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PHYTOCHEMICAL SCREENING STUDIES ON LEAF EXTRACT OF *SESBANIA GRANDIFLORA* (L)

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Abstract

Sesbania grandiflora Linn belonging to family Fabaceae is a well-recognized medicinal plant in numerous countries like India, Srilanka and Southeast Asia. The present study examines the phytochemical screening of leaves of *Sesbania grandiflora.* Preliminary phytochemical analysis revealed the presence of nine compounds such as alkaloids, flavonoids, phenol, quinones, saponins, steroids, proteins, tannins and terpenoids. A comparative antimicrobial activity of dried leaf extract was evaluated against four pathogenic strains names *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae.* The phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, quinones, steroids and proteins. Twenty compounds were observed in extract of petroleum ether and about thirty compounds were observed in extract of ethyl acetate by GC-MS. The result suggest that leaves are a rich source of valuable primary and secondary metabolites exhibiting the antimicrobial activity.

Key words: *Sesbania grandiflora, antibacterial, phytochemical, GC-MS.*

1. INTRODUCTION

For many centuries, plants have always been used as an important source of medicine for treating diseases in many societies. The knowledge of the various healing properties of plant has been transmitted in an empirical way (Thoshiki and Asaduzzaman, 2018). The medicinal properties shown by different medicinal plants are due to the phytochemicals/bioactive compounds present in the plant. Different phytochemicals have an extensive range of activities, which helps to enhance the immune system and give resistance against long term diseases to protect the body from harmful pathogens (Khalid et al, 2018).

Antioxidant, antimicrobial and anti-inflammatory properties of various extracts from many plants have recently been of great interest both in research and food industry, because of their possible use as natural additives to replace synthetic antioxidants and antimicrobials with natural ones. Drugs derived from plants already form a major part of the pharmacopeia of modern medicine. Thus, medicinal plants play an important

role in the development of newer drugs due to their less side effects and relatively low cost when compared to synthetic drugs (Neethu and Dhanyaraj, 2015). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti – infective agents (Amani et al., 1998).

Sesbania grandiflora is an Indian medicinal plant commonly known as Agathi is a widely available plant which belongs to family Fabaceae. It is an open branching tree tall up to 15 m and 39 cm in South or west India. Bark, leaves, gums and flowers have medicinal potential (Padmalochana and Dhana, 2014). Ethnobotanical investigations have shown that some species of *Sesbania* are used frequently and widely in traditional medicine to treat gastrointestinal infections, cardiovascular diseases and have antibacterial and anti-viral activities (Kalpana et al., 2012).

Various parts of *Sesbania grandiflora* are utilized in the treatment of various disorders, viz., bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox, sores, sore throat, anemia, bronchitis, ophthalmia, nasal catarrh, inflammation, leprosy, gout and rheumatism, stomatitis, antiulcer, swellings and tumors (Sarasu et al., 2016). *Sesbania grandiflora* possess rich source of phytoestrogens including flavonoids and other phyto-compounds such as beta-carotene, cyanidin, glycosides, leucocyanidin, neoxanthin, oleanolic acid, sesbanimide, tannin, violaxanthin, vitamins, zeaxanthin and saponins which on hydrolysis gave an acid sapogenin. The pharmacological properties, viz. anticancer, antiurolithiatic, hepatoprotective, anxiolytic, anticonvulsive, cardioprotective, anti-inflammatory, hypotensive, depressant, diuretic, hypoglycemic and hemolytic activity.

Considering these medicinal properties, the present study was intended to explore the presence of various phytochemicals and its antimicrobial activity of *Sesbania grandiflora* leaf against selected pathogenic bacterial strains like *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*, phytochemical screening including alkaloids flavonoids, phenol, quinones, saponins, steroids, tannins, terpenoids and protein using standard protocols and identification of the extracted compounds using GC-MS profiling.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The leaf from the mature plans of *Sesbania grandiflora* was collected from different localities of Thiruvananthapuram District, Kerala state during January- February months of 2022. They were washed with tap water 2-3 times followed by distilled water. The washed leaves were dried under shade. The dried leaves were made to fine powder with the help of mechanical blender. They are stored in air tight plastic container for the further studies with proper labelling. These leaf samples were subjected to phytochemical screening, antimicrobial screening and GC-MS.

2.2 Antibacterial Activity

2.2.1 Bacterial culture

Four strains of bacteria were used as the test microorganisms for the antibacterial study. They are Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* obtained from Dianova Microbiology Laboratory, Kottayam. All the strains were very carefully identified using standard microbiological methods. The cultures were sub cultured weekly and maintained in slants for further use.

2.2.2 Extraction by Soxhlet apparatus

The powdered leaves of Sesbania *grandiflora* were used for Soxhlet extraction process. The bioactive compounds from the dried leaves were extracted by using various solvents like petroleum ether, ethyl acetate, ethanol, methanol and water in Soxhlet extraction apparatus. A known amount of seed powder (50 g) was weighed, packed and placed in the Soxhlet apparatus along with 400 ml of different solvents (petroleum ether, ethyl acetate, ethanol, methanol and water) at their corresponding boiling points and run continuously for 4-5 hours (until almost no plant residues was left in the recycled solvents) respectively in order to get the bioactive compound **(**Reji and Rexin, 2013). After 5 hours of Soxhlet extraction the crude extract was collected by distillation and stored at 4^0 C. These stored plant extracts were tested for antimicrobial activity against four bacterial strains mentioned earlier.

2.2.3 Testing of antibacterial activity for plant extracts

Uniform quantity of sterilized nutrient agar medium was transferred into sterile petri dishes and allowed to solidify. Overnight broth cultures of each bacterial strain (1.0 ml) were added to the petri plate. Wells were made using cork borer on the solidified medium. These wells were filled with 50 µl of original crude extraction of each extract obtained by Soxhlet extraction methods. For control, different pure solvents like petroleum ether, ethanol, methanol, ethyl acetate and water used in place of leaf extracts. The Petri dishes were inverted and incubated at 37^0C for 24 hours. The diameters of the inhibition zones were measured in millimeters and their means were calculated with standard deviation.

2.3 Phytochemical Screening

2.3.1 Test for Alkaloids

To 2 mL extract, 2 ml of concentrated HCl was added followed by few drops of Mayer's reagent. Presence of yellow precipitate indicates the presence of alkaloid (Santhi and Lakshmi, 2011).

2.3.2 Test for Flavonoids

To 2 mL of extract, 1 mL of 2N sodium hydroxide was added. Presence of yellow colour indicates the presence of flavonoids (Evans and Trease, 2002).

2.3.3 Test for Phenols

To 1 mL of extract, 2 mL of distilled water followed by few drops of 10% Ferric chloride was added. Formation of blue or green colour indicates the presence of phenols (Pradeep et al., 2014).

2.3.4 Test for Quinones

To 1 mL of extract, 1mL of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones (Evans and Trease, 2002).

