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Research Article

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Antibacterial Efficiency and Phytochemical Assessment of Stereospermum Chelonoides (L.F.) DC Flower and Leaf Mixed Powder Extracts

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Abstract: Aim of the present study is to evaluate antibacterial efficiency of mixed powder preparation from the leaf and flower of *Stereospermum chelonoides* and its phytochemical evaluation. Bacterial species tested were isolated from UTI cases and showed multidrug resistance property. Stereospermum *chelonoides* is one of the medicinal trees worshiped as Holy plants in Thyagaraja Swamy Temple in Thiruvarur. It belongs to the family Bignoniaceae commonly called Sivappu Pathiri. All parts of the plant show a biological efficiency evidenced through various ethnobotanical databases. Traditional healers use this plant as a heal for vomiting, eructation, piles, acidity, diarrhoea, gonorrhoea, loss of taste, malaria and other fevers. Antimicrobial activity was assessed by making use of two bacterial isolates by disc diffusion and drug dilution method. Zone of inhibition, MIC, MBC, Percentage inhibition and IC_{50} were calculated using standard textual methods. Qualitative and quantitative phytochemicals were assessed by making use of textual procedures. Active principle of the SCMPEE was evaluated by the column chromatography, TLC, LC-MS and NMR methods. Aqueous (SCMPAE) and ethanolic (SCMPEE) extracts produced good antibacterial activity against all the test organisms with efficient MIC and MBC. Percentage of inhibition ranges from 53.1% to 84.2%. *Stereospermum chelonoides* extracts inhibited the growth of gram negative urinary bacterial isolates with 274.4 µg/ml IC50 for Klebsiella pneumoniae by SCMPEE. Qualitative phytochemical analysis showed the presence of tannins, saponins, terpenoids, triterpenoids, flavonoids, anthraquinones and polyphenols in both the extracts. Active principle assessment indicated the presence of quercetin 3 -O-Glucoside, which could exert antibacterial efficiency via protein precipitation mechanisms.

Keywords: Bacterial pathogens, MIC, MBC, Antibacterial activity, IC_{50}, Percentage inhibition, *Stereospermum chelonoides,* flower and leaf mix, phytochemistry.

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1. INTRODUCTION

Assessing biopotentials in relation to antibacterial and phytochemicals of natural medicine is need of the hour as most of the bacterial species isolated from the clinical specimens are found to have multiple virulence properties and multidrug resistance property. This could lead to new diseases from non-common microbial species like *Escherichia fergusonii* and *Klebsiella pneumoniae*[1]. In this study bacterial species were isolated from urine samples. in this study MDR urinary pathogens are assessed for antibacterial efficiency of medicinal plant of religious and regional importance. Indians uses these plants as medicine since time immemorial. It has been noted that about 24 species of Stereospermum are known for their biological activities. All these species were found in South, Central and South Maharastra of Western Ghats. One among the species is *Stereospermum chelonoides* (L.F.) DC (Syn. *S. suaveolens*). This plant is worshiped as thalavirusha at Thyagaraja Swamy Temple in Thiruvarur, Tamil Nadu and also Lord Patalisuvar at the Tirupatirippulyur Temple in Cuddalore District. It is called as sivappu Pathiri tree in Tamil[2]. Root of this plant is one of the ingredients of classical Ayurvedic preparation Dashmularishta. This plant belongs to the family Bignoniaceae. Parts like flower, leaf, root and stem bark of this plant is known for its biological activities like antimicrobial, antiprotozoal and anti-inflammatory properties. Ethnobotanical leaves and flowers of this plant have been used by traditional healers and rural communities for the treatment of vomiting, eructation, piles, acidity, diarrhoea, gonorrhoea, loss of taste, malaria and other fevers[3]. Flowers are used with honey to cure Hi-Cough[4,5,6]. Though this kind of research is by various researchers across the country, this study uses mixed powder preparations from leaf and flower of this plant. Phytochemicals are the metabolic products having multipotential biological efficiency, medicinal plants are the factory of phytochemicals which act on microorganisms and enhance all kinds of healing activities when used in the human body[7, 8]. Having known the importance of this plant, mixed preparation of flower and leaf and phytochemical features of medicinal plants, the present study was used to assess antibacterial efficiency on multidrug resistant urinary isolates along with phytochemical potentials of the test plants.

2. MATERIALS AND METHODS

2.1 Preparation of plant material

Flower and leaves of *Stereospermum chelonoides* were collected as wild from Thyagaraja Swamy Temple in Thiruvarur, Thiruvarur District, Tamilnadu. The collected plant materials were air dried and subjected for extraction. Plant materials were collected during summer and authenticated by Dr. John Britto, Director, Rabinath Herbarium, St. Joseph's College, Tiruchirappalli (Accession Number: 3210).

2.2 Extraction of plant powder

Active components of the plant materials were extracted using ethanol and water[7]. The filtrate was obtained by means of a vacuum filter pump. Filtering was repeated three times with the same plant material until the solution was clear. The filtrate was evaporated in a weighted flask, with a water bath set at 40°C. Extracts were reconstituted by re-dissolving in DMSO. The final filtrate was filter-sterilized by using a syringe filter with a pore size of 0.45µm. Sterile extracts obtained were stored separately in labelled, sterile capped bottles in a refrigerator at 4°C[9].

2.3 Preparation of disc with extracts

Aqueous and ethanol extracts (20µl) obtained were injected into a 6mm sterile disc (Hi-Media, Mumbai) and the standard antibiotics chloramphenicol used as positive control. Discs injected with water and ethanol acts as a negative control[10].

2.4 Preparation of Microorganism Culture

Two urinary isolates were selected from our epidemiological study of UTI infection. Urine samples were processed as per methodology[11]. On the basis of colony morphology on selective cum differential media like Mac Conkey agar and EMB agar, isolates *Escherichia fergusonii* and *Klebsiella pneumoniae* were selected and identified using biochemical tests. This organism's antibiotic susceptibility pattern was also checked with disc diffusion method[10]. Finally, identity of the test organisms was confirmed with 16srRNA sequencing and the sequences were submitted to GenBank and obtained accession number. The bacterial cultures were maintained in nutrient agar slants at 37°C. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

2.5 Antibacterial Susceptibility Assay

Escherichia fergusonii (MK598698) and *Klebsiella pneumoniae* (MK598697) were the significant urinary isolates isolated from UTI cases and subjected for antimicrobial assay. Inoculum of the isolates were obtained by inoculating a loop full of organisms into Nutrient broth (Hi-Media, Mumbai) and incubated at 30±0.1°C for 24 h. Mueller Hinton Agar (Hi-Media, Mumbai) was used for antibacterial activity. Spread 0.1ml of respective cultures of bacteria (10^5 bacteria per ml). Discs injected with extracts were placed on the solid agar medium by pressing slightly (Özçelik, 1992; Collins et al., 1989; Bradshaw, 1992). After Petri dishes obtained were placed at 4°C for 2 h, plates inoculated with bacteria were incubated at 35 ± 0.1°C for 24 hours. At the end of the period, inhibition zones formed on the medium were evaluated as millimetres. These studies were performed in triplicate. Sterilized distilled water and other solvents used in preparation of extracts were used as negative control. Chloramphenicol was used as a standard antibiotic (i.e. positive control)

2.6 Assessment of MIC, MBC and IC_{50}

It was performed by making use of the method of Kowser and Fatena[15] with few modifications.

2.7 Preparation of Turbidity standard for inoculum

Inoculum for the assay of MIC and MBC were prepared at 0.5 level of Mcfarland standard. The approximate cell density corresponding to 0.5 Mc Farland is 1×10^8 CFU/ml.

2.8 Inoculum preparation

Overnight Mueller Hinton broth cultures of *Escherichia fergusonii* (MK598698) and *Klebsiella pneumoniae* (MK598697) were prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of McFarland 0.5 standard and then further diluted to 1: 40 in Mueller Hinton broth. The inoculums thus expected to obtain 10^3CFU/ml.

