for several diseases and hence the therapeutic approach is to target the downstream signaling pathway of KRAS and the suggested route is RAF→MEK→ERK signaling[40-42]. In order to work on downstream target, Kinsey *et al.* found that xenografts in the NOD/SCID mice of human pancreatic cancer cell lines (BxPC3and Mia-PaCa2) or the tumor tissue obtained from the PDAC of patients were rather resistant to single agent trametinib (MEK inhibitor) or chloroquine/hydroxychloroquine (autophagy inhibitor) but combining both inhibitors were highly sensitive. In addition, a partial disease response was achieved for the combination of trametinib and hydroxychloroquine in a patient with metastatic PDAC[43-46]. These results of Kinsey *et al.* are consistent with the research works published by Bryant *et al.* The research groups found that the genetic or pharmacologic inhibition of regulators in autophagy enhances the ability of ERK inhibitors to mediate the antitumor activity in the KRAS-driven PDAC[47-49]. These data shows compelling evidence that the inhibition of ERK signaling pathway drives PDAC cells to become acutely dependent on autophagy and becomes highly sensitive to the inhibitors of autophagy[50]. Autophagy serves as a protective and an adaptive response for the inhibition of RAS→RAF→MEK→ERK signaling in cancer[51]. Autophagy is particularly active during metabolic stress (a process that often occurs in solid tumors and tumor microenvironment). Inhibition of ERK inhibition leads to a limited degree of apoptosis in the pancreatic cancer cells *with the mutants of KRAS* but the process of cell death is significantly increased by the combined inhibition of ERK and autophagy[52]. Meantime, it is also worth to mention that there is a debate on the roles of autophagy in tumor cells: there are compelling evidences emphasizing that activation of autophagy could cause tumor suppression in some cases and tumor growth in some cases. In this background, the roles of autophagy in cancer cells have not yet been well-established at present juncture of multiple and parallel pathways regulating the fate of cancer cells in human

beings.

V. ERK SIGNALING AND CELL DEATH

Induction of cell death by chemotherapeutic agents involves the participation of mediators, triggers, initiators, and executioners with a complex network of intersections and interactions[53]. In this context, MEK→ERK signaling pathway seems to be a major player in the signaling junction. ERK1/2 was found to act as a switch between the necroptotic and the apoptotic cell death[54]. They also behave as a regulator of the cell death occurring as a result of the action of the proapoptotic ether lipid edelfosine in glioblastoma cells. The activation of ERK1/2 diverts the cytotoxic action of the ether lipid to necroptotic or survival responses. However the inhibition of MEK→ERK signaling pathway potentiates edelfosine-induced apoptosis in glioblastoma cells and switches the type of edelfosine-induced cell death from necrosis to apoptosis. Inhibition of MEK→ERK signaling potentiates the apoptotic action of additional agents with antitumor activity like the alkaloid berberine in melanoma cells. Thus, the signaling of MEK→ERK appears to constitute a part of a major signaling junction in which there were different routes leading to necroptosis or apoptosis.

VI. CONCLUSION

It seems that cancer cells are forced and driven to autophagy for survival and hence autophagy became a major target for the development of cancer therapeutics. Hence, the combination therapy of MEK→ERK signaling inhibitors and autophagy blockers would pave a way to efficiently combat the cancer cells, especially PDAC, in near future.

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SP-20

COMPARATIVE ANALYSIS OF PROBIOTICS AND FISH OIL AGAINST DSS INDUCED COLITIS MODEL IN RODENTS

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ABSTRACT

The Inflammatory bowel diseases (IBD) of the mammalian gastrointestinal tract was due to the susceptible bacterial contamination through the toxins from their fodder and unsterile shelter lead to Crohn's Disease (CD) and Ulcerative colitis (UC).The complexity of these IBD pathogenesis, delineate underlying molecular mechanisms and evaluate the capacity of human therapeutics. This study tried on the impact of Probiotics and Omega- 3 fatty acids (fish oil) on rodent model of Ulcerative colitis induced by Dextran sodium sulphate (DSS). A blend of Probiotics and Omega-3 fatty acids was seen as dynamic against DSS induced colitis in rodents by diminishing or hindering the inflammation. In this paper the effect of colitis treatment was 27.15% with probiotics, 47.59% with Organic Salmon omega and 52.84% in combination of both treatments. This demonstrates the Omega- 3 fatty acids with probiotics are successful against the colitis inflammation.

KEYWORDS: ***Inflammatory bowel diseases, Ulcerative colitis, Dextran sodium sulfate, Probiotics, Omega- 3 fatty acids.***

INTRODUCTION

Ulcerative colitis is a transmittable zoonotic inflammation of the colonic lamina propria with resulting damage and interruption of the mucosal barrier[1]. Animal models have been introduced to help in studying the disease process. The rodent model in which UC can be induced using DSS, is advantageous[2]. The restriction of UC to the colon together with the microbiological influence makes it suitable to examine probiotic/prebiotic arrangements. Prebiotic treatment with hardly developed probiotics, for example, *Clostridium butyricum* and bolstering with anaerobic bacterial antigen had all the earmarks of being advantageous in the DSS model of UC[3-4].

In a case study on *Homo sapiens,* it is proposed that a probiotic readiness had the option to keep up the respite of patients over sensitivity to the standard treatment with 5-aminosalicylic acid[5]. Based on epidemiologic, clinical and lab investigations, dietary supplementation with (n-3) polyunsaturated unsaturated fats (PUFA) could be a fascinating methodology for the administration of IBD[6-11].The objective of this work was to validate the Dextran sodium sulphate induced rodent models of inflammatory bowel disease by studying the activity of *Probiotics, Organic Salmon omega and mixture of Probiotics and Salmon omega* for Ulcerative Colitis and also to analyse the level of myeloperoxidase activity in response to inflammation and treatment.

MATERIALS AND METHODS

A. **DSS induction:** Chemically induced colitis rodent models have been developed and extensively used for elucidation of pathogenic mechanisms of inflammatory bowel diseases. One of the widely used experimental models resembling human ulcerative colitis has been developed by treatment of rodents with DSS in drinking water for 6 to 10 days[2].
B. **Animal maintenance:** All the studies were conducted in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA no. 971/bc/06/CPCSEA), Government of India and approved by the Institute of Animal Ethics commission. All the studies were conducted as per the norms of the committee for the purpose of supervision of experiments on animals. Female wistar rodents weighing 140-170g were used for the study. All these rodents were housed 2/cage and kept in the animal house for one week for proper acclimatization before starting the experiment under controlled conditions of illumination (12 h light/12 h darkness) and

temperature ranging from 20-25°C. They were housed under the above laboratory conditions, maintained on standard pellet diet and water.

C. **DSS Preparation:** DSS (mol wt 40,000; ICN) at a final concentration of 2 % (wt/vol) were prepared in rodent's drinking water.

D. **Induction of Colitis:** Colitis was induced by the method previously reported by Kitajima *et al*., (1999) [12]. After one-week quarantine, the rodents were divided into 5 groups composed of 5 rodents each. In the control group (Group 1), rodents were given fresh water and micro fluidized pellets *ad libitum*. In the DSS group (Group 2), DSS (2%) in drinking water was given one week to induce colitis. In the other three groups viz 3, 4, 5 Probiotics, fish oil and a mixture of probiotics and fish oil (50:50)was administered p.o and Group 6 was dosed with Dexamethasone (0.1 mg/kg), which remained as standard control. In this experiment 2% DSS drinking water was given to all groups, except the control group 1. The body weight and feed intake of each rat was recorded from day 1 to 7.