2.3.5 Test for Saponins

To 2 mL of extract, 2 mL of distilled water was added and shaken in a graduated cylinder for five minutes length wise. Formation of 1 cm layer of foam indicates the presence of saponins (Pradeep et al., 2014).

2.3.6 Test for Steroids

To 1 mL of extract add 1mL of chloroform and few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of Phyto steroids (Khan and Hussain, 2010).

2.3.7 Test for Tannins

To 1mL of extract, 2 mL of 5% Ferric chloride was added. Formation of dark blue or greenish black colour indicate the presence of tannins (Yusuf et al., 2013).

2.3.8 Test for Terpenoids

0.5 mL extract was treated with 2mL of chloroform and concentrated sulphuric acid. Formation of red brown colour indicates the presence of terpenoids (Siddiqui and Verma, 2009).

2.3.9 Test for Proteins

To 1 mL of extract, 1 mL of biuret reagent was added. Formation of violet colour indicates the presence of protein (Santhi and Lakshmi, 2011).

2.4 Gas Chromatography -Mass Spectrometry (GC-MS)

Based on the antibacterial analysis, the extracts which showed single, maximum peak was selected for GC- MS analysis. The GC-MS analysis was done at Department of Biosciences, MG University, Kottayam using GC-column Shimadzu model Qp2020.

Shimadzu's GC-MS QP2020 series with electron impact ionization mode and a Bpx5 GC column (length, 30 m; thickness, 0.25 m; diameter, 0.25 mm) were used to analyze the samples. Helium gas was used as carrier gas (99.999%) at a constant flow rate of 1 mL/min and an injection volume of 1 µL in size ratio of 10:1. The ion source temperature was 200 °C, while the injector temperature was 250 °C. The oven temperature progressed from 60 °C, maintained for 1 min, to 300 °C and stayed elevated for 30 min at a rate of 15 °C/min. The solvent delay was adjusted from 0 to 45 min, and the mass spectrophotometer was configured in positive electron ionization mode with an ionization energy of 70 eV. A scan interval of fragments from m/z 35 to 500 Dm was fragmented. The peak area/total peak area ratio was used to compute the relative percentage of each component. Lab Solution was the program utilized, while NIST Coin 4.0

(National Institute of Standards Technology) served as the library. The GC-MS analysis was done in School of Bioscience, MG University, Kottayam using Shimadzu model QP2020.

3. RESULTS

3.1 Antibacterial activity of leaves of *Sesbania grandiflora* under in vitro condition

The distilled solvent free crude plant extracts (50µ) were used for the study of antibacterial activity. The same amount of pure solvents was used as control. The four selected bacterial strains, *viz. Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used through the entire study. The inhibition zone measured in centimetres and the mean of inhibition zone was calculated using standard protocols.

3.1.1 Petroleum ether extract

The petroleum ether leaf extract of *Sesbania grandiflora* found to be effective against all tested bacterial strains indicated that it contains compounds that can inhibit the growth of all the strains. The maximum zone was showed by *Klebsiella pneumoniae* and has higher inhibition zone of 2.3 ± 0.2 cm and about 1.7 ± 0.5 cm inhibition zone was found in *Escherichia coli*. But both the strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed lowest inhibition zone of 1.6 ± 0.2 cm (Table 1 and Fig. 1).

3.1.2 Ethyl acetate extract

The ethyl acetate extracts also found to be effective against all tested bacterial strains and revealed the presence of compounds that can inhibit the growth of all the strains. The maximum zone was showed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* and was found to be 0.9 ± 0.35cm, while the *Escherichia coli* showed about 0.3 ± 0.1 cm. But *Klebsiella pneumoniae* showed lowest inhibition zone of 0.2 ± 0.05 cm (Table 1 and Fig. 1).

