



ANTIMICROBIAL ACTIVITY OF ORGANIC LEAF EXTRACT OF *SESBANIA SESBAN* AGAINST GRAM NEGATIVE PATHOGENIC BACTERIA

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ABSTRACT

Sesbania sesban, belonging to family Leguminosae, is a well-recognized medicinal plant. It is widely distributed in our country. *Sesbania sesban* is an erect, branched small tree up to 6 m tall with soft wood and paripinnate leaves. Flowers are yellow with brown streaks on the corolla. Fruits are sub cylindrical and shortly beaked. Seeds are green or brown and usually mottled. The plant is used as carminative, antihelmintic, astringent, anti-inflammatory, antimicrobial, antifertility, demulcent and purgative. It is also given as a medicine against fever and ulcers. The medicinal value of plants depends up on the chemical compounds that produce in the human body during a specific reaction. chemical substances responsible for such reactions are called secondary metabolites. The most important of these bioactive compounds are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds. By keeping these properties of bioactive compounds of *Sesbania sesban* in view the present investigation was undertaken to screen phytochemical constituents. Preliminary phytochemical screening of leaf in three different extracts (ethanol, methanol and aqueous extract) revealed the presence of carbohydrates, tannins, saponins, glycosides, steroids, flavonoids, phenols, terpenoids, alkaloids and glycosides which indicated antimicrobial activity of the *Sesbania sesban* leaves. The other objective was to investigate antimicrobial activity of three solvent extracts against gram negative pathogenic bacteria viz, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Among the three extracts, ethanol showed the maximum activity. Zone of inhibition caused by ethanol against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at (50µl, 100µl, 150µl) is 25%, 24.5% and 18.8% higher, respectively than caused by methanol and aqueous extracts of *Sesbania* leaves. Ethanol extract of *Sesbania* leaves potentially act as antimicrobial agent.

KEYWORDS: *Sesbania sesban*, phytochemical, antimicrobial agent, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.



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INTRODUCTION

The World Health Organization (WHO) estimates that about 80% of people living in developing countries rely almost exclusively on traditional medicines for their primary health care needs. India is essentially a herbarium of the world. The herbal medicines / traditional medicaments have therefore been derived from rich traditions of ancient civilizations and scientific heritage¹. *Sesbania sesban* Linn is outstanding restorative plant., It is very much circulated all through the fields of India and other tropical nations . *Sesbaniasesban*, normally known as 'Egyptian sesban' is one of the six types of class *Sesbania* which is generally grown in tropical locale of india. The plant is grown for its nitrogen fixing capacity. *Sesbania* species are widely used as fertilizer in different agricultural systems as it improves soil fertility, soil organic matter, water infiltration and holding capacity. *Sesbania* species belong to Leguminosae family. *Sesbania sesban* is one of the exotic multipurpose fodder² trees introduced in the Ethiopian highlands for livestock feeding and soil conservation. *Sesbania* improves intake and digestibility of basal diet and growth rate of animals. it not only improves the digestibility, but also improves rumen fermentation and milk production of animals. Sesban wood is light in weight when compared to other perennial plants in india sesban is oftenly used firewood p and to to make charcoal. Tender parts of this plant is used as fodder to the cattle .The soft wood is not durable but thine fibers can be obtained from inner barkand can be used Gunny bags. The branches and pods consider as litter. The branches can be used for construction of temporary sheds and huts. Sesban has ability to fix atmospheric nitrogen through symbiotic interaction with bacteria, and is able to stabilize soil. Sesbenia is also used as fencing purpose to shade crops like coffee, tea and few other plants. Flowers of sesban are known to be consumed by humans in some regions, perhaps mainly as a decorative element. Medicinal uses of sesban were also recorded. Antibiotic resistance is currently a serious and widespread problem in both developing and developed countries as it causes high mortality every year³. Despite the use of advanced antibiotics, the infectious diseases remain an important cause of morbidity and mortality. Moreover, the synthetic drugs expose the patients to the risk of harmful side effects. Medicinal plants have been used for centuries as remedies for human diseases. They contain components, usually their secondary metabolites, which have various