E. **Evaluation of Colitis**

1. **Measurement of Colon Length, Weight and Estimation of MPO Activity in Colon Tissue:** After the end of 7 days, all rodents were killed by cervical dislocation and the large intestines without the cecum were removed. After washing in ice-cold phosphate-buffered saline (PBS), they were placed on filter papers to measure their length, after which they were opened to remove the content and weighed. Colonic mucosa samples, scraped off by razors, were put into 0.5mL of ice-cold PBS and homogenized on ice for 15s. The homogenates thus obtained were centrifuged at 1900 g for 15 min and stored at -80^{o} C till estimation of MPO was done.
2. **Method- Myeloperoxidase assay:** The myeloperoxidase activity in the colon was assessed by Bradley *et al*., 1982. The colon tissue (50 mg) was homogenized in 1ml of 50mM phosphate-buffered saline (PBS) pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide (HETAB). The homogenate was subjected to three cycles of freezing (-30^{o}C) and thawing (37^{o}C) with intermittent brief periods (15s) of sonication and centrifuged at 12,000 X g for 15min at 4^{o}C. Supernatant (0.1 ml) was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml O-dianisidinedihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 470nm was then measured within 5 minutes using a Beckman spectrophotometer (Beckman DU 640B). The MPO activity was calculated as

$$\text{MPO} = \frac{X}{\text{wt of the piece of tissue (50mg) taken}}$$

$$\text{Wheareas } X = \frac{10 \times \text{change in absorbance}}{\text{wt of the Volume of supernatant taken in the final reaction piece of tissue taken}}$$

STATISTICAL ANALYSIS:

The results are expressed as mean ± S.D from 5 rodents per group. For statistical analysis, ANOVA followed by Dunnett test was used. A $P<0.05$ was considered statistically significant using graph prism version 5.0.

RESULT&DISCUSSION

DSS treated rats displayed signs of moderate colitis. This was indicated by a decreased food intake (Fig:2.) Results in body weight reduction (Fig:1.), shortening of the colon (Fig:3.), increased colon weight (Fig:4.), and increased MPO activity (Fig:5) compared to normal rats. In the present study short-term pretreatment with probiotics did not protect against subsequent induction of colitis by DSS in rats. Contrary to what we hypothesized, the administration of fish oil tended to decrease the condition of inflammation in comparison with Dexamethasone. The mixture of fish oil and probiotic induced changes were significant in comparing to fish oil and probiotic given individually. The administration of 2% DSS for 6 days could only cause loose stool in rats and no obvious macroscopic damage was observed at any time. Probiotic, fish oil and its mixture could reduce acute injury that could be observed at 24hr and 3d, but with no obvious change at 1 week. For rats treated with DSS, the mucosal injury was still present for 1 week. The data collected confirms that the DSS produced more severely acute injury in the distal colon than that induced by DSS. Therefore, it seems the activated immune response after administration of DSS and the disruption of the superficial epithelium are essential to enable the consequent induction of a more

severe inflammatory reaction. Colitis may be a result from a deregulated response of the mucosal immune system toward intraluminal antigens of bacterial origin.[13-14] The DSS-induced model of colitis is associated with a significant decrease in colon length. When colon weight was expressed as a proportion of colon length, mixture of fish oil with probiotics administration resulted in a significantly lower colon weight in comparison to dexamethasone administration in DSS treated rats.The MPO levels (Table.1. Figure.5.) in normal animals were 0.2µg units/gm of tissue.

Tissue MPO activity in the DSS treated animals was 696.74µg units/gm of wet colon. Probiotics and Fish oil significantly decreased the MPO levels to 507.6 and 365.16µg units/gm, which show 61.25% & 56.09%. The mixture of probiotic and Fish oil decreased MPO levels which are 328.61 µg units/gm shows 46.90% better than probiotic and fish oil. Dexamethasone significantly inhibited the MPO activity of 85.75% which was significantly lower than DSS treated animals. Several models of experimental colitis have been reported previously, none of these showed the optimum characteristics. In recent years, some kinds of knockout (KO) mice have been reported.[15]Unfortunately, these strains of mice are not widely available, thus limiting their usefulness. The most widely used models are induced by administering toxic chemical such as TNBS or DSS.

In this study it is found that the intracolonic administration of the "barrier breaker" after 5% DSS treatment resulted in a rapid development of severe ulceration and inflammation of the distal part of rat colon. These features are similar to what happens in human UC were the major symptoms include diarrhoea, rectal bleeding and weight loss. Macroscopic finding showed the damage was characterized by marked ulceration and haemorrhage and that the diseased site was limited to the distal colon. Moreover, dysplasia was commonly found in DSS group. The most important clinical issue in the management of patients with IBD is an increased risk for development of dysplasia and neoplasia. Taken together, these features indicate that DSS induced colitis and human UC shares many similar clinical and morphological aspects.

DSS can induce reproducible acute colitis in rodents when given at a concentration of 5% for more than 6 d[2]. The exact mechanism by which DSS causes inflammation is not fully elucidated. It appears that the potential roles of DSS in induction of colitis may be: (a) direct cytotoxicity; (b) interference with the normal interaction between intestinal lymphocytes and epithelial cells; (c) DSS causes a change in the intestinal microflora, and particularly an increase in the number of Gram-negative anaerobes [2]. Administration of DSS also activates the immune response and stimulates chemokine production by epithelial cells.[16] DSS at a lower concentration up regulates cytokines, although it does not cause bloody diarrhea and ulceration in colon.

It is also found that aggregated lymphocytes in the colonic mucosa after the administration of DSS for 3d. This fact indicates the administration of lower concentration of DSS may also activate the immune response.The administration of 5% DSS for 3 d could only cause loose stool in rats and no obvious macroscopic damage was observed at any time. Thirty percent ethanol treatment induces acute injury that could be observed at 24 h and 3 d, but with no obvious change at 1 week. For rats treated with DSS, the mucosal injury was still present at 1 week. Our data confirmed that the DSS produced a more severely acute injury in the distal colon than that induced by DSS.

Therefore, it seems the activated immune response after administration of DSS and the disruption of the superficial epithelium are essential to enable the consequent induction of a more severe inflammatory reaction. Colitis may be a result from a dysregulated response of the mucosal immune system towards intraluminal antigens of bacterial origin.[13-14] Several advantages of this model make it a useful one for the study of pathophysiology and therapy of UC, especially in developing countries. First, the animal used is the wistar rat, which is inexpensive and widely available. Second, the concentration of DSS is 5% and the duration of administration is 2 d, thus the model is relatively inexpensive. Third, this model is relevant in that it has several features of human UC. Finally, the inflammation is easy to be induced and very reproducible.