2.9 Determination of MIC [15]

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. A MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Various concentrations of the extracts, antibiotics were prepared as illustrated below. Fourteen tubes were placed in a rack and labelled each 1 through 11, one tube marked as B (blank), one tube was labelled as AC (Antibiotic Control) and other tube was labelled as G.C (Growth Control). Different volumes of Mueller Hinton broth were added in each test tube as described in table 4.3 and plugged with cotton. All the tubes were sterilized at 121°C for 15 minutes. 100µl of 0.1% antibiotic solution was added to test tube A.C. 100µl of plant extract from stock was added to the tube no. 1 and mix properly. Similarly, the volume of extracts ranging from 90µl to 5µl for the tube no.2 through 11 was added. The tube G.C received no extracts and served as a growth control. A.C labelled test tube served as an antibiotic control. Each tube was inoculated (including the growth control except blank and antibiotic control) with 100µl of the culture of the respective organism. All the tubes were incubated at 37°C for 24 hours. The tubes were examined for visible growth (cloudy) and recorded visible growth as (+) and no growth as (-). The concentration at which no visible growth was described as the MIC of the extract.

2.10 Determination of MBC[15]

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium completely. It can be determined from broth dilution of minimum inhibitory concentration (MIC) tests by sub-culturing MIC positive tubes are used to determine MBC based on the visible growth. Last two clear tubes to the first two visible tubes were selected to screen MBC. 1ml of culture (grown in Mueller hinton Broth) from MIC tube were serially diluted upto 1:1000 dilution and inoculated 1:100 and 1:1000 diluted samples into Mueller Hinton agar containing Petri plate by spread plate technique. GC tube containing culture was serially diluted upto 10^{-8} and plated 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} diluted materials on respective properly labelled nutrient agar plate to check total viable count of the initial inoculums used to determine percentage inhibition. The number of bacterial colonies in each plate were counted properly and recorded. The dilution at which counted no colonies was considered as MBC of the extract.

2.11 Determination of % inhibition[15]

It is a calculation of inhibitory effect of extracts at particular concentration by making use of total viable count value of GC tube and dilution tubes. It was calculated by making use of the following formula.

$$\frac{\text{Number of colonies in tube GC} - \text{Number of colonies in dilution tube}}{\text{Number of colonies in tube GC}} \text{X} 100$$

2.12 Determination of IC_{50}[15]

According to the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition *in Vitro*. It is obtained from the % inhibition and the concentration of extract used. IC_{50} was calculated by using the formula.

$$\frac{\text{Concentration of Extract}}{\text{\% inhibition}} \text{X } 50$$

2.13 Phytochemical analysis

Preliminary phytochemicals like Tannins, Saponin, Flavonoids, steroids, Terpenoids, triterpenoids, alkaloids, anthraquinones, Polyphenols, Cardiac glycoside, Coumarins and anthocyanins were carried out by using standard textual procedures[16-18].

2.14 Active Phytochemical Fraction assessment

Active phytochemicals were assessed by making use of various standard methods. Here alcoholic extracts were subjected for the analysis.

2.15 Column Chromatography

Initially phytochemicals were separated as fractions using Column Chromatography[19]. Air-dried ethanol extract was first defatted and used for column separation. The 15x4 cm column was packed with 40µ silica gel, which is activated in the oven at 120°C for 2hours. A well-stirred suspension of silica gel (100–150 g in petroleum ether at 60°C–80°C) was poured into a column and the extract was mixed with silica gel slurry and applied to the top of the column as evenly as possible and distortion of the column packing avoided as this would lead to distorted bands. The column was successively eluted with

hexane, chloroform, methanol and their mixtures of increasing polarity. Elution with MeOH afforded a yellow colour.

2.16 Thin Layer Chromatography

Thin layer Chromatography of the fractions were assessed by making use of a method illustrated in Harborne[18]. The presence of flavonoid was detected by the formation of yellow colour spots in the plate, a positive reaction by exposure of ammonia[20].

2.17 UV and FT-IR Spectroscopic analysis

The collected fractions from the column was scanned in the wavelength ranging from 200-900nm using the Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FT-IR analysis was performed using the Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks ranging from 400-4000 cm-1 corresponding to their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation[21].

2.18 NMR Spectroscopy

The NMR experiment was carried out in a BRUKER-AMX400 MHz instrument with 5mg of purified compound in DMSOd6. Tetra Methyl Silane is used as the internal standard and chemical shifts are expressed in ppm. Experimental parameters were done as per the methods illustrated by Martina et al.,[22] with few modifications.

2.19 Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

The chromatographic separation of the flavonoids compound was carried out on a MicroTOF QIII Bruker Daltonic system (Bruker, Germany) equipped with an electrospray ionization (ESI) source according to the method described by Bataglion et al.,[23].

3. STATISTICAL ANALYSIS

All the resulting data (mean values) were statistically analysed with the SPSS analysis of variance (SPSS version 12.0 for Windows, SPSS Inc., Chicago, IL, USA). The significance for all comparisons was determined at the $P < 0.05$ level.

4. RESULTS

Antibacterial potential and phytochemical pattern of the *Stereospermum chelonoids* showed effective potentiality of the plant material as a good antibacterial agent. Two strains used in this study were isolated from urine samples of UTI cases and the organisms UTIEF2 and UTIKN1 were confirms using selective cum differential media, biochemical tests as well as using 16srRNA sequencing, which confirmed organism's identity as *Escherichia fergusoni* and *Klebsiella pneumoniae* respectively. The 16S rRNA sequence was submitted to GenBank and obtained accession numbers as MK598698 for *Escherichia fergusonii* and MK598697 for *Klebsiella pneumoniae*. Nowadays physicians are struggling for the efficient treatment of infections caused by variable multipotent bacterial species. One of the reasons could be virulence and antibiotic resistance. Due to various reasons bacterial species developed resistance to commonly used antibiotics and did not respond to common commercial antibiotics. In this line organisms used in this study also showed MDR status and were subjected for the antibacterial screening against UTI isolates. Though many of the researchers use type strains for antibacterial assay but possess stable characteristics, whereas isolated strains from the clinical samples may be exposed to variable environmental factors and showed variable characteristics. If we assessed wild strains for the study it reflects the original data on susceptibility pattern of natural isolates.

Table 1 - Antibiotic sensitivity pattern of Urinary isolates

S. No.	Antibiotics	UTIEF2	UTIKN1
1	Nalidixic Acid (NA)	S	R
2	Gentamycin (G)	R	S
3	Ceftazidime (CAZ)	R	R
4	Cefpodoxime (CPD)	R	S
5	Cotrimoxazole (COD)	S	R
6	Cefotaxime (CTX)	S	S
7	Erythromycin (E)	R	R
8	Kanamycin (K)	R	R
9	Cefixime (CFM)	R	R
10	Penicillin (P)	R	R
11	Norfloxacin (NX)	S	R
12	Ceftriaxone (CTR)	R	R
13	Ampicillin (AMP)	R	R
14	Nitrofurantoin (NIT)	S	R
15	Chloramphenicol (C)	S	S

Table 1 indicated antibiotic resistance pattern of the test isolates. These two isolates were resistant to multiple number of antibiotics like Ceftazidime (CAZ), Erythromycin (E), Kanamycin (K), Cefixime (CFM), Penicillin (P), Ceftriaxone (CTR) and Ampicillin (AMP). *Klebsiella pneumoniae* is resistant to 12 antibiotics among 15 antibiotics tested. On the other hand, *Escherichia fergusonii* was resistant to 10 antibiotics out of 15 tested. Studies given by the scientists from different parts of the world revealed that biofilm formation and beta lactamase production are the major factors associated with antibiotic resistance. This could be due to misuse or overuse of antibiotics in a community[24, 25, 26, 27]. Out of various studies revived only by Guillermo *et al.*,[28] reported low degree of antibiotic resistance among uropathogens. Sensitive antibiotics control bacterial infection whereas MDR strains cause virulent infection, which cannot be cured by using current antibiotics, scientists need to develop new generation antibiotics to overcome these issues. How many people are working on developing antibacterial substances from medicinal plants. In this line, this study uses mixed leaf and flower preparation of Stereospermum *chelonoides* for screening antibacterial activity against UTI MDR isolates.

