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Phytochemical and antibacterial studies on seeds of *Sesbania grandiflora* Linn.: A novel report

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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 constituents and antimicrobial activity of seed of *Sesbania grandiflora.* The quantitative examination of numerous phytochemicals was analyzed in different solvent systems including Petroleum ether, Ethyl acetate, Ethanol, Methanol and Water. 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 seed extract was evaluated against four pathogenic strains names *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae.* Among the extracts studied, petroleum ether and ethyl acetate show highest antimicrobial activity. 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 the seeds are a rich source of valuable primary and secondary metabolites exhibiting the antimicrobial activity and no further studies were reported yet.

Keywords: *Sesbania grandiflora*, antibacterial, phytochemical, GC-MS

Introduction
Medicinal plants continue to be an important source of lifesaving drugs for humankind, especially in the developing nations since pre-historic times and continue to play an essential role in health care. The World Health Organization has estimated that more than 80% of the world population in developing countries depends primarily on herbal medicine for basic health care (Vines 2004) [1]. They synthesise hundreds of chemical compounds for functions including defence against insects, fungi, disease and herbivorous mammals. Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind; a great deal of effort has therefore focused on using available experimental techniques to identify natural antioxidants from plants (Krishnaiah *et al*., 2011) [2]. Though numerous phytochemicals with potential or established biological activity have been identified, the use of single plant which contains widely diverse phytochemicals, the effects are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety. Medicinal plants are used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine.

Phytochemicals are a wide variety of non-nutritive naturally occurring chemical compounds found in plant foods and are biologically active. They which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's colour, aroma and flavour (Koche *et al*., 2016) [3]. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant (Gibson *et al*., 1998) [4].

The present work has been undertaken to investigate the phytochemical properties and medicinal importance of the genus *Sesbania grandiflora*. The plant is commonly known as vegetable hummingbird, katurai, Agati or West Indian pea, is a small leguminous tree native to Maritime Southeast Asia and Northern Australia. It has edible flowers and leaves commonly eaten in Southeast Asia and South Asia (Orwa *et al*., 2009) [5]. *Sesbania grandiflora* is a fast-growing perennial, deciduous or evergreen legume tree, grows up to 10-15 m height.

Its lifespan is about 20 years. Its roots are heavily nodulated and some floating roots may develop in waterlogged conditions. The trunk is straight with few branches. The leaves, up to 30 cm long, are pinnately compound. The flowers are white, yellowish, pink or red and borne in axillary racemes. The pods are 50-60 cm long, glabrous and indehiscent and hang vertically. They contain 15 to 50 dark brown seeds, 5 mm long and 2.5-3 mm broad (Orwa *et al*., 2009) [5].

Leaves of *Sesbania grandiflora* can possibly be utilized as a remedy for thrombosis, diarrhoea and inflammatory diseases and against couple of significant bacterial pathogens. The juice of the leaves of *S. grandiflora* has been purportedly utilized in the treatment of bronchitis, cough, vomiting, wounds ulcers, diarrhoea and dysentery. The flowers have revealed antimicrobial activity (Julie *et al*., 2016) [6]. Powdered roots of this plant are mixed in water and applied externally as a poultice or rub for rheumatic swelling. The leaves are traditionally used to treat nasal catarrah, nyctalopia and cephalagia. Studies demonstrated that, *S. grandiflora* have antioxidant, antiuroithiatic, anticonvulsive, anti-ligament, anti-inflammatory, anti-helminthic, antibacterial and anxiolytic activity (Julie *et al*., 2016) [6].

Considering the above facts, the main objectives of the study are: to check the antimicrobial activity of seed extract of *Sesbania grandiflora* against selected bacterial pathogenic species like *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa;* to extract chemical constituents using solvents like petroleum ether, ethyl acetate, ethanol, methanol and water; Phytochemical screening of seed extract of *Sesbania grandiflora* including alkaloids flavonoids, phenol, quinones, saponins, steroids, tannins, terpenoids and protein using standard protocols and identification of the extracted compounds using GC-MS profiling. This is the first report on the seeds of *Sesbania grandiflora*.

Materials and Methods

The plant *Sesbania grandiflora* (L.) belongs to the family Fabaceae is used for the study. The seeds of the plant were collected from localities of Thiruvananthapuram District, Kerala, India during 12 June 2022. The seeds were subjected to phytochemical and antimicrobial screening and GCMS.

Antibacterial Activity

Bacterial culture

Four strains of bacteria were used as the test microorganisms for the antibacterial study. The bacterial strains included are Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were obtained from Dianova microbiology laboratory, Kottayam. All the strains were identified carefully using standard microbiological methods. The cultures were sub cultured weekly and maintained in slants for further use. The bacterial strains were maintained on nutrient agar medium (HiMedia). Overnight cultures of the bacterial strains were prepared in nutrient broth, and then incubated for 24 h at 37 °C.

Extraction by Soxhlet apparatus

The seeds of *Sesbania grandiflora* were air dried under shade at normal temperature then it was powdered in a mixer grinder and used for Soxhlet extraction process. The known volume of seed powder (58 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 & Rexin 2013) [7]. After 5 hours of Soxhlet extraction the crude extract was collected by distillation and was stored at 40 C. These stored plant extracts were tested for antimicrobial activity against four bacterial strains are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia.*

Testing of antibacterial activity for plant extracts

The sterilized medium was transferred into sterile petri dishes and allowed to solidify under a laminar air flow. From the overnight broth cultures 1ml. of each bacterial strain was added to the Petri plate. Wells were made using corn borer on the solidified medium. Prepared wells are filled with 50 µl of original crude extraction of each extract obtained by Soxhlet extraction methods. For control, the solvents were used instead of plant extracts. The bacterial cultures used are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Incubation and measurement of zones

The Petri plates were inverted and incubated at 370 C for 24 hours. The diameters of the inhibition zones were measured in millimeters and their means were calculated.