Table.1. Myeloperoxidase assay (µg/mL)

Groups	Mean	SD	% Inhibition
Normal	0	0	0.00
DSS Treated	696.74	32.06	0.00
Probiotic	507.6	26.2	27.15
Fish oil	365.16	24.48	47.59
Probiotic+ Fish oil	328.61	27.28	52.84
Dexamethasone 0.1mg/kg	117.95	21.53	83.04

%inhibition= [(Diseased activity-inhibited activity)/(Diseased activity)]*100%

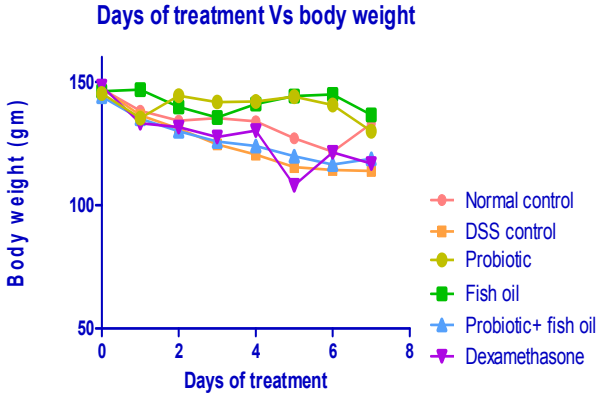

Fig:1 Days of treatment Vs. Body weight.
All the values are expressed as Mean ± S.D where n=5 using Graph pad Prism 5.0

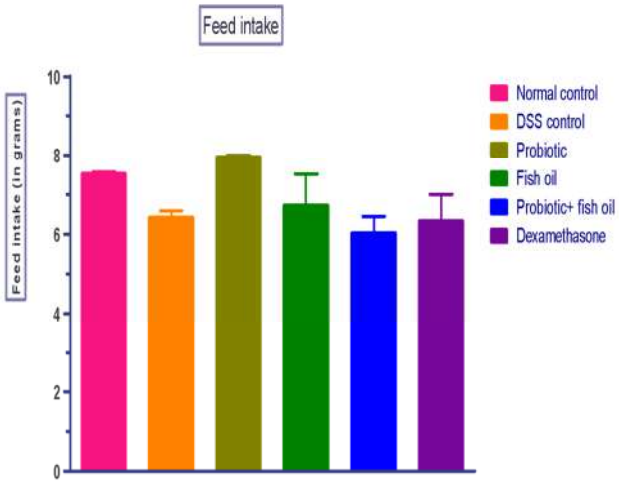

Fig:2. Feed Intake in Grams.
All the values are expressed as Mean ± S.D where n=5 using Graph pad Prism 5.0

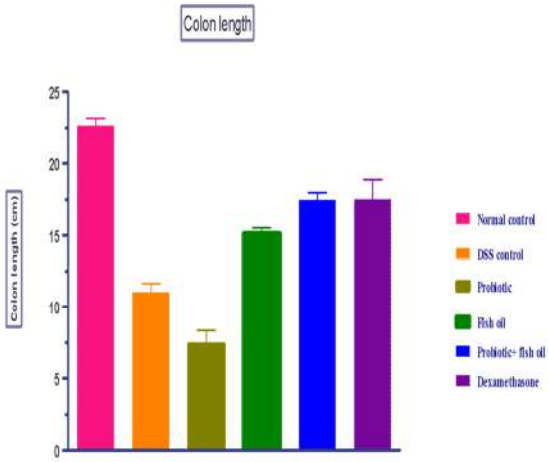

Fig: 3. Colon length in cm Mean ± S.D.

All the values are expressed as Mean ± S.D where n=5 using Graph pad Prism 5.0

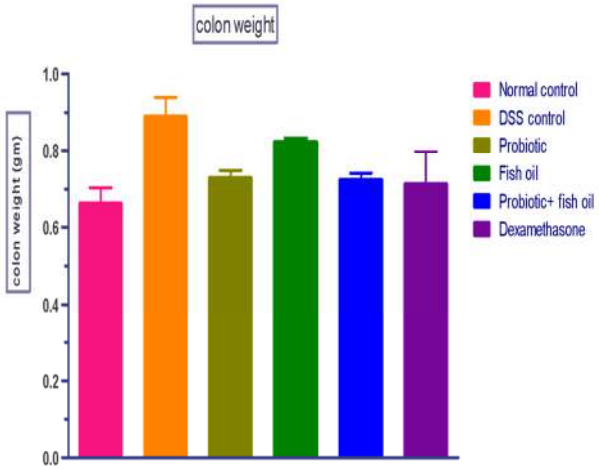

Fig: 4. Colon weight in Grams.

The values are expressed as Mean ± S.D where n=5 using Graph pad Prism 5.0

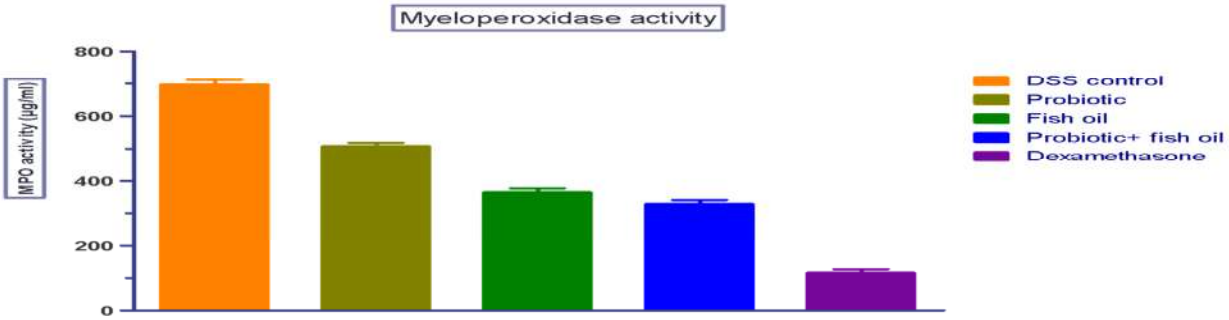

Fig: 5. Myeloperoxidase assay (µg/mL)

CONCLUSION

A mixture of probiotics with fish oilwas found to be very active against DSS induced colitis in rats by reducing or inhibiting the inflammation. Chronic studies and toxicity evaluation will add on to the advantage of the compound in becoming an active molecule in curing the inflammatory diseases. In conclusion, the DSS model is characterized by a clinical course, localization of the lesions and similar to human UC. The model fulfills the criteria set out at the beginning of study and can serve as a useful model in the assessment of future novel drugs for the therapy of UC.

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SP-21

COMPARITIVE STUDY ON BIO FERTILIZERS, VERMI & MICROBIAL COMPOST ON THE GROWTH OF ABELMOSCHUS ESCULENTUS

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ABSTRACT

This study was done to analyse the effect of Integrated Plant Nutrient Supply Systemon growth of Bhendi. Due to the use of chemical fertilizers the soil is polluted so to control the soil pollution an alternate method of biofertilizers with the combination of organic manure is used. The physico-chemical and microbial analysis was done to the experimental pre sowing soil. The experiment was laid out in complete randomized block design with replications of five treatments such as T_1- Control, T_2- Bio fertilizers *(Azospirillum , Pseudomonas, Tricoderma viride,* Phosphobacteria and VAM), T_3- Bio fertilizers + Vermicompost, T_4- Bio fertilizers + Microbial compost (coir compost), T_5- Bio fertilizers + Vermicompost + Microbial compost. The microbial analysis was done by collecting the rhizosphere soil on 15th day and 30th day, from the time period of sowing. The Biometric parameters of bhendi such as plant height and fruit yield of different treatments were observed on 30th and 60th day. The present study revealed that maximum population in microbial analysis was found in T_5 and T_3 gives the maximum height and fruit yield in plants. Thus the combination of Bio fertilizers, Vermicompost and Microbial compost induces the biometric parameters of bhendi, when compare to the other treatments.