Table 2: Antibacterial activity of mixed flower and leaf extracts of *Stereospermum chelonoides*

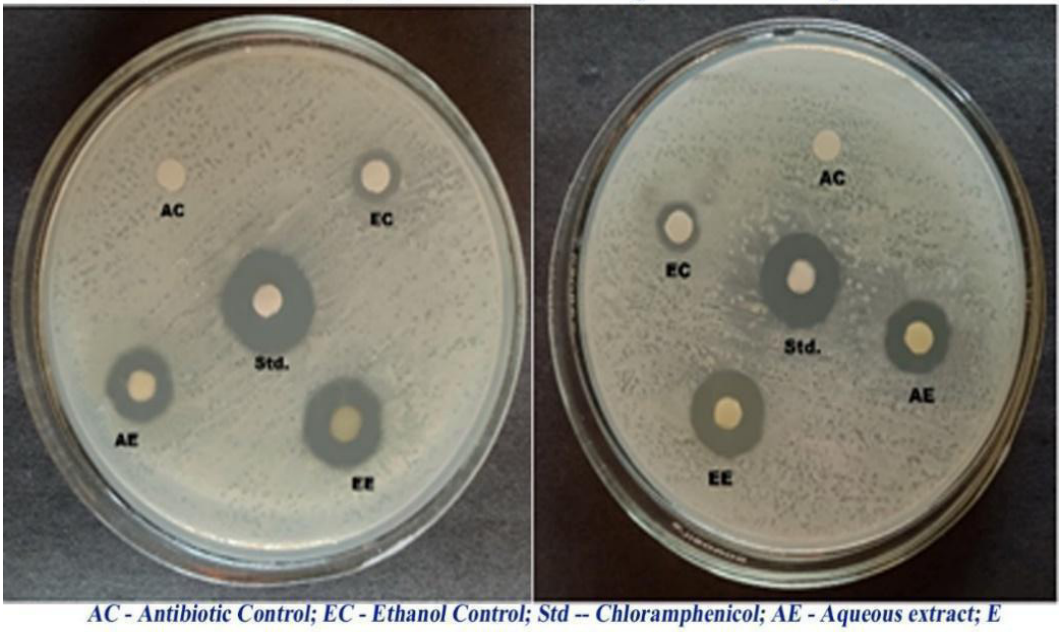
S. No	Clinical isolates	+ve Control	-ve control	Test	
				SCMPAE	SCMPEE
1	UTIEF2	18.85±0.89	7.25±0.08	10.50±0.31	12.70±0.46
2	UTIKN1	18.60±0.88	7.20±0.08	10.10±0.28	12.55±0.45

Values are expressed in mean± SD for 3 observations

Table 2 illustrates the antibacterial efficiency of aqueous and ethanolic extracts of *Stereospermum chelonoides* flower and leaf mixture. SCMPEE produced an effective and good zone of inhibition against *Escherichia fergusonii* and *Klebsiella pneumoniae* (12.55±0.45and 12.70±0.46mm respectively), which was greater than the effect produced by SCMPAE. Negative control used here was solvent used for the extraction whereas for test all the residual ethanol was evaporated under vacuum and used for disc diffusion assay. Hence the effect shown by the extract was the effect of extract on bacterial species not by solvent. Solvent free extract was used for the assay.

Plate I - Antibacterial Activity of *Streospermum chelonoids*

P. Fig. 1 - *Escherichia fergusonii* **P. Fig. 2 - *Klebsiella pneumoniae***



AC - Antibiotic Control; EC - Ethanol Control; Std -- Chloramphenicol; AE - Aqueous extract; E E - Ethanol extract

Plate I with P. Figure 1 and 2 confirmed the efficiency of extracts of *Stereospermum chelonoides* flower and leaf mixture as an ideal antibacterial substance which was evidenced in clear zones of inhibition around extracts injected disc. The organisms tested were MDR strains which were also efficiently controlled by the extracts which was evidenced in table 2. Disc diffusion is better assay due to the easy diffusion nature of the extracts in the surrounding environment.

Table 3: Assessment of MIC and MBC of *Stereospermum chelonoides* mixed extracts

S. No	Clinical isolates	MIC (µg/ml)		MBC µg/ml	
		SCMPAE	SCMPEE	SCMPAE	SCMPEE
1	UTIEF2	716.6±76.0	550.0±50.0	1258.3±123.3	1020.0±69.4
2	UTIKN1	495.4±50.0	416.6±28.8	1035.3±078.5	0896.6±28.8
Values are expressed in mean± SD for 3 observations					

Table 3 supported the efficiency of SCMPAE and SCMPEE with reference to MIC and MBC. Results of MIC revealed that SCMPEE inhibited the growth of bacteria with MIC ranges from 416.6±28.8 µg/ml for UTIKN1 and 550.0±50.0µg/ml for UTIEF2. Mild variable result was noted with SCMPAE against both of these urinary isolates. MIC was assessed by visible inspection of the dilution tubes whereas MBC was performed by inoculating dilution tube medium into the nutrient agar by pour plate method after serial dilution. *Stereospermum chelonoides* mixed flower and leaf preparation needed higher concentration of extracts to kill urinary pathogens. SCMPEE produced 100% growth control of both the bacterial urinary isolates at 0896.6±28.8 µg/ml concentration for UTIKN1 and 1020.0±69.4 µg/ml concentration for UTIEF2. On the other hand, SCMPAE produced 100% growth arrest at higher concentration for UTIEF2 (1258.3±123.3 µg/ml). Disc diffusion assay, MIC and MBC clearly indicated the effectiveness of *Stereospermum chelonoides* mixed plant extracts on urinary MDR bacterial strains. This study also indicated that both the extracts of this plant effectively inhibited the growth of urinary isolates.

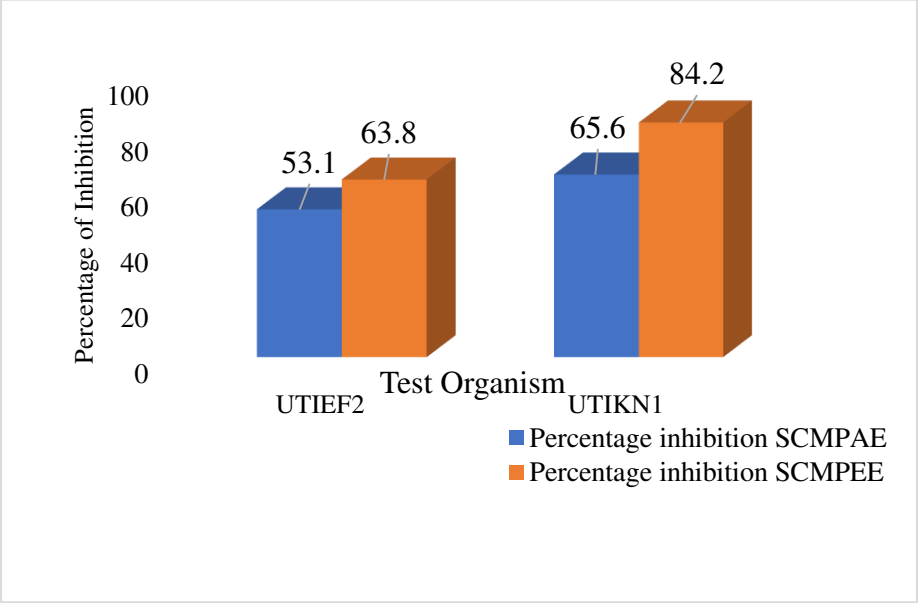


Fig 1 - Effect of *Stereospermum chelonoides* mixed powder extracts with reference to Percentage inhibition

Figure 1 illustrated percentage inhibition performed by the extracts on selective bacterial species. Here MIC positive dilution tube and Growth control tube were subjected for assessing TVC. On the basis of the number of CFU, percentage inhibition was assessed. Results revealed that SCMPEE inhibited 84.2% growth of *Klebsiella pneumoniae* followed by 63.8% growth of *Escherichia fergusonii.* Similarly, SCMPAE also inhibited the growth of urinary isolates effectively (53.1% for Escherichia *fergusonii* and 65.6% for *Klebsiella pneumoniae*) at respective MIC concentrations. Though this is a formula-based description of percentage inhibition, it efficiently indicated performance of SCMP extracts, when the microorganisms are found in the body its growth inhibition is enough to curtail its virulence thereby other immune responses take further action.