pharmacological properties⁴. Some of the secondary metabolites used as drugs are morphine from *Papaver somniferum* L. (Papaveraceae) for pain⁵ and colchicine from *Colchicum autumnale* L. (Colchicaceae) for treatment of pericardial disease⁶. Therefore, searching for therapeutic agents from plants is an alternative way to solve these problems. Indeed, certain plant extracts show significant potential against drug resistant bacteria⁷. Therefore, the main objective of the present study is to test the presence of carbohydrates, alkaloids, saponins, phenol, flavonoid and terpenoids extracts of *Sesbania sesban* leaves. The other main objective was to investigate antimicrobial activity of sesbenia extract against Gram negative pathogens viz, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Sesbania sesban* were collected from open fields of Chittor district. Leaves were washed thoroughly to remove contaminants. Then leaves were air dried under shade. Dried leaves were coarsely powdered and packed in airtight containers for further use.

Preparation of extracts

About 15 g of coarsely powdered leaf was mixed with 50 ml of distilled water, methanol and ethanol separately in 100 ml conical flask and was plugged. Flasks were shaken at 12 rpm for 30 min for 24 hrs. Extracts were filtered using Whatman No.1 filter paper. Leaf extracts were used for preliminary phytochemical screening and assessment of *in vitro* antimicrobial studies.

Bacterial cultures

Pathogenic bacteria viz. *Escherichiacoli* MTCC 63, *Pseudomonas aeruginosa* MTCC 424, and *Klebsiella pneumonia* MTCC 39 were obtained from IMTECH Chandigarh and assessed for antimicrobial activity.

Test for carbohydrates

Carbohydrate test was done using standard protocol⁸.The presence of carbohydrates in three different solvent extracts was determined by different methods such as, Fehling's test, Benedicts test, Molisch's test and iodine test.

Fehling's test

It is used to determine the presence of reducing sugar and aldehyde. Equal volume of Fehling's reagent A and B mixed together and 2ml of this was added to plant extract. Followed by gentle heating, the mixture turns Brick red color.

Benedict's test

2ml of Benedicts solution added to crude plant extracts followed by gentle boiling gives reddish brown precipitate.

Molisch's test

To 2ml of leaf extract, 1 drop of Molisch's reagent was added and 1-2ml of Con. H₂SO₄ was added which develops violet ring at the interphase.

Iodine test

1-2 drops of iodine solution was added to the leaf extract, dark blue colour is observed.

Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures⁹

Alkaloids

Leaf extracts were dissolved in diluted HCL and filtered to get the pure extract.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Flavonoids

The identification of flavonoids was carried out using the sodium hydroxide test. 5ml of plant extract was mixed with few magnesium chips and 2 drops of concentrated hydrochloric acid were added and warmed.

Tannins

Tannins were identified using the Bromine Water test. To 5 ml of plant extract, 20 ml of 50% alcohol was added and then filtered. A few drops of bromine water were added to the resulting filtrate the formation of a buff/white precipitate indicated the presence of tannins.

Saponins

Saponins were identified by frothing test. 3ml of the leaf extract was added to 10 ml distilled water and shaken vigorously for 30 sec. Froth formation indicates the presence of saponins.

Terpenoids

The identification of terpenoids identified using Noller's test. The leaf extract was warmed along with a tin piece and 3 drops of thionyl chloride. Purple colour indicates presence of terpenoids.

Steroids

Steroids were detected by Libermann-Burchard test. 2ml of leaf extract was mixed with 2 drops of chloroform and 2ml of acetic anhydride followed by 1ml of concentrated sulphuric acid added to the sidewall of the test tube. The formation of a reddish ring at the contact zone of the two liquids and a greenish colour in the separate layer indicates the presence of steroids.