KEYWORDS: ***Biometric parameters, Complete Randomized Block Design, Integrated Plant Nutrient Supply System***

I. INTRODUCTION

Abelmoschus esculentus or Okra, an annual or perennial crop the grows up to 0.5m-4m tall, heat and drought tolerant is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. Among the young vegetables, okra is one of the major in cultivation and consumption in Tamil Nadu. It is well for its antioxidants and provides an important source of vitamins, calcium, potassium and other minerals. Application of chemical fertilizers results in harmful residual effects in the plant products that cause health hazards to human beings. Besides, it is a vital concern in preventing environmental pollution particularly in drinking water. The depletion in soil fertility is due the imbalanced and unscientific use of chemical fertilizer and it is one of the major constraints in improving crop productivity[1]. Increased use of chemical fertilizers over a long period of time has led to contamination of food particles. Biofertilizers have shown a good promise and has emerged as an important component of IPNS. Biofertilizers improve the soil physical properties, organic carbon, and soil tilth and soil health in general and enhance nutrient utilization, efficiency and grain quality. They are cheaper and pollution free and their production are based on the renewable energy sources[2]. IPNS as an idea and farm management strategy embraces and transcends from single season crop fertilization efforts to arising with and management of plant nutrients in crop rotations and farming systems on a long-term basis for enhanced productivity, profitability and sustainability[3]. In recent years the use of different organic fertilizers and biofertilizers are being recommended not only to minimize the use of hazardous chemical inputs but also for sustainable crop production particularly in vegetables[4-6].Integrated plant nutrient supply system techniques, a viable alternative and an eco-friendly method of cultivation can boost quality production and thereby increase exports and earn foreign exchange. Organic production of Bhendi could help the growers to get premium price in the world market than conventional growers.The present study focuses on, application of IPNSS on growth of Okra and to find the comparative yield of Okra using Bio fertilizers, Vermicompost, Microbial compost.

II. MATERIALS AND METHODS

A. Design: An investigation was carried out to study the effect of organic nutrient sources and IPNSS for growth of Bhendi.The experiment was laid out in a Complete Randomized Blocks Design (CRBD) with four replications. (Table 1)

B. IPNSS Treatment: Treatments of present experiment deals with IPNSS of Bhendi through organic sources of nitrogen, phosphorous, potassium with vermicompost, microbial compost, VAM and bio fertilizers like *Azospirillum*, *Pseudomonas, Phosphobacteria* and *Tricoderma viride.* The organic sources of nutrients *viz.* nitrogen, phosphorous and potassium and recommended dose of farm yard manure was applied for all treatments. The treatment 5 includes only organic nutrient sources. The detailed information about the treatments is furnished as below.

(i). T_1- Control
(ii). T_2- Bio fertilizers *(Azospirillum, Pseudomonas, Phosphobacteria, Tricoderma viride, Vesicular arbuscular mycorrizae)*
(iii). T_3- Bio fertilizers +Vermicompost.
(iv). T_4- Bio fertilizers + Microbial compost (coir compost).
(v). T_5- Bio fertilizers + Vermicompost + Microbial compost.

C. Cultivation: Bhendi is adaptable to a wide range of soils from sandy loam to clayey loam. Planting will be done throughout June - August and February. Seed rate for normal varieties is 8.0 kg / ha and for hybrid seeds it is 2.5 kg / ha. The seed treatment is done with *Tricoderma viride* at 4 g/kg or *Pseudomonas fluorescens* at 10 g/ kg of seeds and again with 400 g of Azospirillum using starch as adhesive and dried in shade for twenty minutes. Plough the land 4 - 5 times and apply FYM 10 kg, neem cake 1 kg, rock phosphate 100 g and patent khali during last ploughing. Ridges and furrows at 45cm apart are formed.

Apply FYM at twenty five t/hour angle as basal before last plowing. Form raised beds of associate degree twenty} cm dimension at an interval of thirty cm. Install the drip irrigation with main and sub main pipes and place lateral tubes at the center of the each bed at an interval of 1.5 m. Place the drippers in lateral tubes at associate degree interval of sixty cm and fifty cm spacing with four LPH and three.5 LPH capacities respectively. Before planting, wet the beds using drip system for 8-12 hrs. Sowing to be done at a spacing of ninety x forty five x forty five cm within the paired row system, using ropes marked at 45 cm spacing. Sow 3 seeds per hill at thirty cm apart and so skinny to a pair of plants per hill once ten days. Seeds square measure planted at a spacing of forty five x thirty cm. Gap filling to be done at 7th day after transplanting. Irrigation is done at two days intervals.

Basal dose FYM at twenty five t/ha, N at 20 kg, P at 50 kg and K at 30 kg/ha as basal and 20 kg N/ha at 30 days after sowing is done for normal seed varieties and for hybrids basal dose FYM at 40 t / ha, N at 100 kg, P at 100 kg and K at 100 kg/ha as basal and 100 kg N / ha 30 at days after sowing is done. Harvesting can be done 45 days after planting. Fruits square measure harvested in tender stage at one – a pair of days intervals. The yield of Bhendi will be up to 12 - 15 t/ha.

III. RESULT

The intrepretation of the Physico- chemical analysis of the experimental soil, Microbial analysis of non-rhizosphere soil, Microbial population in rhizosphere soil , Effect of nutrient sources and biofertilizers on plant height (cm) at 30^{th} and 60^{th} day, Effect of nutrient sources and biofertilizers on growth of fruits analysis is given below:

Table 1: Layout.

$R_{44}T$	$R_{11}T$
$R_{45}T$	$R_{13}T$
$R_{41}T$	$R_{12}T$
$R_{43}T$	$R_{14}T$
$R_{42}T$	$R_{15}T$
$R_{33}T$	$R_{21}T$
$R_{35}T$	$R_{24}T$
$R_{32}T$	$R_{23}T$
$R_{31}T$	$R_{25}T$
$R_{34}T$	$R_{22}T$

Table 2: Physico- chemical analysis of the experimental soil.

Parameters	Results
pH	8.1
EC	0.07 (mmhos/cm)
Nitrogen	98 (mg/100 gm)
Phosphorous	12 (mg/100 gm)
Potassium	391 (mg/100 gm)
Organic carbon	0.07 (%)
Organic matter	0.12 (%)

Table 3: Microbial analysis of non-rhizosphere soil.

Microorganism	Population
Azospirillum	2×10^5 cfu/g
Phosphobacteria	3×10^5 cfu/g
Pseudomonas	10×10^5 cfu/g
Tricoderma viride	2×10^4 cfu/g

Table 4: Microbial population in rhizosphere soil.

Treatments	*Azospirillum* ($x10^5$ cfu/g)		*Phosphobacteria* ($x10^5$ cfu/g)		*Pseudomonas* ($x10^5$ cfu/g)		*Tricoderma viride* ($x10^4$ cfu/g)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
sT_1	5	8	3	4	13	15	3	4
T_2	9	12	4	5	19	24	4	5
T_3	17	24	6	7	23	28	5	5
T_4	11	19	4	5	21	24	5	7
T_5	28	38	7	9	29	34	4	5

Table 5: Effect of nutrient sources and biofertilizers on plant height (cm) at 30^{th} and 60^{th} day.

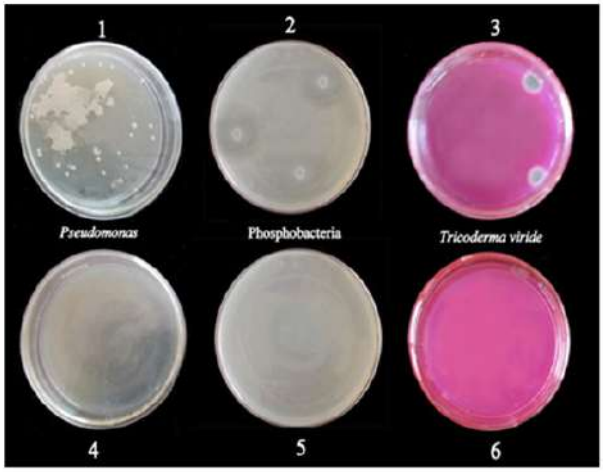
Treatments	Plant height (cm)	
T1	18.1	26.2
T2	19.6	30
T3	21.0	39.5
T4	20.5	29.4
T5	22.8	42.75

Table 6: Effect of nutrient sources and biofertilizers on growth of fruits.