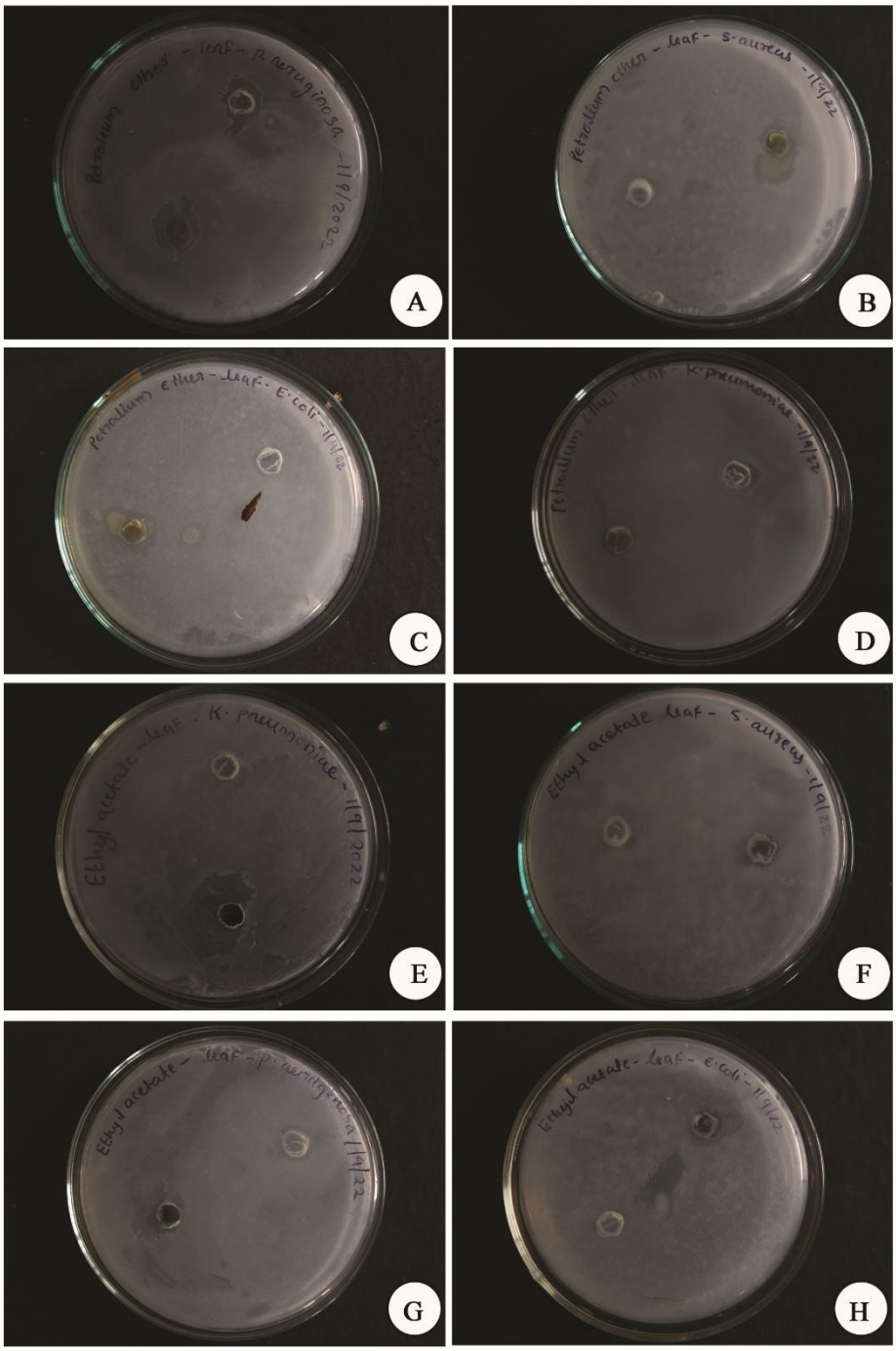


Figure 1: The antibacterial activity of petroleum ether and ethyl acetate extract of leaf against different bacterial strains. (A). petroleum ether extract of *S. aureus,* (B). petroleum ether extract of *P. aeruginosa*, (C). petroleum ether extract of *E. coli*, (D). petroleum ether extract of *K. pneumoniae*, (E). ethyl acetate extract of *S. aureus*, (F). ethyl acetate extract of *P. aeruginosa*, (G). ethyl acetate extract of *E. coli* and (H). ethyl acetate extract of *K. pneumoniae*.

3.1.3 Ethanol extract

The ethanol extract also inhibited the growth of all the strains. The maximum zone was showed by *Escherichia coli* and *Klebsiella pneumoniae* and was found to be 2.5 ± 0.1cm, while *Pseudomonas aeruginosa* showed a zone of inhibition 2.3 ± 0.2 cm. But *Staphylococcus aureus* showed lowest inhibition zone of 1.1 ± 0.2 cm (Table 1 & Fig. 2).

3.1.4 Methanol extract

Methanol extract of *Sesbania grandiflora* showed maximum inhibition zone about 2.6 ± 0 cm by *Staphylococcus aureus*. While *Klebsiella pneumoniae* showed 2.3 ± 0.05 cm and *Escherichia coli* showed a zone of inhibition of 2.0 ± 0.05 cm. But *Pseudomonas aeruginosa* showed the lowest inhibition zone of 1.0 ± 0.55 cm (Table 1 and Fig. 2).

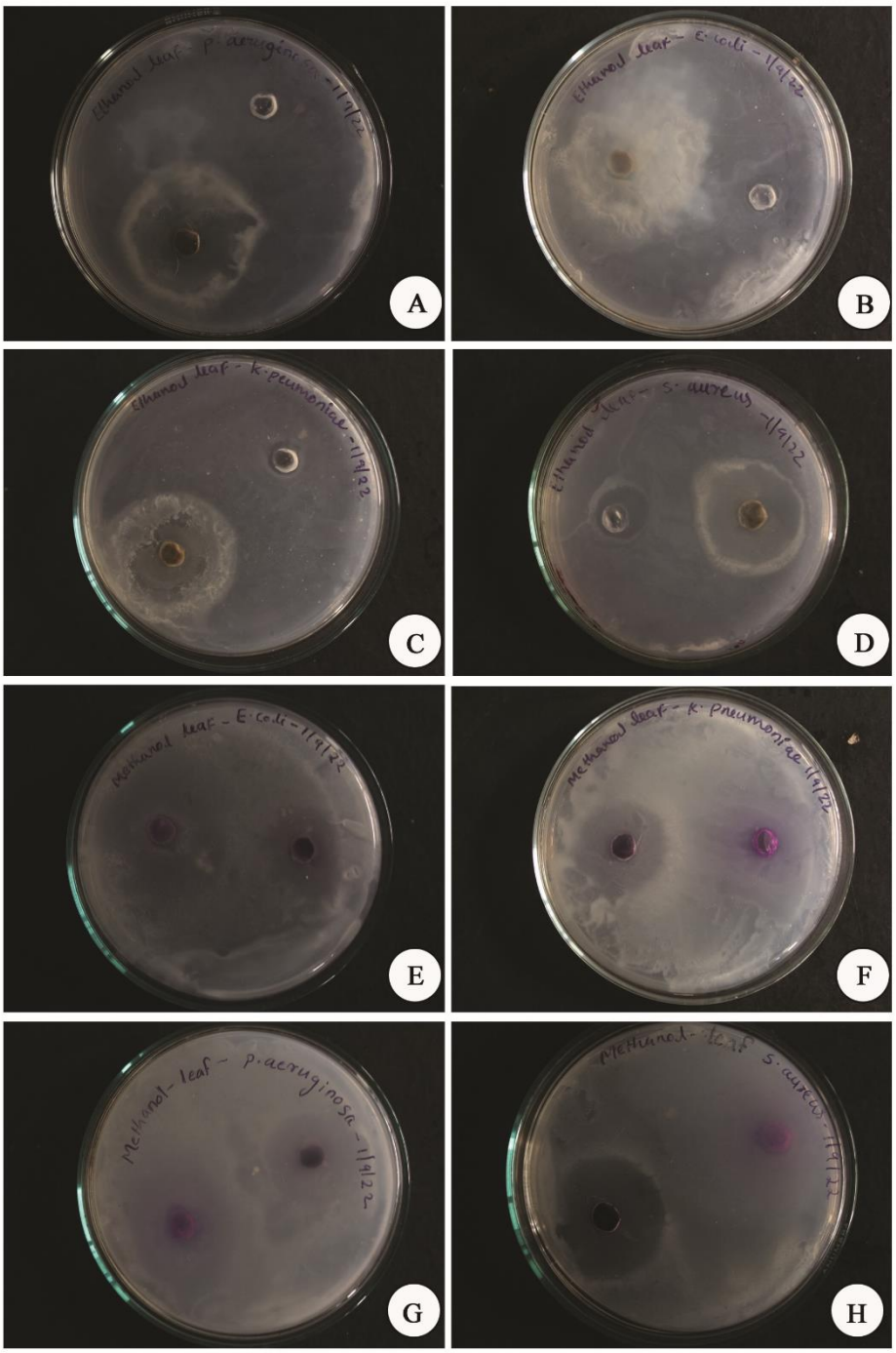


Figure 2: The antibacterial activity of ethanol and methanol extract against four different bacterial strains. (A). ethanol extract of *S. aureus*, (B). ethanol extract of *P. aeruginosa*, (C). ethanol extract of *E. coli*, (D). ethanol extract of *K. pneumoniae*, (E). methanol extract of *S. aureus*, (F). methanol extract of *P. aeruginosa*, (G). methanol extract of *E. coli* and (H). methanol extract of *K. pneumoniae*.

3.1.5 Water extract

While testing water extract, the maximum zone of inhibition was showed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* and was found to be 1.5 ± 0.1cm each, while the other two strains (*Escherichia coli* and *Klebsiella pneumoniae)* showed lowest inhibition zone of 1.2 ± 0.1cm each (Table 1 and Fig. 3).

Table 1: Diameter of zone of inhibition of different extracts against selected pathogenic strains.