Test for glycosides

2ml of chloroform, 2ml of acetic acid were added to leaf extract and allowed to cool, followed by addition of 2ml of Conc. H₂SO₄. The colour of the extract changes from violet to blue and then into green. This indicates the presence of steroidal nucleus that is glycone - portion of glycoside. In another way, the available cardiac glycosides are tested by adding 2ml to the leaf extract, 2ml of chloroform, 2ml of acetic acid and allowed to cool. This was followed by addition of 2ml of Conc. H₂SO₄. The colour of the extract changes from violet to blue and then into green. This indicates the presence of steroidal nucleus. To the leaf extract 1-2 drops of glacial acetic acid and 2% of FeCl₃ solution is added followed by 2ml of H₂SO₄. These give brown ring in the interphase which indicates presence of cardiac glycosides.

Qualitative analysis of reducing sugars

1 ml of extract was hydrolyzed by boiling with 5 mL of dilute hydrochloric acid and it was neutralized with sodium hydroxide solution. The Fehling's test was performed using the similar procedure as mentioned earlier. Brick-red precipitate indicates the presence of reducing sugars.

Assessment of antimicrobial activity of leaf extract against selected pathogenic bacteria

Antimicrobial activity of three different leaf extract viz aqueous extract, methanol extract and ethanol extract was used to determine antimicrobial activity

against Pathogenic bacteria. 50 μ l, 100 μ l and 150 μ l of aqueous extract was introduced on discs measuring 0.5mm diameter and placed in Petri Plates with nutrient agar media containing *E.coli*, *P.aeruginosa* and *Klebsiella pneumonia* in separate plates. Similarly 50 μ l, 100 μ l and 150 μ l of methanol and ethanol extract was introduced on discs measuring 0.5mm diameter¹⁰ and placed in Petri Plates with nutrient agar media containing *E.coli*, *P.aeruginosa* and *Klebsiella pneumonia* in separate plates.

STATISTICAL ANALYSIS

Statistical analysis was performed using Analysis of Variance (ANOVA) and Duncan's new multiple range tests at $P \leq 0.05$ using SPSS 13 version.

RESULTS

The three different leaf extracts of *Sesbania sesban* are screened for the presence of various phytochemical compounds. The presence of carbohydrates was confirmed by Fehling's, Molisch's and benedict's test. Iodine test confirms presence of phenol and tannins by developing green and black colour. Similarly Mayer's test revealed the presence of alkaloids by giving green colour. Presence of Flavonoids was confirmed by sodium hydroxide test which showed pink colour. Bromine water test showed positive for tannins by producing white precipitate, frothing test showed positive towards the presence of Saponins. Libermann –

Burchard test revealed the presence of steroids by producing reddish ring. Antimicrobial activity of three different extracts (methanol, ethanol and aqueous) against selected pathogenic bacteria are shown in Figure 1,2,3. All the three selected concentrations of ethanol / methanol/ aqueous (50 μ l, 100 μ l, 150 μ l) showed significant inhibition of *E.coli*, *P. aeruginosa* and *Klebsiella pneumoniae*. But in each case the inhibition caused by ethanol extract of the plant was noticed to be significantly higher than that caused by methanol and aqueous extract. In each case the magnitude of inhibition is directly proportional to the level of concentration. However the zone of inhibition caused by 50 μ l / 100 μ l/ 150 μ l of ethanol extract is higher than that caused by methanol extract. For instance, the zone of inhibition against *E.coli* caused by ethanol extract at 50 μ l, 100 μ l and 150 μ l (Fig 1.a) is 19.7 % , 41.6% and 22% higher, respectively than that caused by methanol extract (Fig 2.a) and 46.50% , 27.2% and 40% is higher respectively than that caused by aqueous extracts (Fig 3.a). Similarly, the zone of inhibition against *P.aeruginosa* caused by ethanol at (50 μ l, 100 μ l, 150 μ l) is 32.5%, 20% and 18% higher respectively than that caused by methanol and 9.2%, 16% and 11.2% higher respectively than caused by aqueous extract. Zone of inhibition against *Klebsiella pneumoniae* caused by ethanol at (50 μ l, 100 μ l, 150 μ l) is 25%, 24.5% and 18.8% higher respectively than that caused by methanol and 16%, 17.5%, 6.3% higher respectively than that caused by aqueous extracts of *Sesbania* leaves.