Statistical Analysis

All the values of the experimental results were expressed as mean±standard error of mean (SEM).

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 & Lakshmi, 2011) [8].

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 2002) [9].

Test for Phenol

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

Test for Quinones

To 1 mL of extract, 1 mL of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones (Evans 2002) [9].

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) [10].

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 & Hussain, 2010) [11].

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) [12].

Test for Terpenoids

0.5 mL extract was treated with 2 mL of chloroform and concentrated sulphuric acid. Formation of red brown colour indicates the presence of terpenoids (Siddiqui & Verma, 2009) [13].

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 & Lakshmi, 2011) [8].

Gas Chromatography-Mass Spectrometry (GC-MS)

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 1µL 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.

Results

Antibacterial activity of seeds of *Sesbania grandiflora* under *in vitro* condition

The distilled solvent free crude plant extracts were used for the whole study and 50 µL of extracts were used subsequently for checking the antibacterial activity. Pure solvents (50 µL) were used as control. The four selected bacterial strains *viz. Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used in the study for checking the antibacterial activity.

Petroleum ether extract

The petroleum ether extract of *Sesbania grandiflora* seeds found to be effective against all tested bacterial strains. The results revealed that the petroleum ether extract contains compounds that can inhibit the growth of all the strains. The maximum inhibition zone 2.3±0.4 cm was showed by *Pseudomonas aeruginosa*, while the other two strains (*Klebsiella pneumonia* and *Escherichia coli*) showed a zone of inhibition of 2.1±0.6 cm and 2.0±0.05 cm respectively. But *Staphylococcus aureus* showed lowest inhibition zone of 1.9±0.35 cm (Table 1 and Fig. 1).

Ethyl acetate extract

The ethyl acetate extracts also found to be effective against all tested bacterial strains. The results revealed that the ethyl acetate extract contains compounds that can inhibit the growth of all the strains. The maximum inhibition zone was showed by *Pseudomonas aeruginosa* and was found to be 3.1±0.6 cm, while the other two strains (*Escherichia coli* and *Klebsiella pneumoniae)* showed an inhibition zone of 2.75±0.65 cm and 2.95±0.15 cm respectively. But *Staphylococcus aureus* showed lowest inhibition zone of 2.55±0.90cm (Table 1 and Fig. 1).

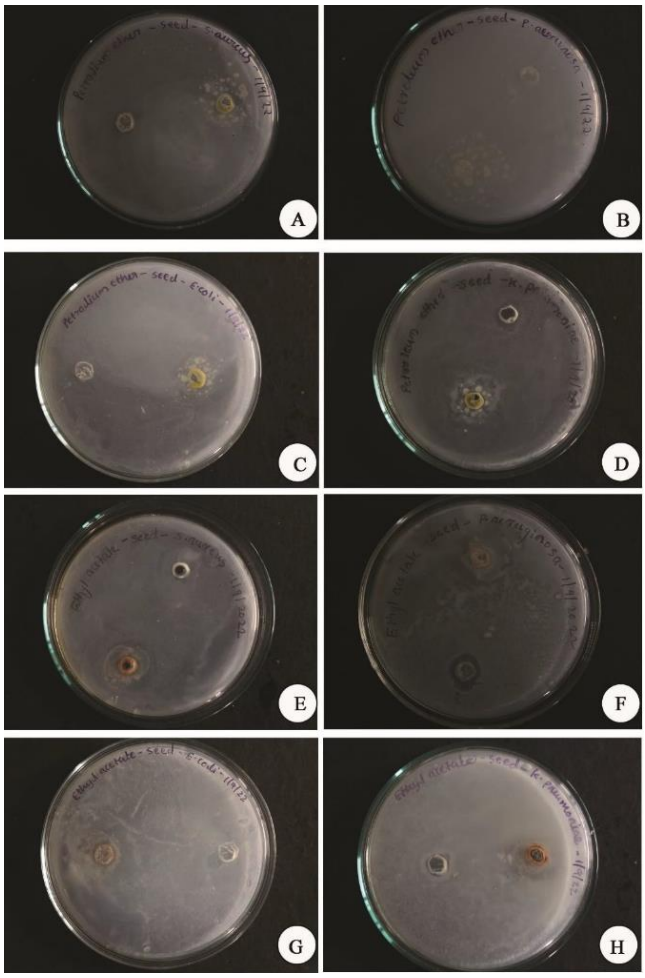


Fig 1: The antibacterial activity of petroleum ether and ethyl acetate extract against different bacterial strains. (A), petroleum ether extract of *S. aureus*, (B), petroleum ether extract of *P. aeruginosa*, (C), petroleum ether extract of E. coli, (D), petroleum ether extract of *K. pneumoniae*, (E),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

Ethanol extract

The ethanol extract also revealed that it contains compounds that can inhibit the growth of all strains tested. The maximum zone was showed by *Escherichia coli* and was found to be 3.8±1.3 cm, while the other two strains (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) showed a zone of inhibition 2.6±2.60 cm and 2.3±0.05 cm respectively. But *Staphylococcus aureus* showed lowest inhibition zone of 2.05±0.77 cm (Table No. 1 and Fig. 2).