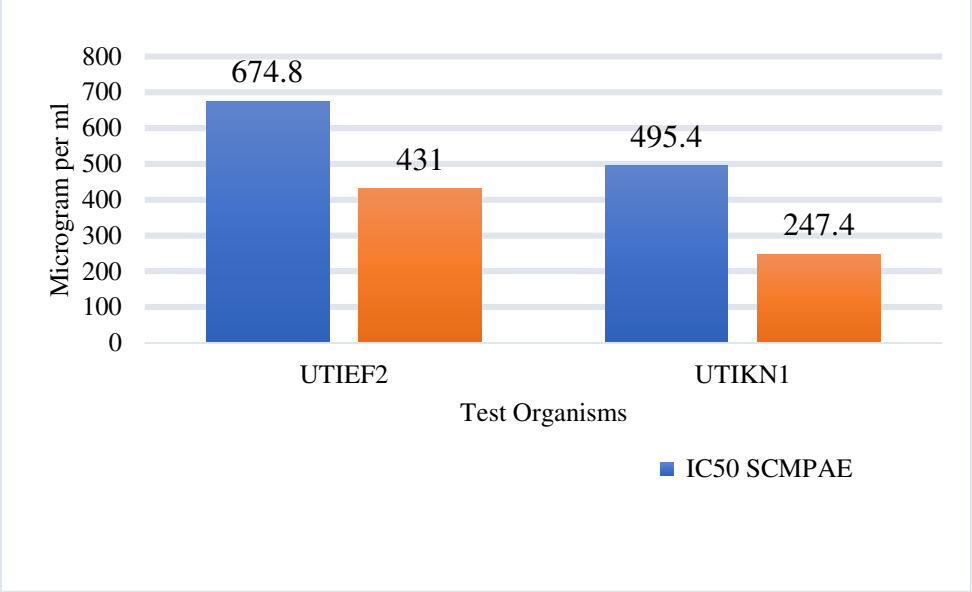


Fig 2 Efficiency of *Stereospermum chelonoides* mixed powder extracts with reference to IC_{50}

Figure 2 effectively showed concentration required to kill 50% of the bacterial population at a specified condition. Results revealed that both extracts inhibited the urinary isolates at specified IC_{50} Concentration ie., 247.4µg/ml and 431 µg/ml by SCMPEE and 495.4 µg/ml and 674.8µg/ml by SCMPAE against *Klebsiella pneumoniae* and *Escherichia fergusonii* respectively. Already in table 1 it was demonstrated that all the microbial populations were inhibited with reference to zone of inhibition, active bacterial population only able to cause a infection in an individual, if herbal preparation is regularly used phytocompounds are found in circulation it not only enhance host immune response whereas reduce virulence properties and activity of bacteria too, thereby extracts reduce microbial burden.

Table 4 - Phytochemicals qualitative analysis of aqueous and ethanolic extracts

S. No	Phytochemicals	Extract	
		Ethanol	Aqueous
1	Tannin	++	++
2	Saponin	+	++
3	Flavonoids	++	++
4	Steroids	++	+
5	Terpenoids	++	++
6	Triterpenoids	++	+
7	Alkaloids	+	-
8	Anthroquinone	++	+
9	Polyphenol	++	++
10	Glycoside	++	++
11	Coumarins	++	++
12	Emodins	++	-
13	Anthocyanins	-	-

(-) Absent, (+) Present and (++) high concentration

Table 4 clearly depicted preliminary quantitative phytochemicals found in the mixed powder aqueous and ethanol extracts of *Stereospermum chelonoides*. It revealed the presence of multiple polar and non-polar phytochemical constituents. Saponins, tannins, flavonoids, steroids, terpenoids, anthraquinone, polyphenols, glycoside and coumarins are found in both of the extracts tested whereas alkaloids and emodins were found in both of the extracts. Anthocyanins were completely absent in mixed leaf and flower extracts. These phytocompounds play a vital role in all kinds of metabolic activities both in human and microorganisms. Flavonoids and phenolic compounds exert radical scavenging and protein precipitation functions respectively.

Table 5 - Chromatographic Separation and detection of flavonoid

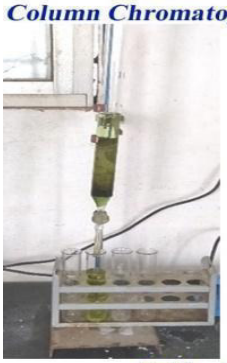
S. No.	Eluents	Number of fraction(s)	Nature of fractions	Availability of flavonoid
1	Hexane (HE)	1	Dark green colour	-
2	Chloroform (CE)	1	Green colour	-
3	Methanol (ME)	1	Dark yellow colour	++

(-) Absent (++) present in higher concentration

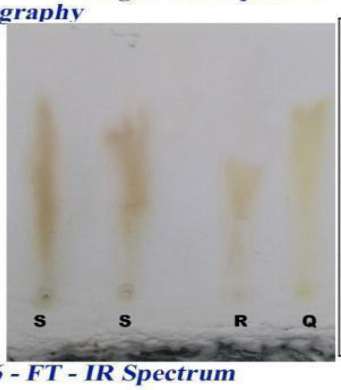
Table 5 showed *Stereospermum chelonoides* mixed powder ethanol extracts phytochemical flavonoid with reference to solvent elute. Three coloured fractions were obtained when eluted with hexane, chloroform and methanol. All the three solvents eluted only three coloured fractions of three different colours (Plate II – P. Fig 3). Dark yellow colour fraction of methanol elute showed the availability of flavonoids.

Plate II - Assessment of Active Principle from *Stereospermum chelonoides* mixed Flower and Leaf extract

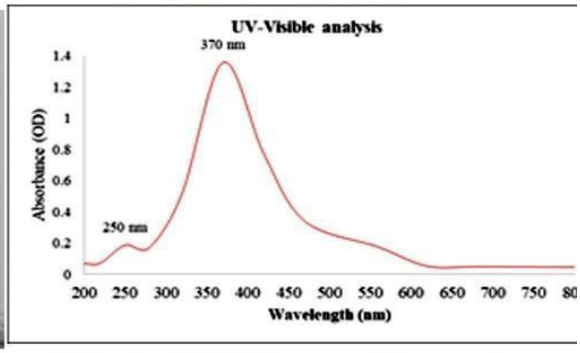
P. Fig. 3 Column Chromatography



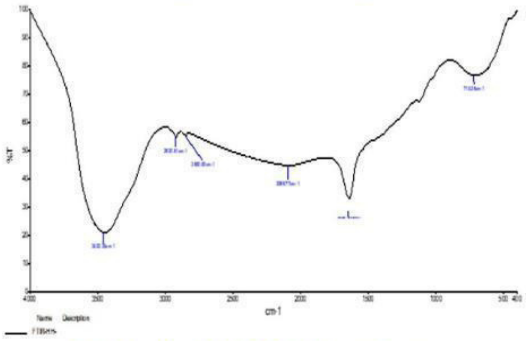
P. Fig. 4 - TLC pattern



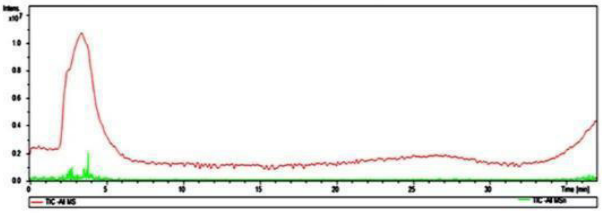
P. Fig. 5 - UV- VIS Spectrum



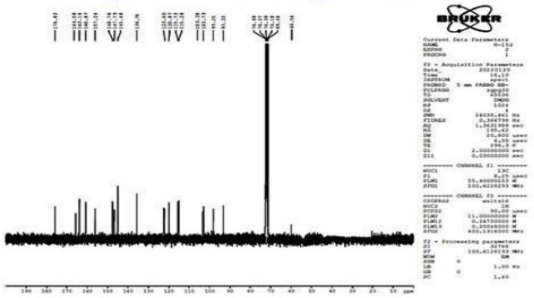
P. Fig. 6 - FT - IR Spectrum



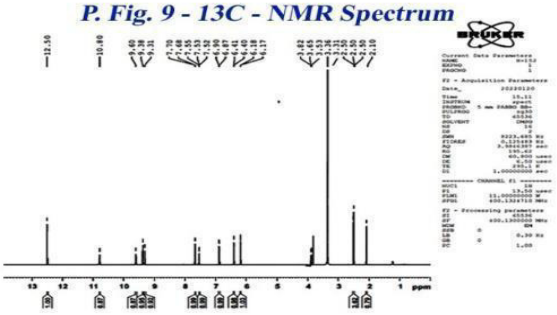
P. Fig. 7 - LC-MS Pattern of Flavonoid

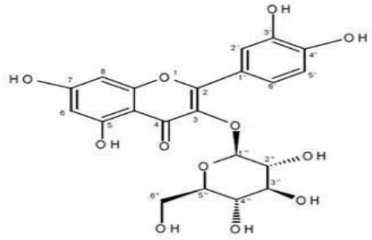


P. Fig. 8 - 1 H NM R Spectrum



P. Fig. 9 - 13C - NMR Spectrum





P. Fig. 10
Structure of Quercetin 3-O Glucoside

Plate Figure 4 illustrates TLC compound detection. Here sample rutin and quercetin were used as a standard. Table 6 also illustrates RF value evaluated through TLC analysis. Results strongly indicated methanol fraction obtained from the column chromatography showed flavonoid and it was compared with standard flavonoids as Quercetin and Rutin. The result of the Rf value of the extract was 0.85 which was matched with the Quercetin Rf value. These results suggested that the isolated compound was Quercetin derivatives. However, this TLC fraction was further confirmed in UV, FT-IR and NMR spectrum also.