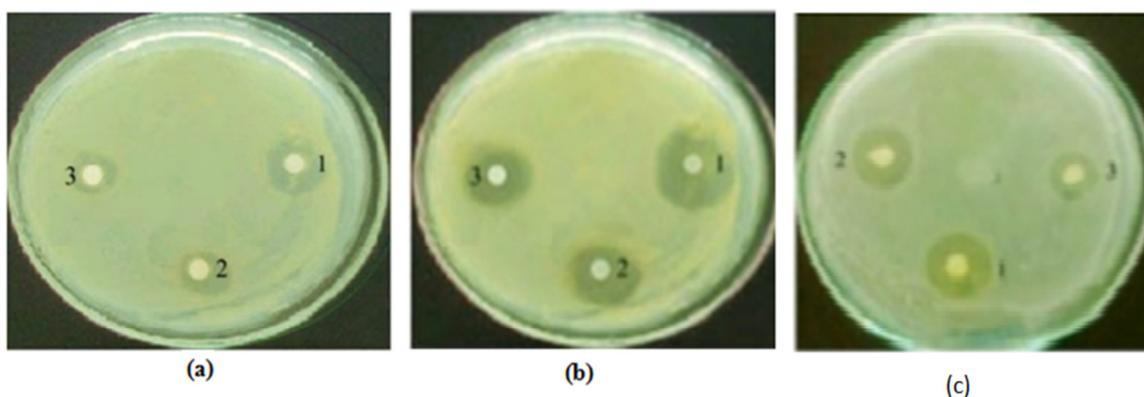


Figure.1
*Antimicrobial activity of ethanol leaf extract [1]50 μ l 2)100 μ l 3)150 μ l against (a) *E. coli* (b) *P. aeruginosa* (c) *K. pneumoniae**

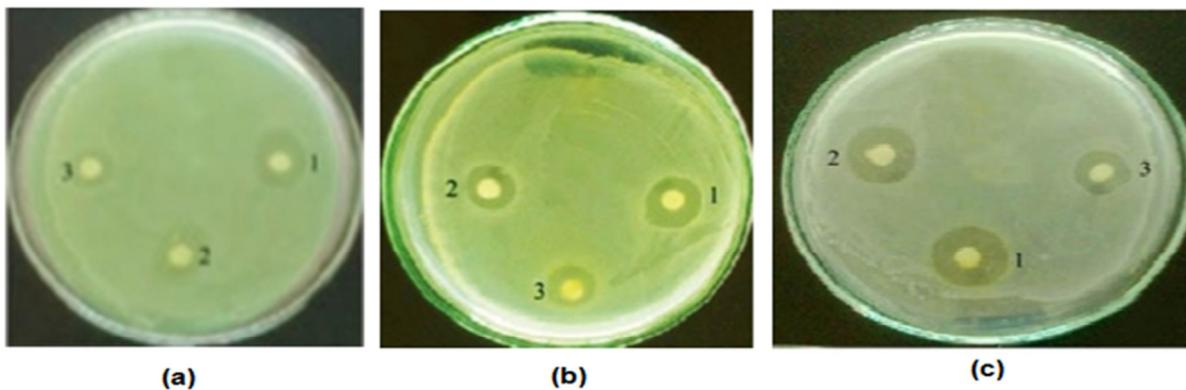


Figure 2
Antimicrobial activity of methanol leaf extract [1)50µl 2)100µl 3)150µl against (a) E. coli (b) P. aeruginosa (c)K. pneumonia