Bacterial strains	Diameter of zone of zone of inhibition (cm)				
	Petroleum ether extract	Ethyl acetate extract	Ethanol extract	Methanol extract	Water extract
Escherichia coli	1.7 ± 0.5	0.3 ± 0.1	2.5 ± 0.1	2.0 ± 0.05	1.2 ± 0.1
Klebsiella pneumoniae	2.3 ± 0.2	0.2 ± 0.05	2..5 ± 0.1	2.3 ± 0.05	1.2 ± 0
Staphylococcus aureus	1.6 ± 0.2	0.9 ± 0.35	1.1 ± 0.2	2.6 ± 0	1.5 ± 0.1
Pseudomonas aeruginosa	1.6 ± 0.2	0.9 ± 0.35	2.3 ± 0.2	1.0 ± 0.55	1.5 ± 0.1

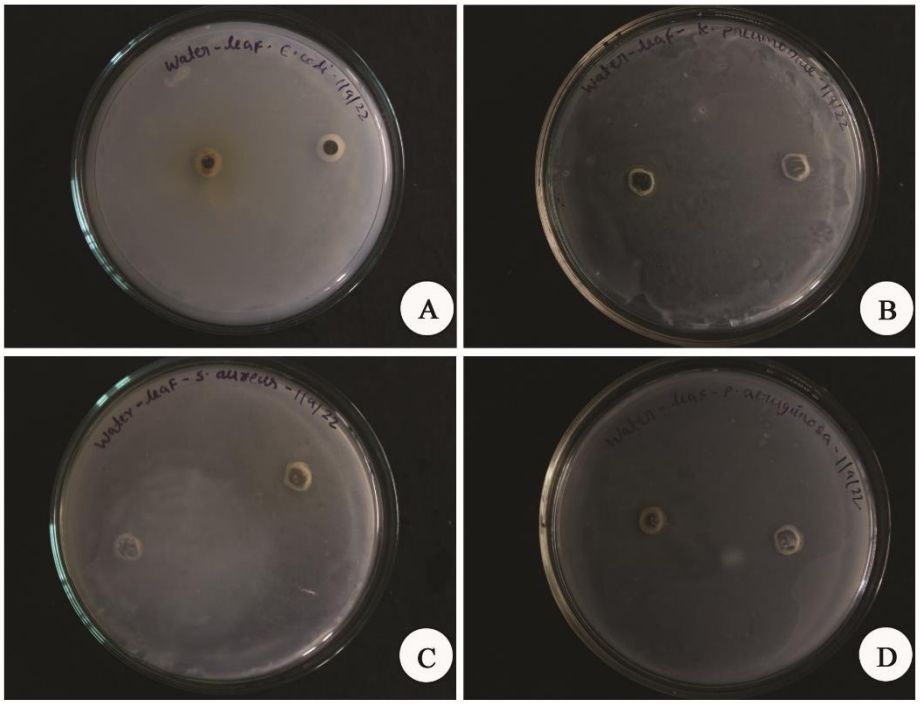


Figure 3: The antibacterial activity of water extract of leaf against four different bacterial strains. (A). *Staphylococcus aureus*, (B). *Pseudomonas aeruginosa*, (C). *Escherichia coli* and (D). *Klebsiella pneumoniae*.

3.2 Phytochemical Screening

The phytochemical screening of leaf extract of *Sesbania grandiflora* showed the presence of alkaloids, flavonoids, phenols, quinones, saponins, steroids, tannins, proteins and terpenoids. The results were shown in Table 2.

3.2.1 Petroleum ether extract

Compounds like alkaloids, flavonoids, phenols, quinones, saponins steroids, tannins, terpenoids and protein were absent in petroleum ether extract of leaf.

3.2.2 Ethyl acetate extract

The phytochemical analysis of ethyl acetate extract revealed the presence of alkaloids, flavonoids, phenols and tannins. While quinones, saponins, steroids, terpenoids and proteins are found to be absent.

3.2.3 Methanol extract

The phytochemical analysis of methanol extract of leaf showed the presence of alkaloids, phenols, quinones, saponins, steroids, tannins, terpenoids and proteins. While flavonoid was absent in it.

3.2.4 Ethanol extract

The phytochemical analysis of ethanol extract showed the presence of quinones, saponins, steroids, tannins, terpenoids and protein. While alkaloids, flavonoids and phenol are absent in it.

3.2.5 Water Extract

The phytochemical analysis of water extract of leaves showed the presence of phenol, quinones, steroids, tannins and terpenoids. While alkaloids, flavonoids, saponins, terpenoids and protein are absent.

Table 2: Results of phytochemical analysis

Test	Observation	Inference
Alkaloids	A yellow colouration observed in each extract indicated the presence of alkaloids.	Ethyl acetate and methanol extract of leaf showed the presence of alkaloids.
Flavonoids	A yellow colouration observed in each extract indicated the presence of flavonoids.	Only ethyl acetate extract of leaf showed the presence of flavonoids.
Phenol	Formation of blue or green colour indicates positive results for the presence of phenol.	Ethyl acetate, methanol extract and water extract of leaves showed the presence of phenol
Quinones	A red coloration indicates the presence of quinones.	Ethanol extract, methanol and water extract of leaves showed the presence of quinones.
Saponins	Formation of 1 cm layer of foam (appearance of creamy miss of small bubbles) indicates positive result for the presence of saponins.	Methanol and ethanol extract of leaf showed the presence of saponins.
Steroids	Appearance of brown ring indicates the presence of steroids	Methanol extract, ethanol and water extract of leaf showed the presence of steroids.
Tannins	Formation of a dark blue or greenish black colour indicates the positive result of tannins.	Ethanol, methanol, ethyl acetate extract and water extract of leaf showed the presence of tannins.
Terpenoids	A reddish-brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.	Methanol and ethanol extract showed the presence of terpenoids.
Proteins	A violet colouration observed in each extract indicated the presence of proteins.	Protein was found in methanol and ethanol extract of leaf.

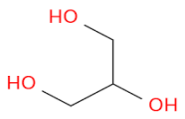
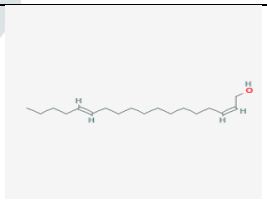
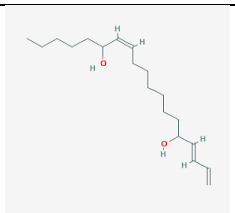
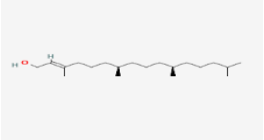
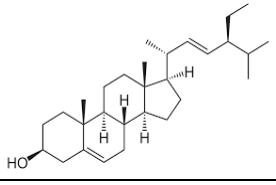
3.3 GC-MS ANALYSIS of *Sesbania grandiflora* Leaf extract