Methanol extract

With methanol extract of *Sesbania grandiflora* seeds recorded maximum inhibition zone 2.1±0.4 cm. with *Staphylococcus aureus* and *Escherichia coli* showed a *m*aximum zone of inhibition of 1.65±0.15 cm. While *Klebsiella pneumoniae* of 1.4±0.1 cm and *Pseudomonas aeruginosa* showed the lowest inhibition zone of 1.3±1.1 cm (Table No. 1 and Fig. 2).

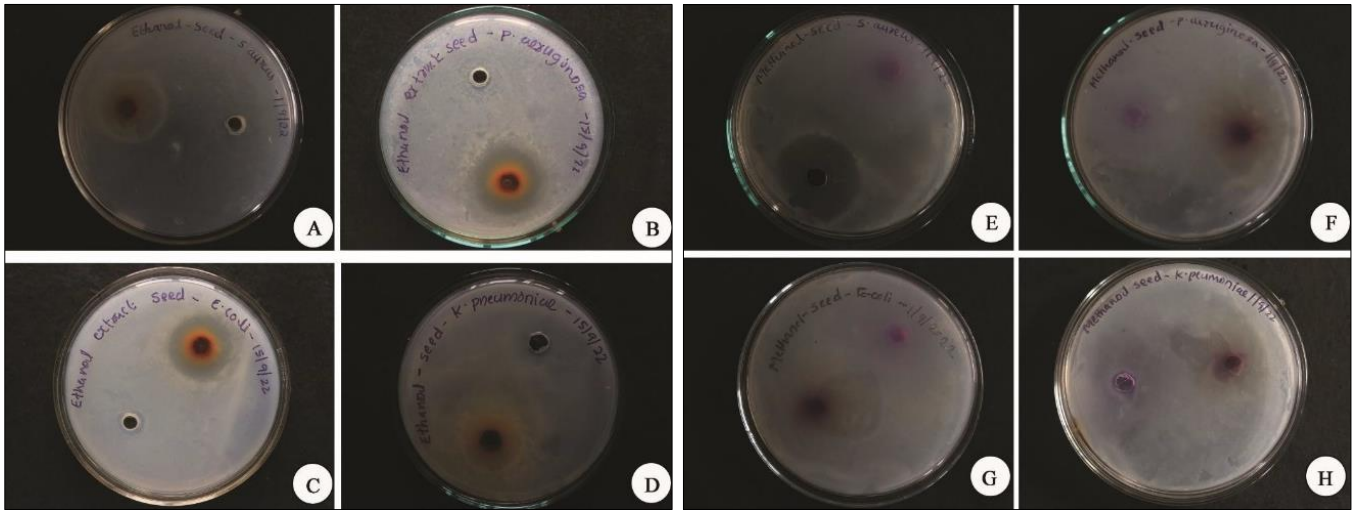


Fig 2: The antibacterial activity of ethanol and methanol extract against four different bacterial strains. (A). Ethanol extract of *S. aureus*, (B). *Ethanol* extract of *P. aeruginosa*, (C). Ethanol extract of *E. coli*, (D). Ethanol extract of K. *pneumoniae*, (E). Methanol extract of *S. aureus*,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

Water extract

With water extracts of seeds of *Sesbania grandiflora*, the maximum zone of inhibition was showed by *Staphylococcus aureus* 2.1±0.1 cm, while the other two strains (*Escherichia coli* and *Klebsiella pneumoniae)* showed a zone of inhibition of 1.6±0.7 cm and 1.3±0.6 cm respectively. On the other hand, *Pseudomonas aeruginosa* showed the lowest inhibition zone of 1.25±0.25 cm (Table No. 1 and Fig. 3).

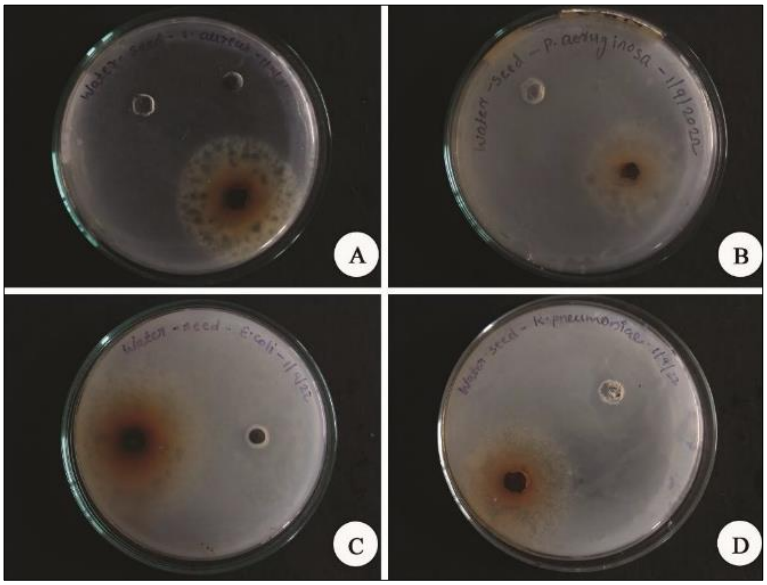


Fig 3: The antibacterial activity of water extract against four different *bacterial* strains. (A). *Staphylococcus aureus*, (B). *Pseudomonas aeruginosa*, (C). *Escherichia coli* and (D). *Klebsiella pneumoniae*.