Table 6-Analysis of flavonoid by TLC

Flavonoid	Rf Value
Sample	0.85
Std. (Rutin and its derivatives)	0.47
Std. (Quercetin and its derivatives)	0.86

. Fig. 5 showed UV spectral data of methanol fraction of mixed powder preparation, the **UV** λ_{max} value of flavonoid was found to be 250 and 370 nm. An investigation conducted by Guvenalp[29] explained that quercetin derivatives showed maximum absorption at 365 to 390nm and 250 to 270nm in UV spectroscopy which is in resemblance to our findings[18]. The results of the UV spectrum study supported the presence of quercetin derivatives. P. Fig. 6 with FT-IR is proved to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts. The FTIR analysis of isolated compounds gave a broad peak at 3452.25cm^{-1} which indicated the presence of phenolic OH stretching. Peaks from 2920.81cm^{-1}represent alkane C–H stretching. It showed strong peaks at 1642.30cm^{-1} representing C=C stretch which indicated the presence of alkenes and C-C stretch (in-ring) attributed to benzene ring present (aromatic ring) whereas 715.28 which indicates aromatic groups [30, 31]. The results of the study supported that quercetin derivatives have aromatic and phenolic groups present.

Table 7- LC-MS screening and identification of bioactive compounds in Stereospermum chelonoids extract

S. No	Name of the Compounds	Rt (min)	Fragments m/z
1	Luteolin 7-O-(2-apiosyl-4-glucosyl-6-malonyl)-glucoside	2.8	665.3
2	1-O-Caffeoyl-5-O-feruloylquinic acid	3.1	529.2
3	3,5-O-Dicaffeoylquinic acid	3.3	515.2
4	Quercetin-7-O-rhamnoside	3.4	447.1
5	Isorhamnetin-3-O-rutinoside	3.7	623.2
6	Quercetin-3-O-glucoside	3.9	463.1

Table 7 indicated the compound possibilities of methanol fraction in LC-MS. The *Stereospermum chelonoides* extract was screened for flavonoid compounds using LC-MS-MS. It has been successfully applied for a quick separation and identification of the flavonoid compounds from *Stereospermum chelonoides* extract (P. Fig 7). The LC-MS profile highlighted the presence of a large group of compounds corresponding to the protonated molecular ions of different flavonoids and caffeoylquinic acids. Individual components were identified by comparison of their m/z values in the Total Ion Count (TIC) profile with those of the selected compounds matching their MS/MS spectra with those reported in a public repository of mass spectral data called Mass Bank30. The six compounds were identified including Luteolin-O-6' malonyl-apiosylglycoside (Rt 2.8min. m/z 665.3), 1-O-Caffeoyl-5-O-feruloylquinic acid (Rt 3.1min. m/z 529.2), 3,5-O-Dicaffeoylquinic acid (Rt 3.3min. m/z 515.2), Quercetin-7-O-rhamnoside (Rt 3.4min. m/z 447.1). 1, Isorhamnetin-3-O-rutinoside (Rt 3.7min. m/z 623.2) and Quercetin-3-O-glucoside (Rt 3.9min. m/z 463.1)[31-,33]. P. Fig. 8 showed signals produced in ^{1}H-NMR spectrum (500MHz, MeOD) the aromatic protons produced signals at C-6 and C-8 appear as δ 6.17 and δ 6.41ppm respectively. Peaks at δ 6.90 (d, H'5'), 7.68(d, H'6') and 7.70 (d, H'2') also confirmed the presence of another trisubstituted benzene. The spectrum in DMSO and its changes after addition of customary shift reagents, suggested that the compound is a flavonol glycoside with free hydroxyl groups at positions C-5 a feature confirmed by the 1H NMR peaks at δ 12.50 (OH-5)[34, 35]. The glucose moiety can be located as a doublet at δ 2.10ppm. The remaining sugar protons appear in the range δ 3.31 - 3.82ppm. Similarly, P. Fig. 9 noted ^{13}C -NMR spectrum of *Stereospermum chelonoids* mixed extract methanol fraction. Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C-NMR data. Due to glycosylation at 3-position, C-2 and C-4 carbons absorb at δ 157.24 and 176.82 ppm respectively. C-1'' absorbs at δ 103.08ppm. The rest of the carbons of the glucose unit appear between δ 3.30-3.80 (6H, m, H-2'', H-3'', H-4'', H-5'', H-6'')[34, 35]. P. Fig. 10 illustrated the predicted molecule structure on the basis of chemical and spectral data evidences have been characterized as isolated compound was 3,3',4',5,7-Pentahydroxyflavone 3-glucoside (Quercetin 3-O-glucoside) from *Stereospermum chelonoids* extract.

5. DISCUSSIONS

Escherichia and *Klebsiella* are equally important bacteria responsible for UTI infection in humans. *Escherichia fergusonii* is one of the emerging pathogens of the wound, Haemolytic uremic syndrome and Cystitis[36]. Klebsiella is associated with about 8-12% of all UTI[37]. In this study these two isolates were isolated

from urine samples and confirmed their identity as *Escherichia fergusonii* and *Klebsiella pneumoniae.* These organisms are able to cause clinically evident UTI in Human[38]. Uropathogens like Escherichia fergusonii showed higher prevalence in UTI since 1985 and are responsible for multiple infections; the species is named to honour William H. Ferguson. This study showed a higher percentage of antibiotic resistance among UTI pathogens like *Escherchia fergusonii* and *Klebsiella pneumoniae*. Similar type of antibiotic resistance was noticed by various scientists all over the world [39,40,41,42]. Organisms showed MDR due to inappropriate use of antibiotics, use of antibiotics in veterinary, use of antibiotics in agricultural services, virulence of organisms and production of enzymes like beta lactamases could be responsible for antibiotic resistance [43, 44, = 47]. Antibiotic resistance is also responsible for millions of deaths among various populations in the world and considered as a global health hazard[48, -, 51]. Pathogens express stx, elt, cxt and eag genes and cause potent UTI infection which affect women more than men[52, 53, 54]. Isolates of this study also belong to MDR isolates and resistant to all traditional antibiotics like amoxicillin penicillin and Gentamycin. To overcome the problems associated with antibiotic resistance and side effects associated with these antibiotics, currently many people believe in the importance of medicinal plants and also the Traditional system of medicine. *Stereospermum chelonoides* root is one of the known components of ayurvedic preparation and flower and leaves are also used in various parts of the country[55-62]. Antibacterial activity of medicinal plants was varied with the usage of extracting solvents, Holistic method of extraction was followed to extract plant materials using water and ethanol. The extracts produced good antibacterial efficiency on MDR urinary isolates. Haque *et al.,*[63] also reported the efficiency of the same plant's stem bark hexane and chloroform extracts. Shantha *et al.,*[64] reported antibacterial efficiency of leaves of this plant chloroform extracts against gram positive as well as gram negative isolates. They reported that this extract produces a zone of inhibition ranging from 6mm to 11mm at 30µg/disc against multiple isolates. Various other scientists from different parts of the country also indicated the role of this plant's genus members as an antimicrobial agent[55 - 62]. Vijaya *et al.,*[61] also indicated antimicrobial roles of this genus members. Phytoconstituents like tannins, phenolic compounds could be responsible for these activities. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues. It forms complexes with protein via covalent type of bonding and inactivate adhesions and enzymes. It also inactivates transport proteins thereby inhibiting microbial growth. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase[65]. Phenols antibacterial effects are largely due to their ability to inhibit enzymes and bind with various soluble proteins present on the bacterial cell membrane. On the other hand, most of the alkaloids are efflux pump inhibitors. Gram positive bacteria are more susceptible than gram negative bacteria because of its nature of cell wall [66-68]. Antimicrobial property of a plant depends on its biologically active phytoconstituents. Tannins also bind to proline rich protein and interfere with protein synthesis.