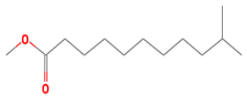
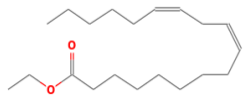
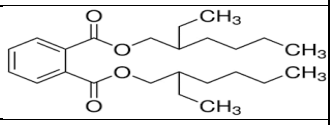
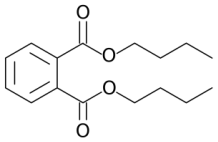
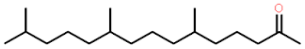
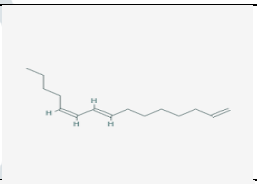
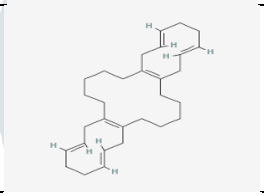
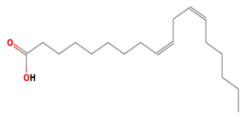
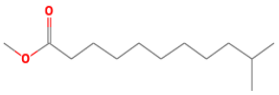
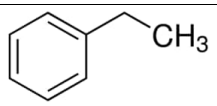
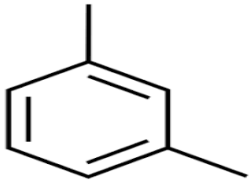
Based on antibacterial activities, the extracts which showed higher antibacterial activity (Ethyl acetate and ethanol) was subjected to GCMS analysis.

3.3.1 Ethyl acetate extract

GC-MS profile of ethyl acetate showed presence of 15 compounds (Table 3), belong to the category of acids, alcohols, terpenes, esters, alkenes and aromatic compounds. The identified compounds are viz, Ethylbenzene, Benzene,1,3-dimethyl, Glycerin, 6-Hydroxy-4, 4, 7-a-trimethyl-5, 6, 77, a, Neophyadiene, 2-Pentadecanone, 6, 10, 14-trimethyl, Undecanoic acid, 10-methyl-methyl ester, E, E, -Z1, 13-12-Nonadecatriene-5, 14-diol, Hexadecenoic acid, ethyl ester, Linoleic acid ethyl ester, Phytol, Tetra decanoic acid, 12-methyl-, methyl ester, (S)-, 9, 12-Octadecadienoic acid, ethyl ester, Neophytadiene, Myristic acid glycidyl ester, Henicosanal, 1, E-8, Z-10 Pendecatriene, Bis (2-ethylhexyl) phthalate, 1HNaphtho [2,1,b] pyron 8(4aH) one, 3 ethenyl decahydro 3, 4, Tricyclo [20.8.0.0(7,16)-triacontane, 1(22), 7(16)-diepoxy-, and Stigmasterol (Table 3 and Fig. 4). In the above, Stigmasterol, Henicosanal, Linoleic acid ester and phytol were reported earlier while others are new compounds reported from leaves.

Table 3: GCMS result analysis of Ethyl acetate

Groups of Phytochemicals	Name of compounds	Molecular Formula	Structure
Alcohols	Glycerin	$C_3H_8O_3$	
	Z, E, 2, 13, Oactadecadien-1-ol	$C_{18}H_{34}O$	
	E, E, Z13, 12 Nonadecatriene 5,14 diol	$C_2OH_{36}O$	
	Phytol	C_2OH_4OO	
	Stigmasterol	$C_{29}H_{48}O$	

Esters	Undecanoicacid,10, methyl, methyl	$C_{13}H_{26}O_2$	
	Linolic acid ethyl ester	$C_2OH_{36}O$	
	Bis (-2ethyl hexyl phthalate	C_6H_4Z	
	Dibutyl phthalate	$C_{16}HZZO_4$	
Terpenes	2, pentadecanone, 6, 10, 14-	$C_{18}H_{36}O$	
Alkenes	1-E-8-Z-10-pentadecatriene	$C_{15}H_{26}$	
	Tr1cyclo [20.8.0.0.0 (7, 16) triocontane, 1(22), 7(16) diepoxy-	$C_{30}H_{52}O_2$	
Acids	9, 12, Octadecadienoic acid	$C_{18}H_{32}O_2$	
	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	
	Tetradecanoid acid, 12-methyl-	$C_{16}H_{33}O_2$	
Aromatic compounds	Ethyl benzene	$C_6H_5CH_3$	
	Benzene,1, 3, dimethyl xylene)	$C_9H_{12}O$	

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GCMS RESULTS

Sample Information

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Analyzed	: 8/24/2022 7:13:12 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: E A EXT LEAF
Sample ID	: E A EXT LEAF
IS Amount	: [1]=1
Sample Amount	: 1
Vial #	: 11
Injection Volume	: 0.10
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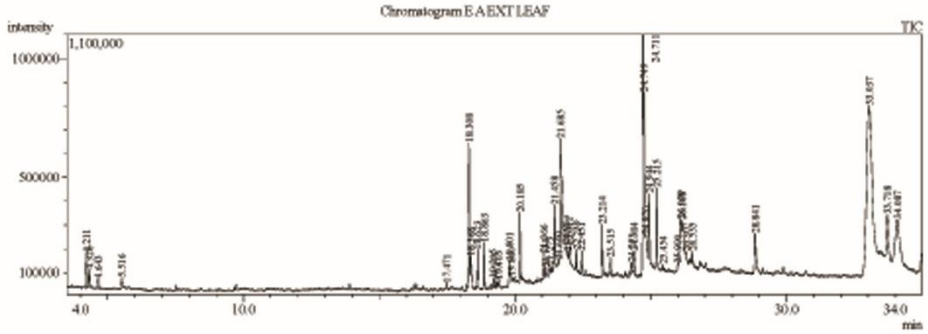


Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Base m/z	Name
1	4.211	285569	0.93	150016	1.72	1.90	91.00	Ethylbenzene
2	4.326	169626	0.55	74274	0.85	2.28	91.00	Benzene, 1,3-dimethyl-
3	4.643	83561	0.27	40591	0.47	2.06	91.00	Benzene, 1,3-dimethyl-
4	5.516	91959	0.30	45402	0.52	2.03	61.00	Glycerin
5	17.471	61896	0.20	27097	0.31	2.28	111.00	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(
6	18.308	1155172	3.76	616660	7.07	1.87	68.00	Neophytadiene
7	18.368	356223	1.16	134113	1.54	2.66	58.00	2-Pentadecanone, 6,10,14-trimethyl-
8	18.623	331694	1.08	163898	1.88	2.02	148.95	Z,E-2,13-Octadecadien-1-ol
9	18.863	400016	1.30	197887	2.27	2.02	81.05	Neophytadiene
10	19.205	91227	0.30	45532	0.52	2.00	148.95	Dibutyl phthalate
11	19.277	61697	0.20	31570	0.36	1.95	58.00	2-Pentadecanone, 6,10,14-trimethyl-
12	19.403	81077	0.26	33769	0.39	2.40	74.00	Undecanoic acid, 10-methyl-, methyl ester
13	19.801	250560	0.82	101161	1.16	2.48	73.00	n-Hexadecanoic acid
14	19.860	66202	0.22	26702	0.31	2.48	148.95	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
15	20.185	523509	1.70	292051	3.35	1.79	88.00	Hexadecanoic acid, ethyl ester
16	21.066	179369	0.58	105772	1.21	1.70	67.00	Linoleyl methyl ketone
17	21.133	65633	0.21	39097	0.45	1.68	55.00	E,E-3,13-Octadecadien-1-ol
18	21.273	94183	0.31	42685	0.49	2.21	67.00	Linolenic acid ethyl ester
19	21.458	497738	1.62	266626	3.06	1.87	71.00	Phytol
20	21.600	59289	0.19	25413	0.29	2.33	74.00	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-
21	21.685	2791905	9.08	533865	6.12	5.23	67.00	9,12-Octadecadienoic acid (Z,Z)-
22	21.863	372823	1.21	87801	1.01	4.25	67.00	Hexadecanoic acid, 2-bromo-
23	21.953	118085	0.38	52977	0.61	2.23	67.00	9,12-Octadecadienoic acid, ethyl ester
24	22.011	160565	0.52	78188	0.90	2.05	79.00	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
25	22.259	169401	0.55	89817	1.03	1.89	88.00	Octadecanoic acid, ethyl ester
26	22.451	167235	0.54	98428	1.13	1.70	68.05	Neophytadiene
27	23.214	386041	1.26	215414	2.47	1.79	57.00	Myristic acid glycidyl ester
28	23.515	139677	0.45	77454	0.89	1.80	82.05	Hexacosanal
29	24.293	104944	0.34	55217	0.63	1.90	67.00	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
30	24.384	217541	0.71	103618	1.19	2.10	82.05	Hexacosanal
31	24.711	2926092	9.52	1576036	18.06	1.86	67.00	9,12-Octadecadienoyl chloride, (Z,Z)-
32	24.749	1686484	5.49	762963	8.74	2.21	129.00	9-Octadecenoic acid (Z)-, oxiranylmethyl ester

Figure 4: GC-MS analysis of ethyl acetate extract of leaf of *Sesbania grandiflora*.

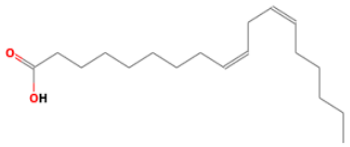
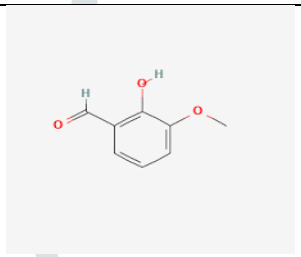
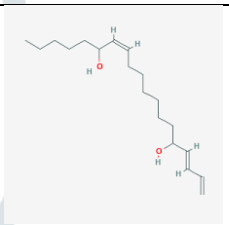
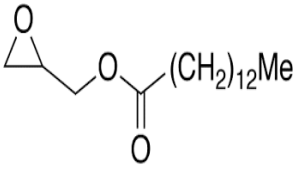
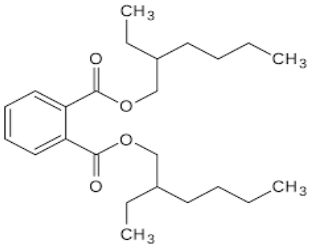
3.3.2 Ethanol extract

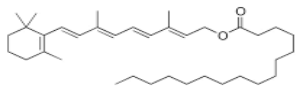
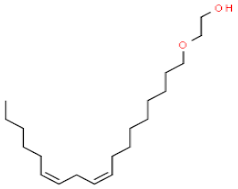
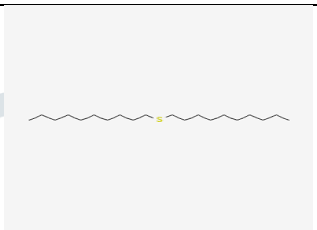
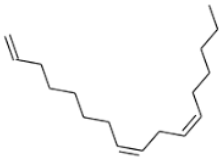
The GCMS analysis of ethanol extract of the leaves showed the presence of 14 compounds. They belong to the category of acids, ethers, alkenes and acyclic hydrocarbons. They are 9, 12- Octadecadienoic acid (Z, Z)-, Myristic acid glycidyl ester, 1,8, 11-Heptadecatriene, (Z, Z)-, 9-Octadecenoic acid (Z), Oxiranyl methyl ester, 9, 12-Ocadecadienoyl chloride, (Z, Z)-, Bis(2-ethyhexyl) Phthalate, Linoleyl palmitate, (9, Z,1, Z, Z)-1-Hydroxy-3-methoxypropan, 2ylOctadeca9, 12die, Decyl Sulfide, Ethanol, 2- (9, 12-Octadecadienyloxyl-(Z, Z) and E, E, Z-1, 3, 12-Nanadecatriene-514 -diol (Table 4 and Fig. 5).

Among the compounds identified, Decyl sulfide, 9, 12-octadecadienol Chloride, Linoleyl Palmitate and Bis(2-ethylhexyl) Phthalate were reported earlier. While the others are new reports.

The GCMS results of ethyl acetate and ethanol extract of leaf of *Sesbania grandiflora* showed the presence of some common compounds that are 9, 12-Octadecadienoic acid (Z, Z)-, Myristic acid glycidyl ester, 9,12-Octadecadienoyl chloride, (Z, Z), Bis(2-ethyhexyl) Phthalate, Linoleyl Palmitate and E, E, Z-1, 3, 12-nanadecatriene-5-14-diol.

Table 4: GCMS result analysis of Ethanol

Group of phytochemicals	Name of compounds	Molecular formula	Structure
Acids	9, 12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	
	9, 12-Octadecadienoyl chloride, (Z, Z)-	$C_{18}H_{28}O_2$	
	(9, Z,1, Z, Z)-1-Hydroxy-3-methoxy propan-2-yl octadeca-9,12-die	$C_{22}H_4OO_4$	
	E, E, Z-1, 3, 12-Nonadecatriene-5-14-diol	$C_{19}H_{34}O_2$	
Esters	Myristic acid glycidyl ester	$C_{14}H_{28}O_2$	
	9, 12 Octadecadinoyl chloride, (Z, Z)	$C_{18}H_{31}CO$	
	Bis(2-ethylhexyl) phthalate	C_6H_{42}	

	Linoleyl palmitate	$C_{34}H_{62}O_2$	
Ether	Ethanol, 2 (9, 12 Octadecadienyloxy) (Z, Z)	$C_2OH_{38}O_2$	
Aromatic hydrocarbon	Decyl sulfide	$C_2OH_{42}S$	
Acyclic hydrocarbon	1,8,11-Heptadecatriene, (Z, Z)-	$C_{17}H_3O$	