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	2.0±0.05	2.75±0.65	3.8±1.3	1.65±0.15	1.6±0.7
Klebsiella pneumoniae	2.1±0.6	2.95±0.15	2.6±2.60	1.4±0.1	1.3±0.6
Staphylococcus aureus	1.9±0.35	2.55±0.90	2.05±0.77	2.1±0.4	2.1±0.1
Pseudomonas aeruginosa	2.3±0.4	3.1±0.6	2.3±0.05	1.3±1.1	1.25±0.25

Phytochemical Screening

The phytochemical screening of seed extract of *Sesbania grandiflora* showed the presence of alkaloids, flavonoids, phenols, quinones, saponins, steroids, tannins, proteins and terpenoids. The results were shown in Table No. 2.

Petroleum ether extract

The preliminary phytochemical analysis of petroleum ether seed extract revealed the absence of all the tested compounds like alkaloids, flavonoids, phenol, quinones, saponins, steroids, tannins, terpenoids and proteins.

Ethyl acetate extract

The phytochemical analysis of ethyl acetate extract showed the presence of alkaloids and flavonoids while the other compounds such as phenol, quinones, saponins, steroids, tannins, terpenoids and protein are absent.

Ethanol extract
The phytochemical analysis of ethanol extract showed the presence of alkaloids, flavonoids, phenol, quinones, saponins, steroids, tannins and terpenoids while protein is absent in ethanolic seed extract.

Methanol extract
The phytochemical analysis of methanol extract showed the presence of flavonoids, saponins, tannins, terpenoids and proteins. All the other compounds like alkaloids, phenol, quinones and steroids are absent.

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

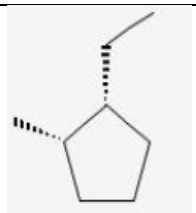
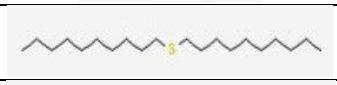
Table 2: Results of preliminary phytochemical analysis

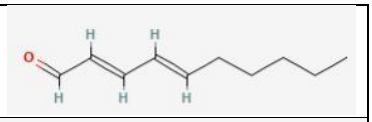
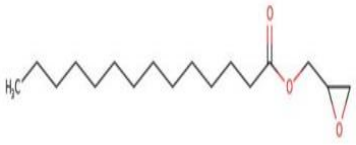
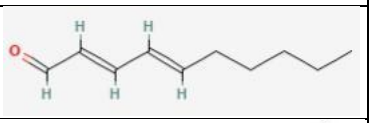
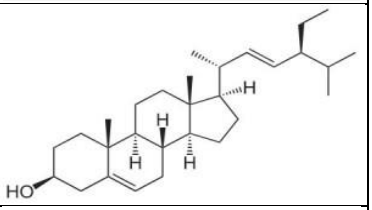
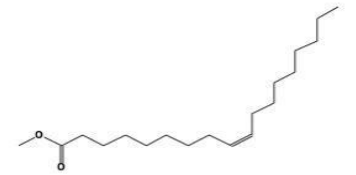
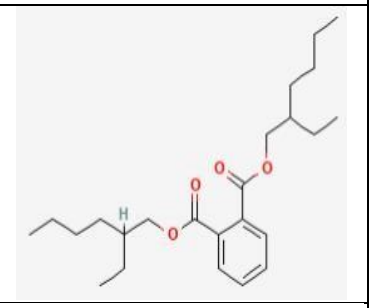
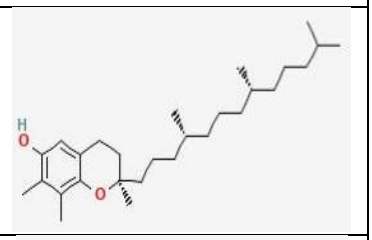
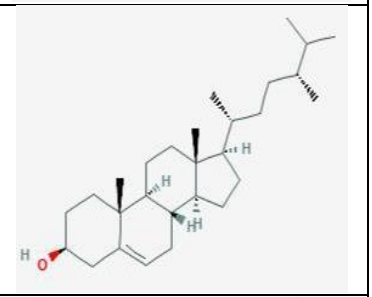
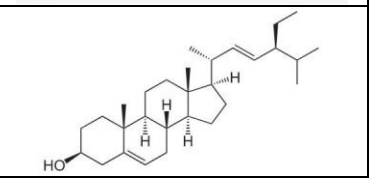
Test	Observation	Inference
Alkaloids	A yellow colouration observed in each extract indicated the presence of alkaloids.	Ethyl acetate, ethanol, and water extract showed the presence of alkaloids.
Flavonoids	A yellow colouration observed in each extract indicated the presence of flavonoids.	Ethyl acetate, ethanol, methanol and water extract showed the presence of flavonoids.
Phenol	Formation of blue or green colour indicates positive results for the presence of phenol.	Ethanol and water extract showed the presence of phenol.
Quinones	A red coloration indicates the presence of quinones.	Ethanol and water extracts shows 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.	Ethanol, methanol and water extracts showed the presence of saponins.
Steroids	Appearance of brown ring indicates the presence of steroids	Ethanol and water extracts showed the presence of steroids
Tannins	Formation of a dark blue or greenish black colour indicates the positive result of tannins.	Ethanol, methanol and water extracts showed the presence of tannins.
Terpenoids	A reddish-brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.	Ethanol and methanol extracts showed the presence of terpenoids
Proteins	A violet colouration observed in each extract indicated the presence of proteins.	Methanol extract showed the presence of proteins.