Treatments	No. of fruits
T1	12%
T2	18%
T3	24%
T4	24%
T5	30%

PLATES

Plate 1: Microbial analysis of experimental soil in 10^5dilution.



1)Pseudomonas 2)Phosphobacteria *3)Tricoderma viride*

Azospirillum

Plate 2: Microbial analysis of *Azospirillum* at 15th and 30th day in 10^5 dilution

Plate 3: Microbial analysis of *Pseudomonas* at 15th and 30th day in 10^5 dilution

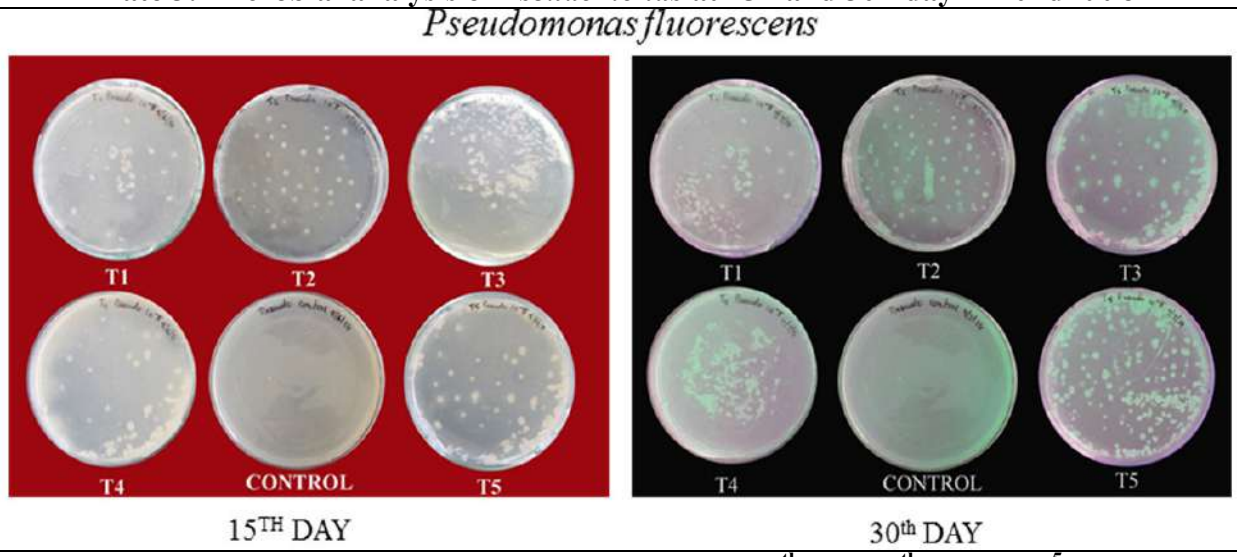


Plate 4: Microbial analysis of Phosphobacteria at 15th and 30th day in 10^5 dilution

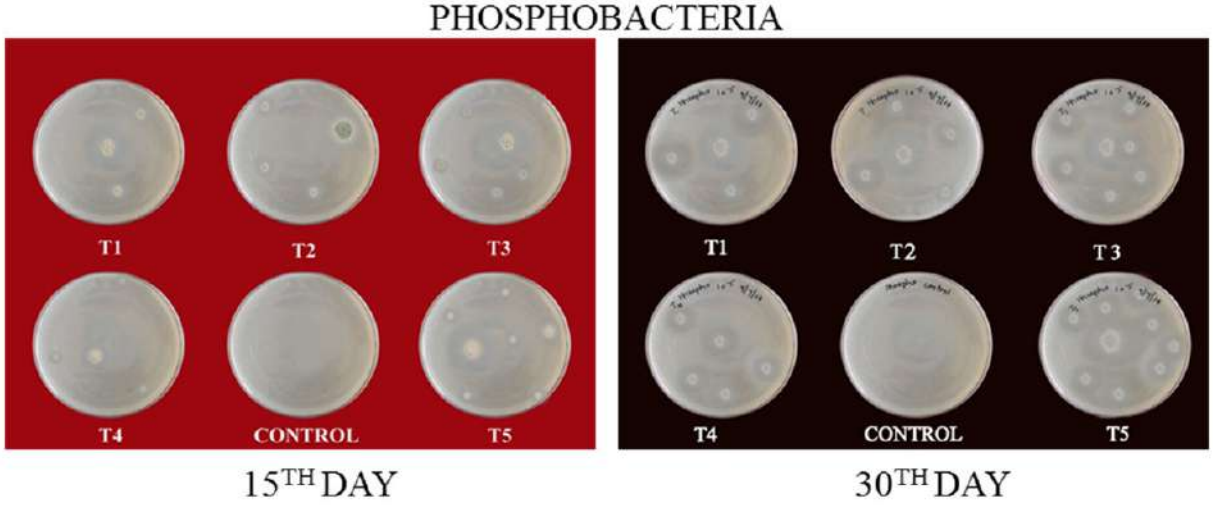


Plate 5: Microbial analysis of *Tricoderma viride* at 15th and 30th day in 10^4 dilution

GRAPH

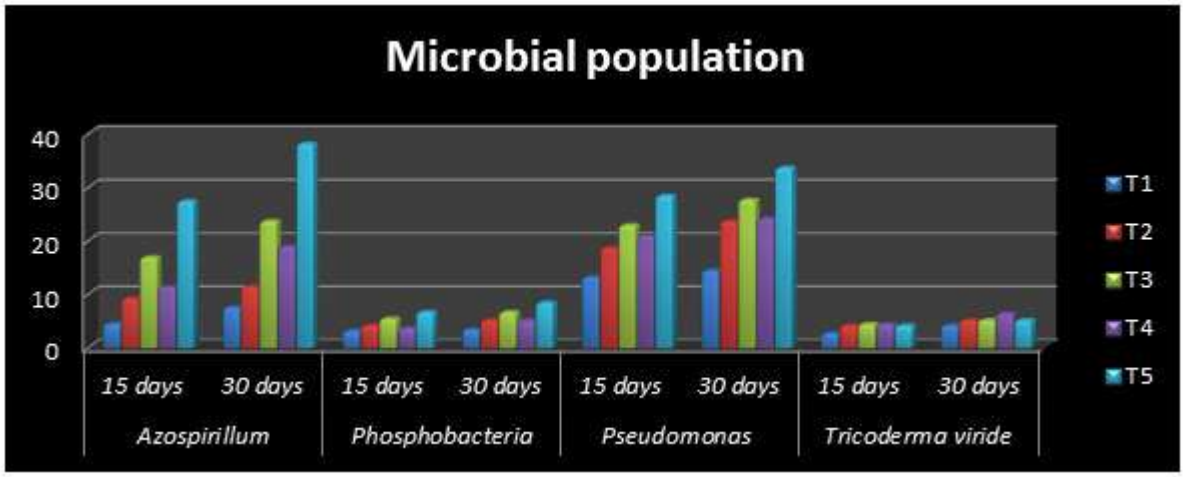


Figure 1. Microbial population of the rhizosphere soil at 15th and 30th day

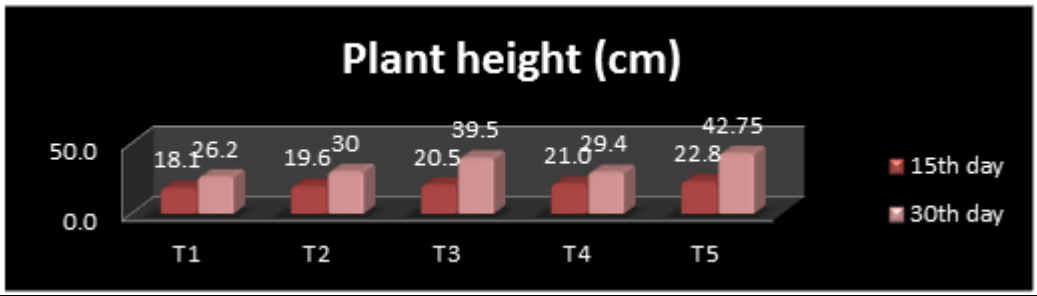


Figure 2. Effect of nutrient sources and bio fertilizers on plant height (cm) at30th and 60th day