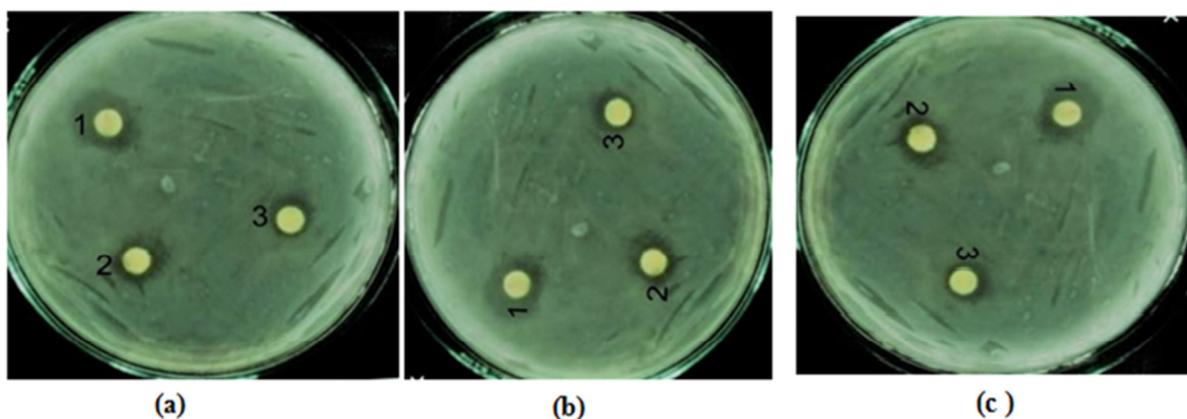


Figure 3
Antimicrobial activity of aqueous leaf extract [1)50µl 2)100µl 3)150µl against (a) E. coliP. aeruginosa (c) K. pneumonia

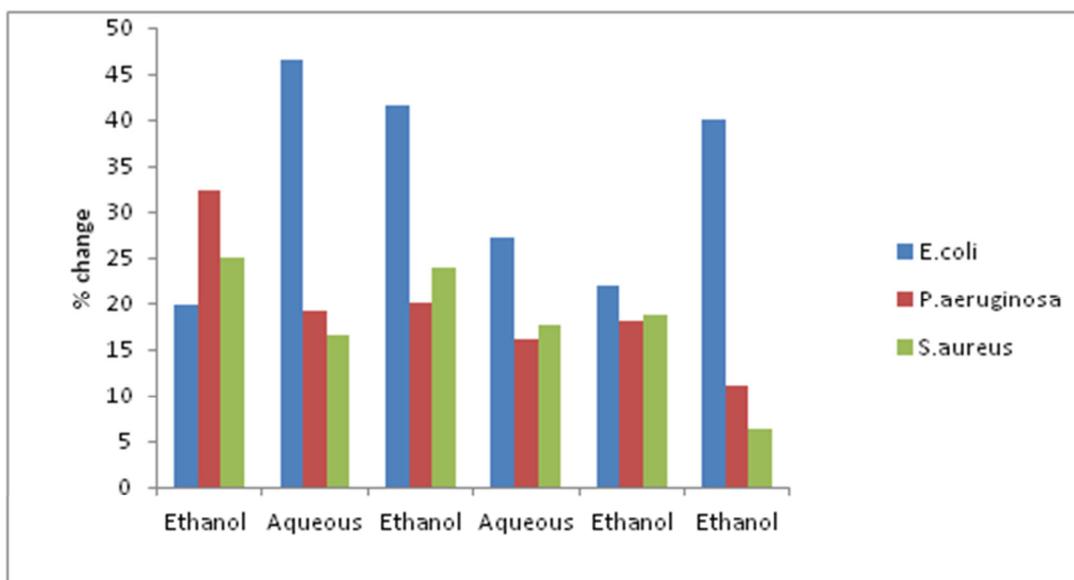


Figure 4
Percentage change in the zone of inhibition caused by ethanol leaf extract compared to methanol and aqueous extract against E. coli , P. aeruginosa and K. pneumonia

Table 1

Zone of Inhibition [mm] of E.coli by Ethanol ,Methanol and Aqueous leaf extract of Sesbania sesban 1) 50 µl 2) 100µl 3) 150µl [values are mean ±S. D of 3 individual observations]

S.NO	LEAF EXTRACT	Aqueous	Ethanol	Methanol
1	50µL	5.2±0.06 ^a	9.1±0.47 ^b	7.6±0.05 ^c
2	100µL	6.6±0.15 ^a	11.9±0.0 ^b	8.4±0.07 ^c
3	150µL	7.0±0.03 ^b	12±0.12 ^c	9.8±0.09 ^b