MAHATMA GANDHI UNIVERSITY

SCHOOL OF BIOSCIENCES

GCMS RESULTS

Sample Information

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Analyzed	: 8/24/2022 6:32:50 PM
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Sample ID	: E EXT LEAF
IS Amount	: [1]=1
Sample Amount	: 1
Vial #	: 10
Injection Volume	: 0.10
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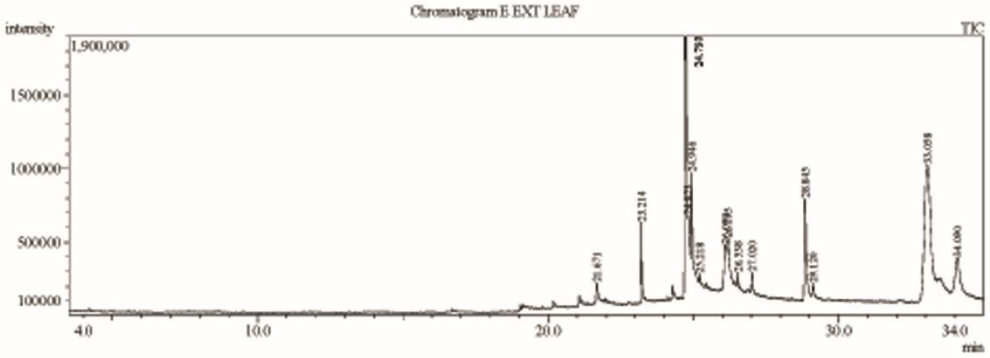
Method File	: F:\Renjith Mahasenan\Auto Injector split 10.qgm



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Base m/z	Name
1	21.671	428505	1.19	129964	1.32	3.30	67.00	9,12-Octadecadienoic acid (Z,Z)-
2	23.214	1045443	2.90	542410	5.52	1.93	57.00	Myristic acid glycidyl ester
3	24.717	6529006	18.10	3403701	34.63	1.92	67.00	1,8,11-Heptadecatriene, (Z,Z)-
4	24.750	4048240	11.22	1862119	18.95	2.17	129.00	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
5	24.823	2313801	6.41	481317	4.90	4.81	67.00	9,12-Octadecadienoyl chloride, (Z,Z)-
6	24.946	1616858	4.48	710890	7.23	2.27	57.00	Myristic acid glycidyl ester
7	25.218	114824	0.32	63655	0.65	1.80	148.95	Bis(2-ethylhexyl) phthalate
8	26.099	1131216	3.14	278724	2.84	4.06	55.00	Linoleyl palmitate
9	26.195	1812895	5.03	294336	2.99	6.16	117.00	(9Z,12Z)-1-Hydroxy-3-methoxypropan-2-yl octadeca-9,12-die
10	26.538	206815	0.57	101260	1.03	2.04	57.00	Myristic acid glycidyl ester
11	27.020	381845	1.06	144991	1.48	2.63	172.95	Decyl sulfide
12	28.845	2330667	6.46	642705	6.54	3.63	67.00	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-
13	29.120	231821	0.64	84855	0.86	2.73	172.95	Decyl sulfide
14	33.058	11552425	32.03	853717	8.69	13.53	67.00	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-
15	34.090	2324931	6.45	234125	2.38	9.93	81.00	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
		36069292	100.00	9828769	100.00			

Figure 5: GC-MS analysis of ethanol extract of leaf of *Sesbania grandiflora.*

4. DISCUSSION

In the present study leaf extract of *Sesbania grandiflora* was subjected to analysis of antimicrobial activity, phytochemical screening and GCMS analysis. The present study has been made an attempt, to analyze different phytochemicals present in leaves while using different solvents. The antimicrobial activity was studied against four different pathogenic strains namely *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus.* The results revealed that the methanol extract has the highest zone of inhibition against *Staphylococcus aureus* (about 2.6 cm of inhibition zone) followed by *Klebsiella pneumoniae* (about 1cm of inhibition zone) (Table 1 & Fig. 1)

Ethanol extract was also found to be effective against all the bacterial strains and the maximum zone of inhibition was showed by *Escherichia coli* and *Klebsiella pneumoniae* (about 2.5 cm inhibition found in each, Table 1 & Fig. 1). The petroleum ether extract also has inhibition against all strains of bacteria, while *Klebsiella pneumonia* has the maximum inhibition (about 2.3 cm inhibition zone) followed by *Staphylococcus aureus*, about 1.6 cm of inhibition zone (Table 1 & Fig. 2). Water extract of leaves also

showed maximum inhibition against the pathogenic strains. Maximum inhibition found in both *Staphylococcus aureus* and Pseudomonas aeruginosa (about 1.5 cm inhibition zone showed by each) (Table 1 & Fig. 3) In Ethyl acetate extract, maximum inhibition was showed by both *Staphylococcus aureus* and *Pseudomonas aeruginosa,* about 0.9 cm inhibition zone but *Klebsiella pneumoniae* showed lowest zone of inhibition compared to all other extractions that was about 0.2 cm (Table 1 & Fig. 1). These results were comparable with some previous research reports.

Anantaworasukal et al., 2011 reported that ethanol extracts of *Sesbania grandiflora* was effective against the pathogenic strains like *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Gandhi et al., 2017 also reported that water extract of *Sesbania grandiflora* was effective against *Staphylococcus aureus*. Lakshmi et al., 2011 reported that ethanol extract of *Sesbania grandiflora* was found to be effective against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* They also reported that water extract was more effective against the pathogenic strains like *Staphylococcus aureus* and *Escherichia coli*.

In this preliminary study, the phytochemical analysis of *Sesbania grandiflora* leaves showed the presence of several phytochemicals. Ethyl acetate extract showed the presence alkaloids, flavonoids, phenols and tannins. Methanol extract showed the presence of alkaloids, phenols, quinones, saponins, steroids, tannins, terpenoids and proteins. Ethanol extract showed the absence of alkaloids, flavonoids and phenols. In water and petroleum ether extract, all the tested phytochemicals were found to be absent (Table 2).

Some previous reports revealed the presence of phytochemicals and it is found that the plant is highly medicinal. Arun et al., 2014 reported the presence of alkaloids in water, methanol and ethanol extract of *Sesbania grandiflora*. They reported the presence of steroids in methanol and ethanol extract, presence of saponins in methanol and ethanol and in water, methanol and ethanol extract showed the presence of tannins. Patil et al., 2021 reported the presence of steroids, alkaloids and terpenoids in petroleum ether extract of *Sesbania grandiflora*. They also reported the presence of alkaloids, flavonoids, tannins and saponins in methanol extract.