GC-MS analysis
Based on antibacterial activities, the extracts (petroleum ether and ethyl acetate) which showed highest antibacterial activity was subjected to GC-MS analysis.

Petroleum ether extract
The GCMS results of petroleum ether extract, showed the presence of twenty compounds. They are Oxirane tetramethyl, Cyclopentane 1-ethyl-2-methyl, 2-Decenal, 2, 4-Decadiebal, (E, E), Eicosane, Myristic acid glycidal ester, 9, 12-Octadecadienoyl chloride, 9-Octadecenoic acid (z), 9, 12-Octadecadien-1-ol (z, z), Glutraic acid, Dis (cis-non-3-enyl) ester, Bis (2-ethylhexyl) phthalate, Decyl sulfide, Ethanol 2-(9, 12-Octadecadienyloxy)-, Gamma-Tocopherol, Campesterol, Stigmasterol and 16-Hentria Contanone. These compounds belong to different secondary metabolites such as fatty acids, esters, aldehydes, alicyclic hydrocarbons, aromatic hydrocarbons and steroids. The results were shown in Table 3 and Fig. 4.
Among these 20 compounds, three compounds *viz*., Gamma-Tocopherol, Campesterol and Stigmasterol are reported earlier in *Sesbania grandiflora.* All other compounds are found to be new to *Sesbania grandiflora*.

Table 3: GCMS result analysis of petroleum ether

Groups of phytochemicals	Name of compounds	Molecular formula	Chemical structure
Cyclic ether	Oxirane tetramethy	C6H12O	
Hydrocarbons	Cyclopentane, 1-ethyl-2-methyl-, cis-	C8H16	
	Decyl sulfide	C20H42S	
Aldehydes	2-Decenal, (E)	C10H18O	

	2,4-Decadienal, (E, E)-	C10H16O	
Alkanes	Eicosane	C20H42	
Fatty acids	Myristic acid glycidyl ester	C17H32O3	
	9,12-Octadecadienoyl chloride, (z, z)	18H31ClO	
	Glutraic acid, di(cis-non-3-enyl) ester	C25H40O4	
	16-Hentriacontanone	C31H62O	
Esters	-Octadecenoic acid (Z)-Oxiranylmethyl ester	C36H68O4	
	Bis(2-ethylhexyl) phthalate	C6H4(CO2C8H17)2	
Dialkyl ethers	Ethanol, 2-(9,12-octadecadienyloxy	C20H42O2	
Vitamin	Gamma-tocopherol	C28H48O2	
Steroids	Campesterol	C28H48O	
	Stigmasterol	C29H48O	