Values similarly marked are not significantly different from each other

*** Indicates significane at (P < 0.01)*

Table 2

Zone of inhibition [mm] of P.aeruginosa by Ethanol, Methanol and Aqueous leaf extract of S.sesban 1)50µl 2)100µl 3) 150µl [values are mean±S.D of 3 individual observations]

S.NO	LEAF EXTRACT	Aqueous	Ethanol	Methanol
1	50µL	7.6±0.02 ^a	11.0±0.12 ^b	8.3±0.04 ^c
2	100µL	8.6±0.05 ^a	12.0±0.0 ^b	10.0±0.15 ^b
3	150µL	9.9±0.09 ^b	13.7±0.27 ^c	11.0±0.23 ^a

Values similarly marked are not significantly different from each other

*** Indicates significane at (P < 0.01)*

Table 3

Zone of inhibition [mm] of K. pneumonia by Ethanol, Methanol and Aqueous leaf extract of S.sesban 1)50µl 2)100µl 3) 150µl [values are mean ± S.D of 3 individual observations]

S.NO	LEAF EXTRACT	Aqueous	Ethanol	Methanol
1	50µL	7.9±0.05 a	11.5±0.04 ^b	9.2±0.03 ^c
2	100µL	9.0±0.14	13.2±0.07 ^c	10.6±0.11 ^c
3	150µL	11.0±0.06c	13.9±0.02 ^c	11.7±0.04 ^a

Values similarly marked are not significantly different from each other

*** Indicates significane at (P < 0.01)*

DISCUSSION

Preliminary phytochemical screening revealed the presence of carbohydrates, tannins, saponins, glycosides, steroids, flavonoids, phenols, terpenoids, alkaloids, glycosides. saponins and they have been extensively used as detergents, pesticides as well as molluscicides in addition to their industrial application such as foaming, surface active agents etc and also found to have beneficial health effects¹¹. Flavonoids plays important role as scavengers of superoxide anions¹². Antimicrobial activity revealed that all the three leaf extracts of *Sesbania sesban* (Ethanol, Methanol and Aqueous) at three different concentration (50µl, 100µl, 150µl) showed zone of inhibition against *E.coli*, *P.aeruginosa* and *Klebsiella pneumoniae*. Among the three extracts tested in the present study, ethanol showed higher zone of inhibition than

methanol and aqueous extracts. Similar result reported by Alcohol extract¹³⁻¹⁵ showed maximum zone of inhibition against *Klebsiella pneumoniae* and *E.coli* when compared to chloroform extract¹⁶. Further the magnitude of inhibition of assessed pathogens by ethanol followed order of *Klebsiella pneumoniae*>*P.aeruginosa*>*E.coli* (Table 1.1, Table 1.2 & Table 1.3). Methanol and aqueous extract also showed similar pattern of inhibition against their pathogenic species. Based on the magnitude of inhibition, it is clear that methanol and aqueous extract of leaves are next to ethanol^{17,18}. The organic extracts of *Sesbania* could be further exploited as source of phytochemical compounds for pharmaceutical industry. Phenols are reported as anti tumour agents and exhibit antioxidant properties¹⁹The results of the study supports to a certain degree, the use of traditional medicinal plants in human and animal disease

therapy and reinforce the concept of ethno botanical approach in screening plants as potential sources of bioactive substances.

CONCLUSION

Herbals which form a part of our nutrition and provide us an additional therapeutic effect are in demand and *Sesbania* is one such plant. The first step towards this goal is the In Vitro antibacterial activity assay and in the recent years several reports have been available on the antibacterial activity of plant extracts on human pathogenic bacteria. Phytochemicals of leaf extract have antibiotic activity. The broad antibacterial activities could be as a result of the plant secondary metabolites like alkaloids, flavonoids, tannins, phytosterols etc., present in the extracts. Tannins had been widely used topically for sprains, bruises and superficial wounds as such, it could be probable that tannins and other plant phenols from the extract were responsible. In the present study, ethanol extract showed maximum zone of inhibition on all the selected pathogens. Based on the present study,

ethanol extract of *Sesbania* leaves potentially act as antimicrobial agent.