Plants contain various types of bioactive compounds having different polarities and their separation still remains a big challenge in the process of identification and characterization of bioactive compounds. The organic solvent contain various bioactive compounds determined by means of several separation techniques such as extraction and isolation of bioactive constituents by using thin-layer chromatography, column chromatography, UV-Vis, FTIR and NMR techniques are used in prediction of the chemical nature and structure of the isolated compound [34,35]. In the present study, an isolated compound from extract was identified through UV spectrum, FT-IR spectrum, 1H-NMR, ^{13}C -NMR spectrum as Quercetin 3-O-glucoside. Ultraviolet and visible spectroscopy was one of the earliest techniques routinely used for flavonoid analysis due to the existence of two characteristic UV/Vis bands in flavonoids, band I in the 300 to 550 nm range, arising from the B ring, and band II in the 240 to 285 nm range, arising from the A ring. For examples, while the band I of flavones and flavanols lies in the 240 – 285 nm range, that of flavanone (no C ring instauration) lies in the 270 – 295 nm range; conversely, the band II of flavones an d flavanone (no 3-OH group) lies around 303 –304 nm, and that of 3-hydroxylated flavanols is centred around 352 nm. Shift reagents, such as sodium methoxide and aluminium chloride, lead to shifts in the maximum wavelength of these bands due to methoxide-induced deprotonation of OH groups or Al 3+ complexation by OH groups, were also routinely used to study flavonoid structure. Nowadays these techniques are not routinely used but still continue to be applied in some cases, in particular to HPLC eluates - a hyphenated LC-UV-MS has been developed using post-column UV shift reagents for the flavonoid analysis of crude extracts. The results of phytochemical study confirmed the availability of multipotent phytochemicals in mixed preparation from flower and leaf extracts. It contains flavonoids, coumarins, polyphenols, terpenoids and a bioactive agent. Sofowora[16] indicated that phytochemicals are the contributors of medicinal and physiochemical activity in medicinal plants. Various reports showed the activity of phytochemicals. The alkaloids could be responsible for analgesic activity[69] and anaesthetic activity[70]. The tannins are for antibacterial and anti-inflammatory property[71]. Flavonoids can do multiple functions, it enhances the efficiency of vitamin c and acts as antioxidants. Flavonoids reduce the burden of liver toxins, tumours, viruses etc. it also exhibits anti-allergic, anti-cancer, antioxidant, anti-viral. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. The flavonoids prevent the oxidation of low-density lipoprotein. Glycosylation of flavonoids takes place at any position of the carbon, mostly it happens at 7th position and are called flavones, isoflavones, flavanones and flavanols. Glycosylation at 3rd position forms quercetin 3 O-glycoside, which is also called isoquercetin [72,-75]. Many flavonoid glycosides possess acylated glycosyl moieties. NMR studies on structural elucidation of isolated compounds revealed the presence of glycosylated quercetin. 1H and 13C NMR experiments also confirmed quercetin in ethanol extracts of this plant. Intramolecular H bonding between the 4-oxo group and the 3- and 5-hydroxy groups, and on intermolecular association [76- 81]. The flavanol-type flavonoid quercetin has increased in popularity because of its ability to modulate signal transduction pathways. Direct antioxidant properties may play a role in the abrogation of both DNA damage, but potentially of more importance quercetin, can also target multiple signalling pathways associated with oncogenesis and tumour progression, which include DNA damage, inflammation and obesity. Quercetin can also upregulate proteins that abrogate free radical damage, such as p53. The concurrent targeting of quercetin's multiple bioactivities presents a potent chemo preventative strategy, but because

bioavailability of quercetin is poor it will be necessary to develop quercetin analogues to maximize the full chemopreventive potential of the compound. This review will explore the structural and mechanistic properties of quercetin as they relate to its ability to act as a chemo preventive compound[78, 79, 80, 81]. O-glycoside derivatives of Quercetin are of two types, they are 3-O-glycoside and other O-glycoside derivatives. O-Glycoside quercetin derivatives represent broad-spectrum biological activities[39].

6. CONCLUSION

The result of this study confirmed antibacterial efficiency of leaf and flower mixed preparation of *Stereospermum chelonoides which* is traditionally used for treatment of UTI infections. Extracts of this plant effectively controlled the growth of MDR UTI pathogens like *Escherichia fergusonii* and *Klebsiella pneumoniae*. Medicinally active constituents in the plant materials prevents microbial growth, which is evidenced bydisc diffusion assay, MIC and MBC analysis. Presence of flavonoids, polyphenols, terpenoids like phytocompounds are responsible for antibacterial efficiency. Flavonoids are the major compounds in this plant's ethanolic extracts which exert antimicrobial activity via protein precipitation mechanism. Further studies on phytochemical screening confirmed bioavailability of Quercetin – 3-O glucoside. This compound could be responsible for the antibacterial efficiency of *Stereospermum chelonoides* leaf and flower mixed preparation.

7. AUTHOR CONTRIBUTION STATEMENT

Mrs. Sangeetha hypothesized the study and performed all experiments, Dr. S. Rajan analysed all data and discussed all study matters and Dr. B. Anandaraj designed all methodology described in the study. All the authors read this article and approved it.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. Savini V, Catavitello C, Talia M, Manna A, Pompetti F, Favaro M et al. Multidrug-resistant Escherichia fergusonii: a case of acute cystitis. J Clin Microbiol. 2008;46(4):1551-2. doi: 10.1128/JCM.01210-07, PMID 18256229.
2. Yoganarasimhan SN.medicinal plants of India, vol. 2. Tamil Nadu. Bangalore: Rotman Research Institute. 2000.
3. Wahab Sab BA, Jacob J, Manjunath GG, Singh VK, Mundkinajeedu D, Shankarappa S. Cycloolivil, A lignan from the roots of Stereospermum suaveolens. Pharmacognosy Res. 2015;7(1):45-8. doi: 10.4103/0974-8490.147198, PMID 25598634.
4. Kritikar KR, Basu BD. Indian Medicinal plants, Dehradun, International book distributors. 1999;2:1848.
5. Masoumeh R, Deokule SS. Deterioration of chemical constituents in roots of drug Stereospermum chelonoides DC. Under storage. Asian J Plant Sci Res. 2013;3(1):111-4.
6. Prema S, Saraswathi A, Chitra K, Gopal V. A Review on Stereospermum colais Mabb: Bignoniaceae. Int J Pharm Sci Rev Res. 2013;21(1):314-7.
7. Meena AK, Yadav AK, Panda P, Preet K, Rao MM. Review on Stereospermum suaveolens DC: A potential Herb. Drug Invent Today. 2010;2(5):238-9.
8. Moniruzzaman M, Kuddus MR, Haque MR, Chowdhury AS, Rashid MA. Stereospermum suaveolens (Roxb.) DC. Shows Potential in vivo and in vitro Bioactivities. Dhaka Univ J Pharm Sci. 2018;17(2):257-63. doi: 10.3329/dujps.v17i2.39184.
9. Farnsworth NR, Chapter 9. Screening of plants for new medicines. In:. In: Wilson EO, editor, Biodiversity. Washington, DC: National Academy Press; 1988.
10. Bauer AW, Kirby W, Sherris TS, Turek M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 1966;36:493-6.
11. Koneman EW, William MJ, Stephen DA, Schreeken B, Washington CW. Laboratory and clinical diagnosis of infectious diseases. In: Introduction to diagnostic microbiology. Philadelphia: J B Lippincott Publishers Company; 1994. p. 1-19.
12. Özçelik S, Kılavuzu GML. Elazığ Fırat Üniversitesi Fen-edebiyat Fakültesi Yayınları, yayın. 1992;1:135.
13. Collins CH, Lyne PM, Grange JM. Microbiological methods. 6th ed. Vol. 1989. Boston: Butterworths & Co, Ltd; 1989. p. 410.
14. Bradshaw LJ USA. Laboratory of microbiology. 4th ed, Saunders College Publishing, Harcourt Brace Javanovich College Publishers; 1992. p. 435.
15. Kowser MM, Fatena N. Determination of MIC and MBC of selected azithromycin capsule commercially available in Bangladesh. The Orion Med J. 2009;32(1):619-20.
16. Sofowara A. Medicinal plants and Traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd; 1993. p. 289.
17. Trease GE, Evans WC. Text book of Pharmacognosy. 12th ed, Belliere Tinad. London; 1983. p. 257.
18. Harborne JB. Phytochemical Methods; A guide to modern techniques of plant Analysis. 2nd ed. London, New York; 1984.
19. Javed I, Mohammad A. Isolation of a flavonoid from the roots of Citirus sinensis. Malasiyan J Pharm Sci. 2009;7(1):1-8.
20. . JHA, . RO, . CCW. Phytochemical Screening of Flavonoids in three Hybrids of Nepenthes (Nepenthaceae) and their Putative Parental Species from Sarawak and Sabah. J Biol Sci. 2002;2(9):623-5. doi: 10.3923/jbs.2002.623.625.
21. Luvincia FM, Abhishek BR, Vivek P. Phytochemical, FTIR and NMR analysis of crude extract of Acacia planifrons seeds. J Pharm Sci Res. 2019;11(5):1960-2.
22. Blunder M, Orthaber A, Bauer R, Bucar F, Kunert O. Efficient identification of flavones, flavanones and their glycosides in routine analysis via off-line combination of sensitive NMR and HPLC experiments. Food Chem.