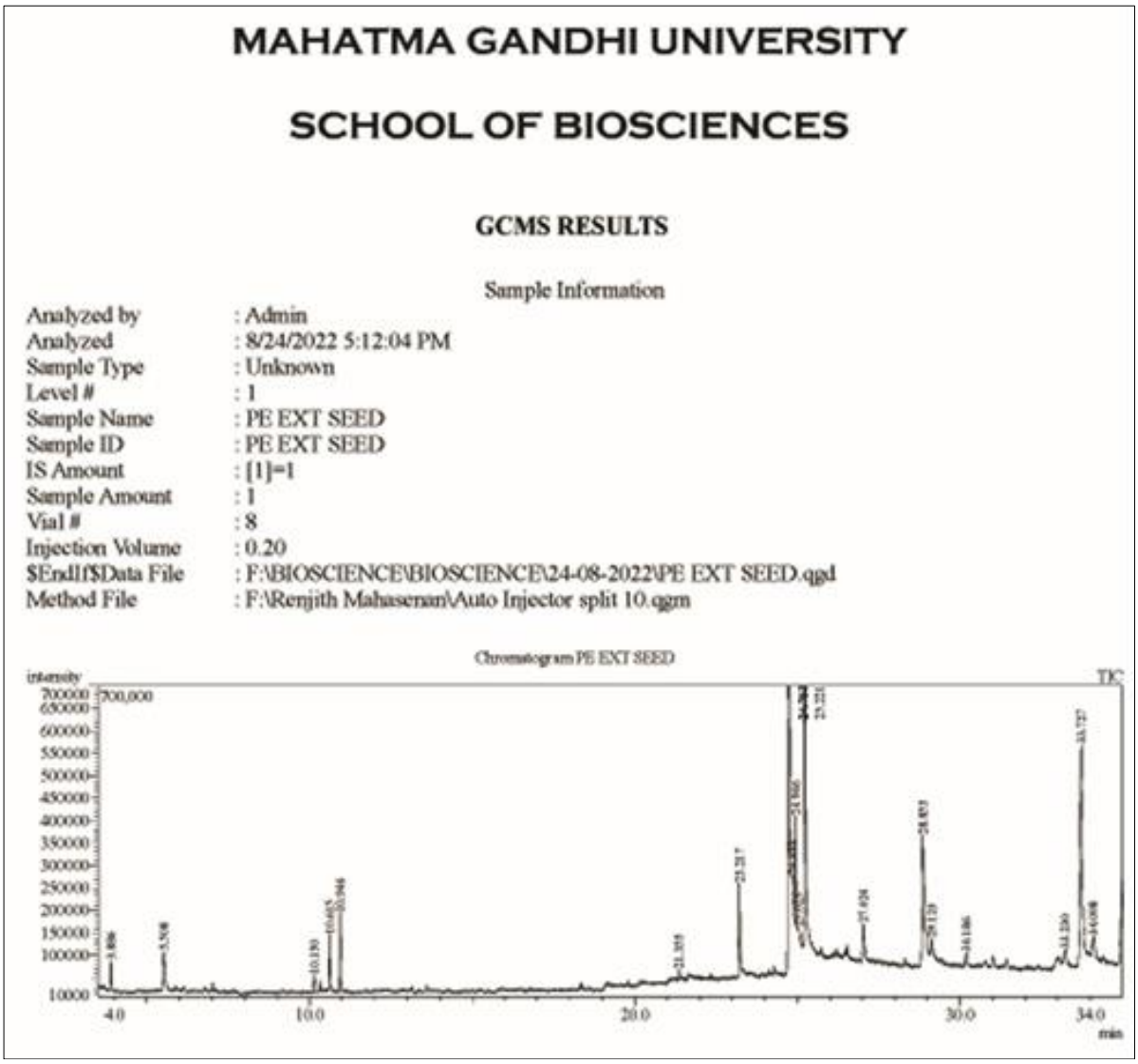
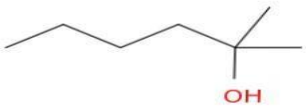
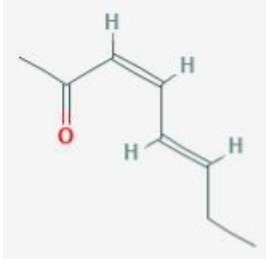
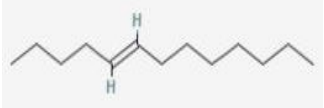
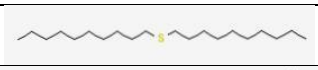
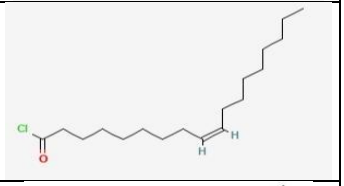
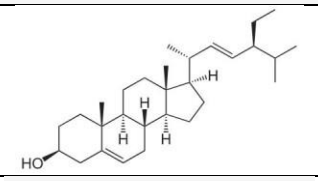
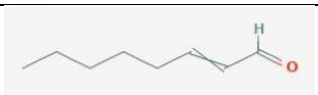
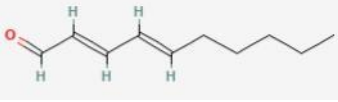
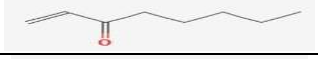
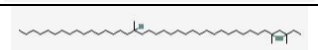
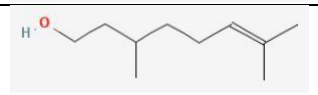
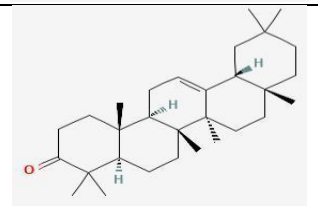
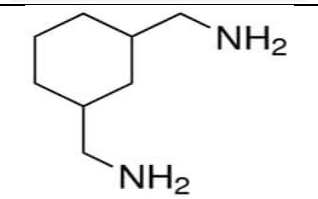
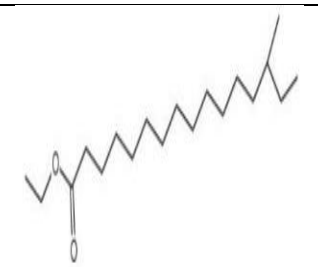
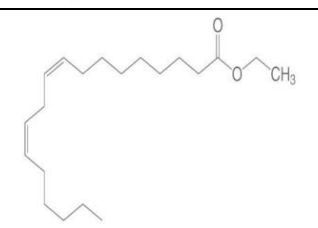
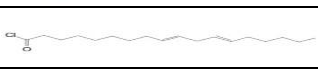
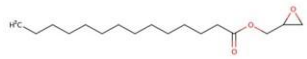
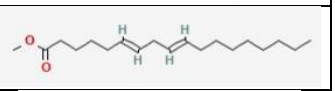
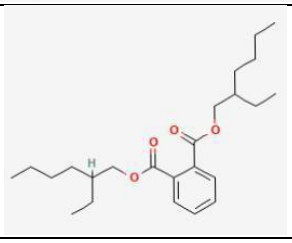
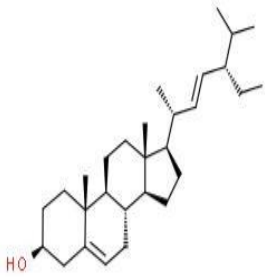