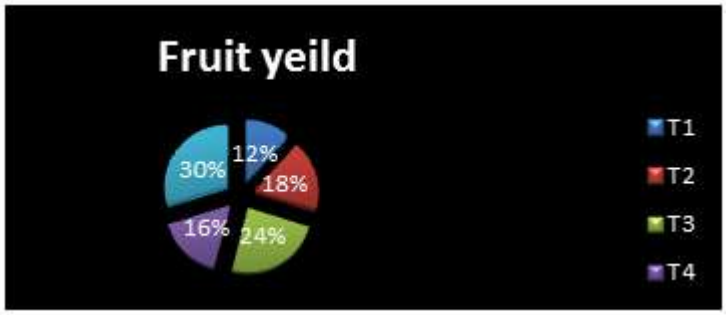


Figure 3. Effect of nutrient sources and biofertilizers on yield of fruits till 60th day

IV. DISCUSSION

The rhizosphere soil is most certainly an area of intense biological activity within the soil environment. It is represented by a diverse group of microbial population. The provision of energy to microbial community by root exudates, dead roots and sloughed cells results in an intense microbial activity and microbial

interactions in rhizosphere over non-rhizosphere soil. With the combination of microbes and the organic manure the plant growth is observed.

The microbial analysis in present study (Table-1-4), (Plates 1-5), Fig.1 revealed that maximum population of microbes were found in T5 (Biofertilizers+ Vermicompost+Microbialcompost) and T3 (Bio fertilizers +Vermicompost) which in turn resembles directly on the biometric parameters in the plant. The activity of bio fertilizer and vermicompost in the treatments might have induced the plant growth and fruit yielding capacity. (Table-5,6)(Fig-2,3) Vermicompost is one of the best organic manure in increasing the crop yield; they aerate and fragment the substrate there by drastically altering the microbial activity. It results variations in plant response such as growth and yield parameters when it is applied[4].The soil enrich with vermicompost provides additional substances that are not found in chemical fertilizers[5]. The fast breakdown of organic wastes by wiggler produces vermicompost having high quantity of total and obtainable Nitrogen, phosphoric, potassium, micronutrients, enzymes and growth regulators[6]which combines with biofertilizers show the maximum height of the plant.

V. CONCLUSION

The study concludes that integrated system in growing plant species shows a high yield which makes it more economically impotant. For further cultivation of plants in a safer way other than the use of chemical fertillizers and in a high profitable manner, the integrated system can be used globally.

ACKNOWLEDGMENT

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SP-22

AUTOMATIC AGRICULTURAL MONITORING AND IRRIGATION SYSTEM USING WSN

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ABSTRACT

In Agriculture there raise many problems in fields. Many researches are taken all round the world for implementing irrigation system by using wireless sensor and mobile computing. Also researches are been done by adding machine learning in agricultural field too commonly "Machine to machine (M2M)" communication is an emerging technology which allows devices, objects etc to communicate among each other and send data to Server or Cloud through the Core Network. So accordingly we here have develop self - organizing method in Automated Irrigation system where sensor data pertaining to soil moisture and temperature captured and accordingly. For deployment of these heterogeneous sensors VorLag algorithm was used which was a little bit energy consuming and Sensor self- organization was not discussed. Deploying of vehicle sensors are based on centralized approaches i.e., powerful cluster head is available to collect the sensor location. It is known from existing works that WSNs were used in outdoor and indoor environments, it is difficult to deploy sensor in outdoor and easy in indoor. Many works were carried out to address energy conservation issue in indoor environment. Required amount of sensors were spaced randomly for monitoring and to ensure redundancy, to design a sensor node for the above problem the sensor should switch between two modes ie., active to sleep and sleep to active mode. Thus algorithm deployed for analyzing the sensor data for prediction towards irrigating the soil with water. This is a fully automated where devices communicate among themselves and apply the intelligence in irrigating.

KEYWORDS: ***M2M, WSN, Sensor.***

1. INTRODUCTION:

Agriculture is the primary spine of Indian Economy[1-4]. Most clean water assets are utilized in Agriculture. In India maximum of the irrigation structures are operated manually which isn't automated[5-9]. In the recent years automated and semi- computerized technology been deployed for irrigating the sector which has changed the conventional Agricultural mechanism[10-12]. The available conventional techniques of irrigation are drip irrigation, ditch irrigation, sprinkler device. This trouble can be without problems rectified through utilizing the automated system in place of the conventional systems. The modern irrigation methodology adopted hire uniform water distribution which isn't always top of the line. So for those reason technologies being implemented toward agricultural monitoring this is required with the aid of farmers.

2 ADVANTAGES:

2.1 IT ENHANCES DEVICE COMMUNICATION:

IoT permits the communication between gadgets. It is a Machine-to-Machine interplay. Though some people say the machine might be hard to handle once it malfunctioned, the relationship of physical devices shows the alternative. There's an available overall precision that supports lessens machines' inefficiencies.

2.2 IT GATHERS USEFUL DATA:

The more considerable the records, the easier it is to make the right choice. The amount of records taken from gadgets that talk with each different will aid the consumer to know what is incorrect with the tool. Meaning, the machines can perform maintenance greater efficiently because they know what's malfunctioning inner their machine. The system-to-machine conversation or M2M helps maintain transparency in the techniques.

2.3 IT FOCUSES ON AUTOMATION AND CONTROL :

Due to physical items getting linked and controlled digitally with wi-fi assist[6], a massive quantity of manipulate and automation is achievable. Without human intervention, the machines can lead a quicker and timely output. Therefore, it fills a number of the disorganized gaps of the gadget and human interactions.

2.4 USEFUL IN MONITORING:

One of the most obvious blessings of IoT is monitoring. It offers a bonus of understanding things earlier. With this, the precise amount of resources, water distribution and consumption, sensible power management, and security distribution gets collected easily. It also takes essential motion in case of screw ups and emergencies.

2.5 IT IS EFFICIENT AND IT SAVES TIME:

The system-to-device interplay offers better time reaction and normal device operation performance. Since the IoT machine produces correct outcomes in an immediately, it allows humans to do different creative jobs. Its speedy-paced function saves a lot of treasured time.

3 HARDWARE SECTION

3.1 RASPBERRY PI

The Raspberry Pi-three is used in my version .Raspberry Pi is a unmarried board pc with Linux or other small running systems[5]. It was evolved via Raspberry Pi foundation in UK for using pc technology training. The second version of the Raspberry Pi is used in my task. This element describes fashions of Raspberry Pi is available. This report will no longer attempt to provide complete specifications but an outline in order to assist in making choice as to which tool it's far required to perform the goals in query. Currently, five Raspberry Pi version do exists. They are: Model B+, Model A+, Model B, Model A and the Compute Module (currently handiest available as a part of the Compute Module improvement package).All these models use the equal SoC (Sytem on Chip blended CPU & GPU), the BCM2835, however other hardware features vary. The raspberry pi board is includes HDMI port is connected to monitor.Usb1 is connected to keyboard and different usb2 is attached to mouse. The board that has electricity supply port and insert memory card.