In the present study, the GCMS results of ethyl acetate and ethanol extract showed the presence of several phytochemicals and is relevant to the current scenario of ayurvedic medicine. The GCMS result of ethanol extract showed the presence of 14 compounds. The compounds belong to the category of acids, ethers, alkenes and acyclic hydrocarbons. These compounds include 9, 12-Octadecadienoic acid (Z, Z)-, Myristic acid glycidyl ester, 1, 8, 11-Heptadecatriene, (Z, Z)-, 9-Octadecenoic acid (Z), Oxiranyl methyl ester, 9, 12 - Ocadecadienoyl chloride, (Z, Z)-, Bis (2-ethyhexyl) Phthalate, Linoleyl palmitate, (9, Z,1, Z, Z)-1-Hydroxy-3-methoxypropan, 2yl Octadeca 9, 12 die, Decyl Sulfide, Ethanol, 2-(9, 12-Octadecadienyloxyl-(Z, Z) and E, E, Z-1, 3, 12-Nanadecatriene-514 -diol. The results were shown in the Table 3 and Fig. 4. Among the compounds identified, Decyl sulfide, 9, 12-octadecadienol Chloride, Linoleyl Palmitate and Bis(2-ethylhexxyl) Phthalate were reported earlier. While the others are new report from leaves.

GC-MS profile of ethyl acetate showed presence of above 15 compounds. The compounds belong to the category of acids, alcohols, terpenes, esters, alkenes and aromatic compounds. The identified compounds are viz, Ethylbenzene, Benzene, 1, 3-dimethyl, Glycerin, 6-Hydroxy-4, 4, 7-a trimethyl-5, 6, 77, a-, The Neophyadiene, 2-Pentadecanone, 6, 10, 14-trimethyl, Undecanoic acid, 10-methyl-methyl ester, E, E,-Z1, 13-12-Nonadecatriene-5, 14-diol, Hexadecenoic acid, ethyl ester, Linoleic acid ethyl ester, Phytol, Tetra decanoic acid, 12-methyl-, methyl ester, (S)-, 9, 12-Octadecadienoic acid, ethyl ester, Neophytadiene, Myristic acid glycidyl ester, Henicosanal, 1, E, 8, Z10 Pendecatriene, Bis (2 ethyl hexyl phthalate, 1H Naphtho [2, 1, b] pyron 8(4aH) one, 3 ethenyl decahydro 3, 4, Tricyclo [20.8.0.0(7, 16) triacontane, 1(22), 7(16) diepoxy-, and Stigmasterol. The results were shown in Table 4 and Fig. 5. In which Stigmasterol, Henicosanal, Linoleic acid ester and phytol was reported earlier while others are found to be new compounds. While compared with both the GC-MS results of ethyl acetate and ethanol showed the presence of some common compounds that are 9-12-Octadecadienoic acid (Z, Z), Myristic acid glycidyl ester, 9-Octadecenoic acid (Z)-Oxiranyl methyl, 9, 12-Octadecadienoyl chloride (Z, Z), Bis(2-ethylhexyl) phthalate, E, E, Z-1, 3, 12-nanadecatriene-5 were found.

Many reports indicate the presence of secondary metabolites in *Sesbania grandiflora*. Hussain and Sukumaran, (2014) reported the presence of 3, 4, 5-trimethoxyphenol, Erucic acid, Phytofluene, 2-furancarboxaldehyde, Nonanoic acid, methyl ester, Acrylonitrile 4-methyloxazole,1-propanol-2-methyl-, 3-hexene-2-one, 3, 4-dimethyl, Urea, Hexadecenoic acid, 9-hexadecenol, dioctyl ester, Malonic acid, Ethyl 1, 3-hexy ester. Sein et al., (2019) reported the presence of 3, 4, 5-Trimethoxy phenol, Erucic acid, 2-Furan carboxaldehyde, Vitamin E acetate, 4-Methyloxazole, palmitic acid, 9-hexadecenol and dioctyl ester are major compounds in leaves of *Sesbania grandiflora* by GC-MS analysis.

5. CONCLUSION

The plant *Sesbania grandiflora* belongs to Fabaceae is a well-recognized medicinal plant in India and Southeast Asia. The plant has numerous medicinal properties and it is effective against several diseases like tumors, swellings, antiulcer, leprosy, Inflammation, bronchitis, nasal catarrh, sore throat anemia, small pox sores, bruises, dysentery, fever, headache, etc.

The preliminary study focused on the antibacterial and phytochemical analysis of leaves of the plant. Leaf extract in ethyl acetate, ethanol, methanol and water extract reported the presences of secondary metabolites like alkaloids, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids and protein, while all of them are absent in petroleum ether extract. The antibacterial activity was evaluated against different pathogenic bacterial strains like *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli.* The maximum inhibition zone was found in methanol extract against *Pseudomonas aeruginosa* (2.6 cm inhibition zone) and lowest inhibition was found in ethyl acetate extract against Klebsiella *pneumoniae* (0.2 cm inhibition zone). Based on this data the extracts were subjected to qualitative phytochemical analysis of alkaloids, flavonoids, phenols, quinones, saponins, steroids. The qualitative phytochemical analysis was further confirmed by GC-MS.

The GCMS was done in ethyl acetate and ethanol extracts and the results revealed the presence of secondary metabolites such as compounds, *viz.*, Ethyl benzene, Benzene, 1, 3-dimethyl, Gycerin, 6-Hydroxy-4, 4, 7-a-trimethy 5, 6, 7, a, Neophyadiene, 2-Pentadecanone, 6, 10, 14-trimethyl, Undecanoic acid, 10-methyl-methyl ester, E, E, -Z1, 13-12- Nonadecatriene-5, 14-diol, Hexadecenoic acid, Ethyl ester, Linoleic acid, Phytol, Tetra decanoic acid, 12-methyl-, methyl ester, (S)-, 9, 12-Octadecadienoic acid, ethyl ester, Neophytadiene, Myristic acid glycidyl ester, Henicosanal, 1, E-8, Z-10-Pendecatriene, Bis(2-ethylhexyl) phthalate, 1H-Naphtho [2, 1, b] pyron-8 (4aH)-one, 3-ethenyl decahydro3, 4, Tricyclo [20.8.0.0 (7,16)-triacontane, 1(22), 7(16)-diepoxy- and Stigmasterol in ethyl acetate and compounds include 9, 12-Octadecadienoic acid (Z, Z)-, Myristic acid glycidyl ester, 1, 8, 11-Heptadecatriene, (Z, Z)-, 9-Octadecenoic acid (Z), Oxiranyl methyl ester, 9, 12-Ocadecadienoyl chloride, (Z, Z)-, Bis (2-ethyhexyl) Phthalate, Linoleyl Palmitate, (9Z, 1ZZ)-1-Hydroxy-3-methoxy propan, 2-yl Octadeca-9, 12-die, Decyl Sulfide, Ethanol, 2-(9, 12-Octadecadienyloxyl-(Z, Z). E, E, Z-1, 3, 12-Nanadecatriene -5-14-diol in ethanol extract. Those compounds are belonging to alcohols, esters, ethers, acids, aromatic compounds, acyclic hydrocarbons, terpenoids, etc.

The results revealed, that the plant is highly medicinal and is effective against various pathogenic bacteria, so it is clear that we can effectively use *Sesbania grandiflora* leaves to treat against various pathogenic diseases. The results also suggested that the leaves are composed of rich source of valuable primary and secondary metabolites exhibiting antimicrobial activities.

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