2017;218:600-9. doi: 10.1016/j.foodchem.2016.09.077, PMID 27719955.
23. Bataglion GA, da Silva FMA, Eberlin MN, Koolen HHF. Determination of the phenolic composition from Brazilian tropical fruits by UHPLC-MS/MS. Food Chem. 2015;180:280-7. doi: 10.1016/j.foodchem.2015.02.059, PMID 25766829.
24. Suman E, Jose J, Varghese S, Kotian MS. Study of biofilm production in Escherichia coli causing urinary tract infection. Indian J Med Microbiol. 2007;25(3):305-6. doi: 10.4103/0255-0857.34788, PMID 17901664.
25. Pramodhini S, Niveditha S, Umadevi S, Shailesh K, Stephen S. Antibiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India, Australias. Med J. 2012;5(7):344-8.
26. Mina Y, Mohammad YM, Naser A, Nasser S, Reza G. Antibiotic resistance patterns of biofilm-forming Pseudomonas aeruginosa isolates from mechanically ventilated patients. Int J Sci Study. 2017;5(5):1-5.
27. Ohadian Moghadam S, Pourmand MR, Aminharati F. Biofilm formation and antimicrobial resistance in methicillin-resistant Staphylococcus aureus isolated from burn patients, Iran. J Infect Dev Ctries. 2014;8(12):1511-7. doi: 10.3855/jidc.5514.
28. Sanchez GV, Master RN, Karlowsky JA, Bordon JM. In vitro antimicrobial resistance of urinary Escherichia coli isolates among U.S. Outpatients from 2000 to 2010. Antimicrob Agents Chemother. 2012;56(4):2181-3. doi: 10.1128/AAC.06060-11, PMID 22252813.
29. Guvenalp Z, Nurcan K, Kazaz C, Yusuf K, Omur DL. Chemical Constituents of Galium tortumense. Turkist J Chem. 2006;30:515-23.
30. Patle TK, Shrivas K, Kurrey R, Upadhyay S, Jangde R, Chauhan R. Phytochemical screening and determination of phenolics and flavonoids in Dillenia pentagyna using UV-vis and FTIR spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2020;242:118717. doi: 10.1016/j.saa.2020.118717, PMID 32745936.
31. Sumanth MV, Yellanthoor MB, Ravikumar K, Ravichandran P. Comparative physicochemical, phytochemical and HPTLC studies on root species used as Patala in Ayurvedic system of medicine. J Pharm Res. 2013;7(9):810-6. doi: 10.1016/j.jopr.2013.09.007.
32. Subramanian SS, Nagarajan S, Sulochana MN. Flavonoids of the leaves of Stererospermum suaveolens. Curr Sci. 1972;41:102-3.
33. Ramachandran AG, Kotiyal JP. Stereolensin: A new flavone glucoside from Stereospermum suaveolens. Indian J Chem. 1979;18B:188-9.
34. Leslie Gunatilaka AA, Dhammika Nanayakkara N, Wazeer MIM. 13C NMR Spectra of some D:A-friedo-oleananes☆. Phytochemistry. 1983;22(4):991-2. doi: 10.1016/0031-9422(83)85038-9.
35. Teng RW, Wang DZ, Wu YS, Lu Y, Zheng QT, Yang CR. NMR assignments and single-crystal X-ray diffraction analysis of deoxyloganic acid. Magn Reson Chem. 2005;43(1):92-6. doi: 10.1002/mrc.1502, PMID 15505818.
36. Baek SD, Chun C, Hong KS. Hemolytic uremic syndrome caused by Escherichia fergusonii infection. Kidney Res Clin Pract. 2019;38(2):253-5. doi: 10.23876/j.krcp.19.012.
37. Marami D, Balakrishnan S, Seyoum B. Prevalence, antimicrobial susceptibility pattern of bacterial isolates, and associated factors of urinary tract infections among HIV-positive patients at Hiwot Fana Specialized University Hospital, Eastern Ethiopia. Can J Infect Dis Med Microbiol. 2019;2019:6780354. doi: 10.1155/2019/6780354, PMID 30881531.
38. Tessema NN, Ali MM, Zenebe MH. Bacterial associated urinary tract infection, risk factors, and drug susceptibility profile among adult people living with HIV at Haswassa University Comprehensive Specialized Hospital, Hawassa, Southern Esthiopia. Sci Rep. 202010(1):10790. doi: 10.1038/s41598-020-67840-7, PMID 32612139.
39. Simmons K, Islam MR, Rempel H, Block G, Topp E, Diarra MS. Antimicrobial Resistance of Escherichia fergusonii Isolated from Broiler Chickens. Journal of Food Protection. 2016;79(6):929-38. doi: 10.4315/0362-028X.JFP-15-575.
40. Singh AK, Das S, Kumar S, Gajamer VR, Najar IN, Lepcha YD et al. Distribution of antibiotic-Resistant Enterobacteriaceae Pathogens in Potable Spring water of Eastern Indian Himalayas: emphasis on Virulence Gene and antibiotic Resistance Genes in Escherichia coli. Front Microbiol. 2020;11:581072. doi: 10.3389/fmicb.2020.581072, PMID 33224119.
41. Bader MS, Loeb M, Leto D, Brooks AA. Treatment of urinary tract infections in the era of antimicrobial resistance and new antimicrobial agents. Postgrad Med. 2020;132(3):234-50. doi: 10.1080/00325481.2019.1680052, PMID 31608743.
42. Bours PHA, Polak R, Hoepelman AIM, Delgado E, Jarquin A, Matute AJ. Increasing resistance in community-acquired urinary tract infections in Latin America, five years after the implementation of national therapeutic guidelines. Int J Infect Dis. 2010;14(9):e770-4. doi: 10.1016/j.ijid.2010.02.2264.
43. Almakki A, Jumas-Bilak E, Marchandin H, Licznar-Fajardo P. Antibiotic resistance in urban runoff. Sci Total Environ. 2019;667:64-76. doi: 10.1016/j.scitotenv.2019.02.183, PMID 30826682.
44. Praveenkumarreddy Y, Akiba M, Guruge KS, Balakrishna K, Vandana KE, Kumar V. Occurrence of antimicrobial-resistant Escherichia coli in sewage treatment plants of south India. J Water Sanit Hyg Dev. 2020;10(1):48-55. doi: 10.2166/washdev.2020.051.
45. Costa EC, Arpini CM. Antibiotic sensitivity profile of enteric bacteria isolated from beach waters and sewage from the municipality of Vila Velha-ES. Braz J Bacteriol Parasitol. 2016;7:3-7.
46. Karkman A, Do TT, Walsh F, Virta MPJ. Antibiotic-resistance genes in waste Water. Trends Microbiol. 2018;26(3):220-8. doi: 10.1016/j.tim.2017.09.005. PMID 29033338.
47. Farmer JJ, Fanning GR, Davis BR, O'Hara CM, Riddle C, Hickman-Brenner FW et al. Escherichia fergusonii and Enterobacter taylorae, Two New Species of Enterobacteriaceae Isolated from Clinical Specimens. J Clin Microbiol. 1985;21(1):77-81. doi: 10.1128/jcm.21.1.77-81.1985, PMID 3968204.
48. Adzitey F. Incidence and antimicrobial susceptibility of Escherichia coli isolated from beef (meat muscle, liver and kidney) samples in Wa Abattoir, Ghana. Cogent Food