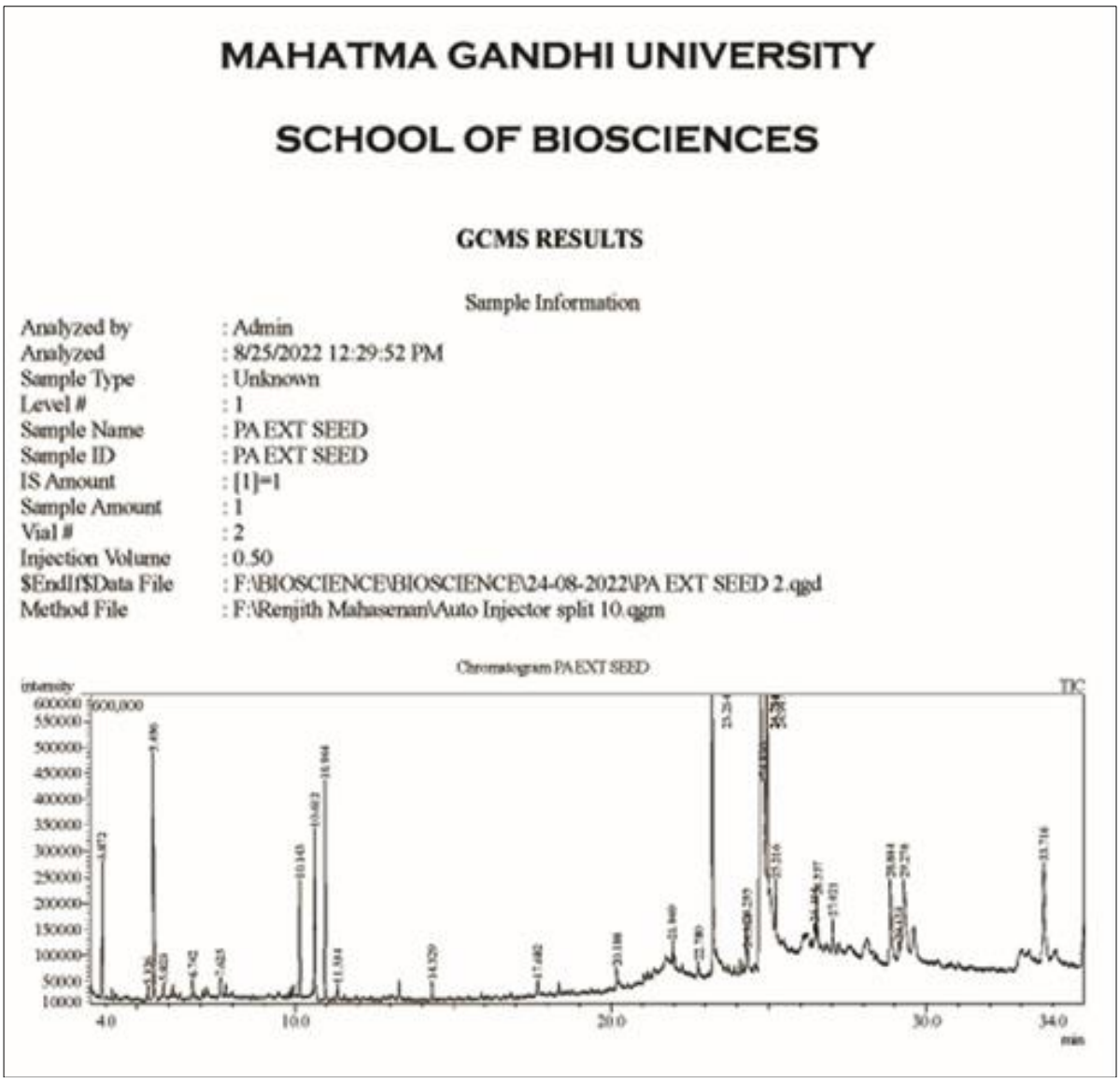
Fig 4: Results of GC-MS analysis of petroleum ether extract of seed of *Sesbania grandiflora*.

Table 4: GC-MS analysis of Ethyl acetate

Group of phytochemicals	Name of compounds	Molecular formula	structure
Alcohol	2-Hexanol, 2-methyl	C7 H16 O	
Hydrocarbons	Cyclopentane, 1-ethyl-1-methyl	C8H16	
	3,5-Octadien-2-o1	C8H12O	
	5-Tridecene, (z)	C13H26	

	Decyl sulfide	C20H42S	
	Oleonyl chloride	C18H33ClO	
	3-Trifluoro methyl benzoic acid, octadecyl ester	C26H40F4O2	
Aldehydes	2-octenal, (E)	C8H14O	
	2-Decenal, (E)	C10H18O	
	2,4-Decadienal, (E, Z) (poly unsaturated fatty aldehydes)	C10H16O	
Unsaturated monocarboxylic acid	1-octen-3-o1	C8H16O	
Alkanes	Tetracontane, 3, 5, 24-trimethyl	C43H88	
Terpenoids	Beta-Citronellol, chlorodifluroacetate	C10H20O	
	Beta-Amyrone	C29H46O	
Amines	Bis(cyclohex-3-enylmethyl) amines	C12H13N3	
Fatty acids	Ethyl 14-methyl-hexadecanote	C18H36O2	
	Linoleic acid ethyl ester	C20H36O2	
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl eater	C18H36O2	
	9,12-Octadecadienoyl chloride, (z, z)	C18H31ClO	

	Cis-13, 16-Docasadienoic acid	C22H40O2	
	Myristic acid glycidyl ester (saturated fatty acids).	C17H32O3	
	6,9-Octadecadienoic acid, methyl ester (unsaturated fatty acids).	C19H34O2	
	Bis (2-ethyl hexyl) phthalate	C6H4 (CO2C8H17)2	
	n-Propyl 9, 12-octadecadienoate	C21H38O2	
Steroids	Stigmasterol	C29H48O	



MAHATMA GANDHI UNIVERSITY

SCHOOL OF BIOSCIENCES

GCMS RESULTS

Sample Information

Analyzed by	: Admin
Analyzed	: 8/25/2022 12:29:52 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: PA EXT SEED
Sample ID	: PA EXT SEED
IS Amount	: [1]=1
Sample Amount	: 1
Vial #	: 2
Injection Volume	: 0.50
$EndIf$Data File	: F:\BIOSCIENCE\BIOSCIENCE\24-08-2022\PA EXT SEED 2.qgd
Method File	: F:\Renjith Mahasenan\Auto Injector split 10.qgm

Fig 5: Results of GC-MS analysis of ethyl acetate extract of seed of *Sesbania grandiflora*.