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AUTHOR'S CONTRIBUTION STATEMENT

M.Nirosha U.Haseena and K.Pavani conceptualized and gathered the data with regard to this work. A. Komala Priya and S. Jyothsna analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Satyavati GAK. Medicinal Plants of India. 2nd Editio. New Delhi: Indian council of Medical Research; 1987. 405-407 p.
2. Evans, D.O., and Macklin B (eds. Perennial sesbania production and use. Nitrogen fixing Tree Association; 1990. 4 p.
3. Eliopoulos GM, Cosgrove SE, Carmeli Y. The Impact of Antimicrobial Resistance on Health and Economic Outcomes. Clin Infect Dis. 2003;36(11):1433-7. DOI: 10.1086/375081
4. Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chem. 2007;103(2):381-8. DOI:10.1016/j.foodchem.2006.07.034
5. Saeio K, Chaiyana W, Okonogi S. Antityrosinase and antioxidant activities of essential oils of edible Thai plants. Drug Discov Ther. 2011;5(3):144-9. DOI:10.5582/ddt.2011.v5.3.144
6. Pace S, Burke TE. Intravenous Morphine for Early Pain Relief in Patients with Acute Abdominal Pain. Acad Emerg Med. 1996;3(12):1086-92. DOI:10.1111/j.1553-2712.1996.tb03365.x
7. Deftereos S, Giannopoulos G, Papoutsidakis N, Panagopoulou V, Kossyvakis C, Raisakis K, et al. Colchicine and the Heart. J Am Coll Cardiol. 2013;62(20):1817-25. DOI: 10.1016/j.jacc.2013.08.726
8. Sadasivam S and TB. Practical Manual (Undergraduate). 2nd Editio. Tamil Nadu Agricultural University, Coimbatore,; 1985.
9. CK. K. A textbook of practical pharmacognosy. 5th Editio. Vallabh Prakashan,; 2015.
10. Padhi L, Panda SK. Antibacterial activity of *Eleutherine bulbosa* against multidrug-resistant bacteria. J Acute Med. 2015;5(3):53-61. DOI:10.1016/j.jacme.2015.05.004
11. Raghvendra, D., Kushagra, D., Yasodha, K.J., Jayaveera KN. Comparative antimicrobial a studies of aqueous methanolic and saponins extract of seeds of *Trigonella foenumgraecum* on Human vaginal pathogens causing UTI infection. Der Pharma Chem. 2010;2(5):84-8.
12. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. J Ethnopharmacol. 1988;23(2-3):345. DOI:10.1016/0378-8741(88)90046-3
13. Packiyalakshmi PS, Premalatha R, Saranya A. Invitro Antimicrobial Activity of Leaf

- extracts from *Sesbania grandiflora*. Int J Curr Microbiol Appl Sci. 2016;5(4):21–7. DOI:10.20546/ijcmas.2016.504.004
14. MA. R. Pharmacological and Phytochemical Screenings of Ethanol Extract of *Sterculia villosa* Roxb. J Biomed Pharm Res. 2013;2(1):9–14.
15. Usman H, Osuji JC. Phytochemical And *In Vitro* Antimicrobial Assay Of The Leaf Extract Of *Newbouldia laevis*. African J Tradit Complement Altern Med. 2008;4(4):476. DOI:10.4314/ajtcam.v4i4.31240
16. Performance standards for antimicrobial disk susceptibility tests; Approved standard. National C. Wayne, USA.: Villanova, P.A. Publication,; 1993.
17. Ajayi IA, Ajibade O OR. Preliminary phytochemical analysis of some plant seeds. Res J Chem Sci. 2011;1(3):58–62.
18. Arjun P RJ. Antimicrobial activity of extracts of *sesbania grandiflora* leaf. :90–3. Available from: https://thepharmstudent.com/issue_2015/9.Patra_and_Joshi.pdf
19. Satish S, Raghavendra MP and RK. Evaluation of the antibacterial potential of some plants against human pathogenic bacteria. Adv Biol Res (Rennes). 2008;2(3–4):44–8.