Agric. 2020;6(1):1718269. doi: 10.1080/23311932.2020.1718269.
49. Meng L, Liu H, Lan T, Dong L, Hu H, Zhao S et al. Antibiotic resistance patterns of Pseudomonas spp. Isolated from raw milk revealed by whole genome sequencing. Front Microbiol. 2020;11:1005. doi: 10.3389/fmicb.2020.01005. PMID 32655503.
50. Adegoke AA, Madu CE, Aiyegoro OA, Stenström TA, Okoh AI. Antibiogram and beta-lactamase genes among cefotaxime resistant E. coli from wastewater treatment plant. Antimicrob Resist Infect Control. 2020;9(1):46. doi: 10.1186/s13756-020-0702-4, PMID 32164766.
51. Morris S, Cerceo E. Trends, Epidemiology, and Management of Multi-Drug Resistant Gram-Negative Bacterial Infections in the Hospitalized Setting. Antibiotics (Basel). 2020;9(4):1-20. doi: 10.3390/antibiotics9040196, PMID 32326058.
52. Ss M. Occurrence of Multiple Antibiotic Resistant E. coli on Surface Water of River Ganga at Allahabad, Uttar Pradesh, India. OFOAJ;8(1). doi: 10.19080/OFOAJ.2018.08.555730.
53. Sandhu R, Aggarwal A, Sayal P, Kumar S. Intestinal carriage of drug-resistant Gram-negative bacteria belonging to family Enterobacteriaceae in children aged 3-14 years: an emerging threat. Int J Health Allied Sci. 2019;8:108-15. doi: 10.4103/ijhas.IJHAS.
54. Igwaran A, Iweriebor BC, Okoh AI. Molecular characterization and antimicrobial resistance pattern of Escherichia coli recovered from wastewater treatment plants in Eastern Cape South Africa. Int J Environ Res Public Health. 2018;15(6):1237. doi: 10.3390/ijerph15061237, PMID 29895735.
55. Kothai S. In vitro synergistic effect of chewing sticks (toothbrush), cinnamon and honey against Streptococcus pyogenes. Int J Sci Innov Discov. 2013;3(1):43-50.
56. Kothai S, Thiyagarajan T. Antimicrobial activity of chewing sticks of Jimma-Ethiopia against Streptococcus pyogens. J Phytol. 2011;3(8):34-7.
57. Van Vuuren SF, Viljoen AM. The in vitro antimicrobial activity of toothbrush sticks used in Ethiopia. S Afr J Bot. 2006;72(4):646-8. doi: 10.1016/j.sajb.2006.03.009.
58. Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku HU et al. An ethnobotanical survey of Bauchi state herbal plants and their antimicrobial activity. J Ethnopharmacol. 2005;99(1):1-4. doi: 10.1016/j.jep.2004.12.025, PMID 15848012.
59. Rashedul IM, Rubina A, Obaidur RM, Ahsanul AM, Muhammad A, Dedarul AK et al. In vitro antimicrobial activities of four medicinally important plants in Bangladesh. Eur J Sci Res. 2010;39(2):199.
60. Lenta BN, Weniger B, Antheaume C, Noungoue DT, Ngouela S, Assob JC et al. Anthraquinones from the stembark of Stereospermum zenkeri with antimicrobial activity. Phytochemistry. 2007;68(11):1595-9. doi: 10.1016/j.phytochem.2007.03.037, PMID 17499823.
61. Vijaya BR, Jerad SA, Kumudha VB, Lata S, Geetha LS, Thirumal M. In vitro antibacterial and antifungal studies of Stereospermum colais leaf extracts. Int J Pharm Technol. 2010;2(3):603-11.
62. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol. 2006;106(3):290-302. doi: 10.1016/j.jep.2006.04.003, PMID 16698208.
63. Haque MR, Rahman KM, Iskander MN, Hasan CM, Rashid MA. Stereochenols A and B, Two quinones from Stereospermum chelonoides. Phytochemistry. 2006;67(24):2663-5. doi: 10.1016/j.phytochem.2006.08.014, PMID 17027879.
64. Shanta MA, Mondal M, Majumder S, Islam MI, Hoque N, Tithi NS et al. Pharmacological investigations of chloroform extract of Stereospermum chelonoides leaves. Int J Pharm Sci Res. 2018;9(10):4256-66. doi: 10.13040/IJPSR.0975-8232.9(10).4256-66.
65. Jaime A, Teixera DS, Abdulla IH. Citrullus colocynthis (colocynth): biotechnological prospectives. Emirates J Food Agric. 2017;29(2):83-90.
66. Patni CS, Kohe SJ, Awasthi RP. Efficacy of botanicals against Alternaria blight (Alternaria brassicae) of mustard. Indian Phytopathol. 2005;58(4):426-30.
67. Karou D, Salvodogo A, Canini A. Antibacterial activity of alkaloids from Sida acuta. Afr J Biotechnol. 2005;4:1452-7.
68. Masika PJ, Afolayan AJ. Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. J Ethnopharmacol. 2002;83(1-2):129-34. doi: 10.1016/s0378-8741(02)00242-8, PMID 12413718.
69. Antherden LM. Textbook of pharmaceutical chemistry. 8th ed. Oxford: Oxford University press; 1969. p. 813-4.
70. Hérouart D, Sangwan R, Fliniaux M, Sangwan-Norreel B. Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of Datura innoxia. Planta Med. 1988;54(1):14-7. doi: 10.1055/s-2006-962320.
71. Duguid JP. A guide to the laboratory diagnosis and control of infection. In collee et al. (eds) Mackie and McCartney Medical Microbiology. 13th ed. Vol. 1. New York: Churchill Livingstone; 1989. p. 163.
72. Li QM, Claeys M. Characterization and differentiation of diglycosyl flavonoids by positive ion fast atom bombardment and tandem mass spectrometry. Biol Mass Spectrom. 1994;23(7):406-16. doi: 10.1002/bms.1200230705, PMID 8068736.
73. Vukics V, Guttman A. Structural characterization of flavonoid glycosides by multi-stage mass spectrometry. Mass Spectrom Rev. 2010;29(1):1-16. doi: 10.1002/mas.20212, PMID 19116944.
74. Ferreres F, Gil-Izquierdo A, Andrade PB, Valentão P, Tomás-Barberán FA. Characterization of C-glycosyl flavones O-glycosylated by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2007;1161(1-2):214-23. doi: 10.1016/j.chroma.2007.05.103, PMID 17602695.
75. Cuyckens F, Claeys M. Determination of the glycosylation site in flavonoid mono-O-glycosides by collision-induced dissociation of electrospray-generated deprotonated and sodiated molecules. J Mass Spectrom. 2005;40(3):364-72. doi: 10.1002/jms.794, PMID 15674860.
76. Fossen T, Andersen OM. Spectroscopic techniques applied to flavonoids. In: Flavonoids – chemistry, biochemistry and applications, Andersen ØM, Markham KR,(37-142). Taylor & Francis. USA; 2005. p. 978-0-8493-2021-7.

77. Olejniczak S, Potrzebowski MJ. So lid state NMR studies and density functional theory (DFT) calculations of conformers of quercetin. Org Biomol Chem. 2004;2(16):2315-22. doi: 10.1039/b406861k, PMID 15305212.
78. Mendoza EE, Burd R. Quercetin as a systemic chemopreventative agent: structural and functional mechanisms. Mini Rev Med Chem. 2011;11(14):1216-21-2221. doi: 10.2174/1389557511091216, PMID 22070678.
79. Alizadeh SR, Ebrahimzadeh MA. O-glycoside quercetin derivatives: biological activities, mechanisms of action, and structure-activity relationship for drug design, a review. Phytother Res. 2022;36(2):778-807. doi: 10.1002/ptr.7352, PMID 34964515.
80. Haneef DM, Budhiraja MV, Budhiraja MP. Antioxidant Potential, Acute Toxicity Profile and Bioavailability Studies of Water Soluble Analogue of Curcumin. Int J Pharma Bio Sci. 2022;13(2):40-50. doi: 10.22376/ljpbs.2022.13.2.p40-50.
81. Sarkar D, Ganguly A. Molecular docking studies with garlic phytochemical constituents to inhibit the human EGFR protein for lung cancer therapy. Int J Pharma Bio Sci. 2022;13(2):1-14. doi: 10.22376/ljpbs.2022.13.2.b1-14.