3.2 SENSORS

The distributed sensor community that consists of soil moisture sensor, temperature sensor, humidity sensor, color sensor and water degree sensor. There are distinct sorts of soil sensor technology and measurement techniques that have been advanced for the dimension of soil moisture content. The generally used soil sensors are based on frequency area reflectometry (FDR), which makes use of capacitance probes to measure the dielectric permittivity of the soil. In this paintings but, we used a resistive soil sensor, which was developed using probes to pass electrical currents into the soil and reads the reaction or resistance to get the moisture content of the soil[7]. The resistive sensor works at the precept that the greater moisture we have within the soil makes the soil to behavior strength easily because of decrease resistance even as dry soil situations makes the soil conducts energy poorly because of higher resistance. Water degree sensors hit upon the level of water. The color sensor is used to measure the best coloration of the leaf.

3.3 AUTOMATIC MOTOR CONROLLER

This module is used to control 3the pump mechanically. The most soil moisture kingdom reaches it robotically is going minimum soil moisture nation. The watering is on whilst the soil get dry, when the watering might be forestall when the soil is moist. The pump might be grew to become on automatically each day at a specific time for an positive soil, right away the pump will turns off. Led is an act as motor. The turning on and rancid of the pump will work no matter the moisture price round the sector vicinity. This automatic mode may be set via sending records to monitor.

4. METHODOLOGIES:

In an existing automated water control device we cannot take decision at that instance by taking exceptional characteristic of agriculture soil. Current computerized devices for irrigation works with a effective parameter at one time. Soil have specific characteristic like soil moisture and temperature, humidity etc. Soil moisture is beneath threshold value then water valve is open for water supply and after right water deliver if

it is going above threshold cost water valve is get near. Existing gadget does not concern about to be had water in reservoir and requirement of water to specific crop. So device does now not have selection energy[4]. It best works on one situation at one time. In the gadget send the facts approximately the boom of paddy plant and sugarcane inside the area. The information are ship information about the each stage in the plant increase in the area. In this painting, the automated irrigation gadget based totally on low power microcontroller became developed and deployed. To triumph over the drawbacks of present machine like high price, difficult in preservation and wired connection, we introduce a brand new gadget so that it will have wireless connection between server and nodes. We introduce a brand new layout of embedded internet server making use of raspberry pi technology and net of things. The automatic irrigation device consists of distributed sensor community built the use of soil moisture sensor, temperature sensor, humidity sensor and water degree sensor and shade sensor. Water degree sensor senses the excess water inside the discipline and the motor automatically pumps the water to the outer area. Irrigation machine makes use of valves to show irrigation ON and OFF[12]. The shade sensor is positioned in the subject is used to recognize the right shade of leaf and offer the pesticide before destroying the plant life[12]. Earlier, farmer faced the problem of sending SMS and making calls, overcoming which we're designing an Desired application which does the work by button clicks, right here the hardware works in 3 modes of operation, ect

5 CONCLUSIONS

Thus tracking system in agricultural fields may be a powerful method on the way to be beneficial in situations wherein usage of water assets is limited. By imposing this undertaking, there can be a powerful and efficient usage of water assets. It may be an indirect mean for the proper development of agricultural yield. The overall performance measures obtained can be maintained in database with the intention to be useful for evaluating unique yields at one of kind climatic situations.

6 FUTURE WORK :

This Agricultural monitoring process can be developed under the concept of Distributed database[6]. It will enhance the performance measure and will be used for improving the crop quality and health of the plant

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SP-23

WIRELESS SENSOR NETWORK IN AGRICULTURAL IRRIGATION USING ZIGBEE

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ABSTRACT

The advanced evolution in wireless sensor networks can be used in controlling various parameters in agriculture. Due to the irregular natural distribution of rainwater, it is very challenging for farmers to monitor and control the distribution of water to the agriculture field in the whole farm or as per the necessity of the crop. There is no ideal irrigation system for all climate conditions, soil structure and variety of crop cultures. Farmers suffer large monetary losses because of the incorrect predictions of weather and incorrect irrigation methods. With the development of miniaturized sensor devices joined with wireless technologies, it is probable to monitor parameters such as moisture, temperature, and humidity. In this document, it is suggested to design, develop and implement a wireless sensor network connected to a central node using ZigBee, which in turn is connected to a CMS-Central Monitoring Station through a GPRS - General Packet Radio Service or a GSM -Global System for Mobile. The system obtains the Global Positioning System (GPS) parameters associated with the field and sends them to a central monitoring station. This system is supposed to help farmers in appraising soil conditions and work accordingly.

KEYWORDS: ***ZigBee, waterlogging , nano-scale , Mesh networking***

INTRODUCTION

In India, where the economy is based on agriculture, the climatic conditions are isotropic and are not able to make the entire use of agricultural resources[1]. The main reason is the shortage of rain and the large usage of land reservoir water. The constant extraction of water from the earth is reducing the water level due to which a lot of lands is getting slowly in the zones of un irrigated land[2].Another very important cause for this is due to the unplanned use of water due to which a vital amount of water goes waste[3].In the latest drip irrigation systems, the most significant advantage is that water is provided near the root zone of the plants drip by drip due to which a huge quantity of water is reserved[4].In the present period, the farmers have been using irrigation techniques in India through the old-fashioned control in which they irrigate the land at regular intervals[5].This process sometimes uses more water or sometimes the water reaches delayed due to which the crops get dried[6].Water insufficiency can be detrimental to plants before visible wilting occurs. Delayed growth rate, lighter weight fruit follows slight water deficiency[7]. This obstacle can be perfectly rectified if farmers use an automated irrigation system by using the ZigBee module.

DIFFERENT TYPES OF IRRIGATION

SURFACE IRRIGATION

Surface irrigation is described as the act of applying certain techniques where water is applied and distributed over the soil surface by means of gravity[8].It is by far the most basic form of irrigation throughout the world and has been followed in many areas practically unchanged for thousands of years[9].Surface irrigation is often referred to as flood irrigation, signifying that the water distribution is uncontrolled and therefore, inherently ineffective[10].In actuality, some of the irrigation practices classified under this name involve an important degree of management (for example surge irrigation). Surface irrigation occurs in three major types; level basin, furrow, plus border strip. The process of surface irrigation can be described using four stages[11].As water is applied to the top end of the field it will run or drive over the field length. The advance phase refers to that length of time as water is applied to the top end of the field and runs or

advances over the field length[12].After the water strikes the end of the field it will either run-off or start to pond[13].The period between the end of the advance phase and the shut-off of the inflow is termed the wetting, ponding or storage phase. As the inflow stops the water will continue to runoff and infiltrate until the entire field is emptied[14].The depletion phase is that short period after cut-off when the length of the field is still submerged (Fig.1). The recession phase describes the period while the waterfront is retreating towards the downstream edge of the field[15].The amount of water applied to any point in the field is a function of the opportunity time, the length of time for which water is present on the soil surface.

Fig.1 Surface Irrigation

DRIP IRRIGATION

Drip irrigation, also recognized as trickle irrigation or micro irrigation or localized irrigation, is an irrigation system which conserves water and fertilizer by allowing water to drip gradually to the plant roots, either onto the soil surface or directly onto the zone of the root, through a network of valves, pipes, tubing, and emitter[16].It is done with the aid of narrow tubes that delivers water directly to the bottom of the plant. Recent drip irrigation has arguably become the world's most appreciated innovation in agriculture since the invention of the impact sprinkler in the 1930s, which offered the first realistic alternative to surface irrigation. Drip irrigation may also use devices called micro-spray heads, which spray water in a small area, rather than dripping emitters. These are commonly used on tree and vine crops with wider root zones[17].A thorough study of the relevant determinants like topography of the land, soil conditions, water, crop, and agro-climatic conditions are required to determine the most suitable drip irrigation system and elements to be used in a specific installation process[18].

Drip tape causes additional cleanup costs after harvest[19].It is necessary to plan for drip tape winding, disposal, recycling or reuse. Loss of water, time & harvest, if not installed correctly[20].These systems require accurate study of all the associated factors like topography of the land, soil, water, crop, and agro-climatic conditions, and suitability of drip irrigation system and its elements[21].The major drawback of surface irrigation is that it tends to lead to waterlogging and soil salinity if there are no provisions for adequate drainage. Another drawback is that it tends to be labor-intensive (Fig.2).Due to surface irrigation overwatering is occurred[22].Due to overwatering can cause waterlogging and reduce root growth by cooling the soil. Hence crop quality. In manual control technique of irrigation moisture level of the soil is not taken into account[23].But in this system, we are providing the water as per the requirement of crops. This avoids the overwatering and its side effects[24].

Fig.2 Drip Irrigation

NEED OF ZIGBEE IN AGRICULTURE FIELD:

ZigBee is an established collection of specifications for wireless personal area networking which is nothing but WPAN, i.e. digital radio connections linking computer systems and its related devices[1].ZigBee gives specifications for devices that produce low data rates, use very low power and are thus characterized by great battery life. ZigBee makes it completely possible for networked homes where all devices can

communicate and be controlled by a single unit[2].

ZIGBEE

ZigBee is a low-cost, low-power, wireless mesh network measure[3].The low cost allows the technology to be broadly used in wireless control and monitoring applications. Low power-usage supports longer life with smaller batteries. Mesh networking provides high reliability and a more broad range[4].ZigBee chip merchants typically sell integrated radios and microcontrollers with between 60 KB and 256 KB flash memory[5]. ZigBee usually functions in the industrial sectors, scientific sectors and medical (ISM) radio bands; 868 MHz in Europe, 915 MHz in the USA and Australia, and 2.4 GHz in most domains worldwide. Data transmission rates differ from 20 to 900 kilobits/second[6].The ZigBee network layer natively supports both star and as well as tree typical networks and generic mesh networks[7].Every network must have one coordinator device, tasked with its creation, control of its parameters and basic maintenance[8].Within star networks, the central node must be the coordinator. Both meshes and trees allows the use of ZigBee routers to expand communication at the network level[9].ZigBee builds upon the physical layer and medium access control defined in the IEEE standard 802.15.4(2003 version) for low-rate WPANs[10].The specification of ZigBee complete the standard by adding four foremost components to it: network layer, application layer, Zigbee device objects (ZDOs) and manufacturer-defined application objects which allow for customization and favor total integration[11].

ZIGBEE APPLICATIONS IN AGRICULTURE

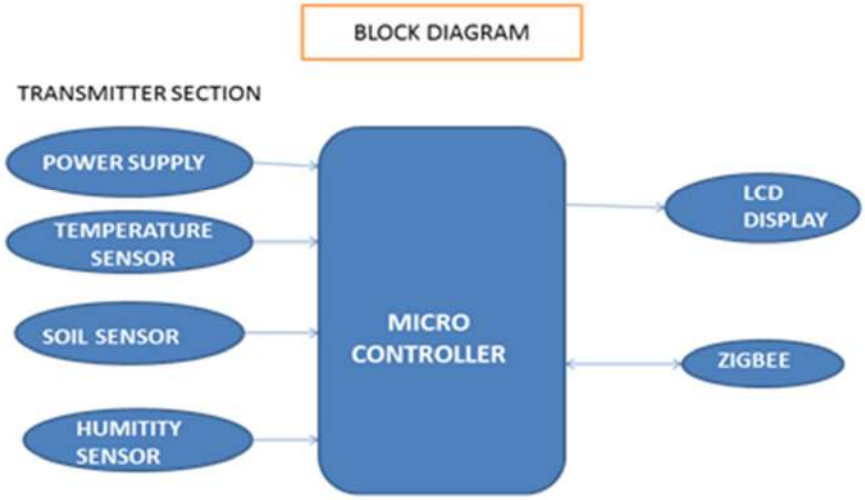

Fig.3 Basic Structure of ZIGBEE

The current implementations of wireless data communication for agricultural engineering purely depend on an architecture composed of one or more sensors for environmental data (temperature, humidity, etc), a signal conditioning block (**Fig.3**), a microprocessor/microcontroller with an external memory chip and a radio module for wireless communication between the sensor nodes and/or a base station[14]. The applications that make use of wireless sensor technology for precision agriculture are briefly explored below.

'SMART FIELDS' MONITORED BY WIRELESS NANOSENSORS

Considering and taking into account the conditions that a farmer may want to monitor the presence of plant viruses or the level of soil nutrients that operate at the nano-scale, and it is possible that the surfaces of the soil can be altered at the nano-scale to bind selectively with particular and suitable biological proteins, sensors with nano-scale sensitivity will be especially important in recognizing the vision of smart fields[15].

SOILNET

A Zigbee based soil moisture sensor network, Soil moisture plays a fundamental role in partitioning water and energy fluxes, in providing moisture to the atmosphere for precipitation, and controlling the pattern of groundwater recharge. Large-scale soil moisture variability is handled by space-time precipitation and radiation model[16]. At local scales, land cover, conditions of the soil, and topography of the land act to redistribute soil moisture[17].

MOBILE PLANTS

Application in association with agriculture deals with the surveillance of mobile plants[18].These plants are not located stationary in beds but in mobile planting bins which are located in varying greenhouses according to the vegetation condition. Sensors to measure air temperature and humidity levels, soil temperature, and, the light intensity is attached to the modules to monitor the plants entirely[19].In the applications detailed above, the measured and digitized data are conveyed wirelessly to a base station, which is attached to a standard personal computer for visualization and storage. Due to the standardized interfaces, the base station can be effortlessly integrated into available climate control systems[20].

CONCLUSION

In this paper, a thorough study and literature survey of several automated irrigation systems have been presented. We have successfully designed a remote control and monitoring system for farmers that will graphically describe the soil condition of the crop distantly relating to ZigBee.The designed system is Power-efficient, cost-effective and user friendly that is efficient enough to monitor the crop condition and remotely control the peripherals of the irrigation system which will make the job of the farmers easier and simpler. This system is scalable, as it allows a number of devices to be added with no larger changes in its core architecture. Currently, the soil humidity, soil moisture sensor, and fire sensor have been successfully interfaced with Atmega32. Wireless transmission between the sensor and the coordinator node is carried out through ZigBee. GSM is used as a remote controller to act remotely if the user is not in range. In the future, the latter points will be covered.

- More sensors will be interfaced such as pressure sensors to know conditions of the weather, Soil temperature sensors, and PH sensors to make the system much more effective.
- The software will be developed on the server which enables the user to view the sensor data and schedule the irrigation schemes remotely.
- It is possible to create a mobile application to retrieve information related to soil and perform actions remotely using Wi-Fi or GPRS.

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