Ethyl acetate extract

The GCMS result of ethyl acetate extract showed the presence of thirty compounds. The compounds include 2-Hexanol, 2-Methyl, Cyclopentane 1-Ethyl-1-Methyl, 2-Octenal (E), 1-Octen-3-ol, 3,5-Octadirn-2-ol, 5-Tridecene (z), 2,4-Decadienal (E, Z), Tetracontane 3,5,24-Trimethyl, Beta citronellol chlorodifluoroacetate, Bis (cyclohex-3-enylmethyl) amines, Ethyl 14-Methyl-hexadecanote, Linoleic acid ethyl ester, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester, Myristic acid glycidyl ester, 6,9-Octadecadienoic acid methyl ester, Oleonyl chloride, 9,12-Octadecadienoyl chlorode (z, z), 9-Octadecenoic acid (z)-Oxiranyl methyl ester, Undec-10ynoic acid but-3-yn-2-yl ester, Myristic acid glycidyl ester, Bis (2-ethylhexyl) phthalate, n-Propyl 9,12-Octadecadienoate, Decyl sulfide, cis-13,16-Docasadienoic acid, 3-Trifluoromethylbenzoic acid octadecyl ester, Beta-Amyrone and Stigmasterol. These compounds belong to hydrocarbons, alcohol, aldehyde, monocarboxylic acid, alkanes, monoterpenoids, amines, fatty acids, esters, terpenoids and sterol. The results were shown in Table 4 and Fig. 5.

Among these 30 compounds identified, only Stigmasterol reported earlier and all other compounds are found to be newly observed.

The GCMS results of petroleum ether and ethyl acetate extract showed the presence of some common compounds that are Myristic acid glycidyl ester, Cyclopentane 1-ethyl-2-methyl, 2-Decenal (E), 9, 12 Octadecadienoyl chloride (z, z), Bis (2-ethylhexyl) phthalate, Decyl sulfide, Stigmasterol and 2,4-Decadienal (E, E).

Discussion

In the present study seed extract of *Sesbania grandiflora* were subjected to antimicrobial activities, phytochemical screening and GCMS analysis and this is the first report. The study has been made an attempt, to analyse different phytochemicals present in the seeds 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 petroleum ether extract has the highest zone of inhibition against *Pseudomonas aeruginosa* (about 2.3±0.4 cm of inhibition zone) followed by *Klebsiella pneumoniae* (about 2.1±0.6 cm of inhibition zone) (Fig. 1). The ethyl acetate extract of *Sesbania grandiflora* seed was also found to be effective against all tested bacterial strains and the maximum zone of inhibition was showed by *Pseudomonas aeruginosa* and was found to be 3.1±0.6 cm followed by *Klebsiella pneumoniae* 2.95±0.15 cm (Fig. 1). The ethanol extract also found to be effective against all tested bacterial strains. The results revealed that the ethanol extract contains compounds that can inhibit the growth of all the strains. The maximum inhibition zone was showed by *Escherichia coli* and is about 3.8±1.3 cm and followed by *Klebsiella pneumoniae* about 2.6±2.60 cm (Fig. 2).

The methanol extract of seeds showed maximum inhibitory zone on *Staphylococcus aureus* and was found to be 2.1±0.4cm, followed by *Escherichia coli* about 1.65±0.15 cm of inhibition zone (Fig. 2). The water extract also contains compounds that can inhibit the growth of all the strains. The maximum zone was showed by *Staphylococcus aureus* and was found to be 2.1±0.1 cm and followed by *Escherichia coli* and was found to be 1.6±0.7 cm (Fig. 3).

These results were comparable with some previous research reports. Anantaworasukal *et al*., 2011 [14] reported that ethanol extracts of stem barks of *Sesbania grandiflora* effective against the pathogenic strains like *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Pachiyalakshmi *et al*., 2016 [15] also reported that ethanol, methanol and water extract of leaf of *Sesbania grandiflora* was more effective against *Staphylococcus aureus.* They also proved that ethanol, methanol and water extract was also effective against the pathogenic strains like *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae.* Gandhi *et al*., 2017 [16] also reported that water extract of leaves of *Sesbania grandiflora* effective against *Staphylococcus aureus.* Lakshmi *et al*., 2011 [17] reported that ethanol extract of leaf 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 the preliminary study, the phytochemical analysis of *Sesbania grandiflora* seeds showed the presence of several phytochemicals except protein. Followed by ethanol, water extract also showed the presence of all compounds except terpenoids and proteins. The methanolic extract showed the presence of flavonoids, saponins, tannins, proteins and terpenoids and alkaloids, phenol, quinones and steroids were found to be absent. The seed extract of ethyl acetate showed the presence of only alkaloids and flavonoids. In the case of petroleum ether all the checked phytochemicals are 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 [18] reported the presence of alkaloids in water, methanol and ethanol extract of leaf 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 [19] reported the presence of steroids, alkaloids and terpenoids in petroleum ether extract of barks of *Sesbania grandiflora.* They also reported the presence of alkaloids, flavonoids, tannins and saponins in methanol extract. Reji & Rexin 2013 [17] also reported the presence of alkaloids, flavonoids, tannins, steroids, terpenoids and proteins in ethanol, water and petroleum ether extract of leaves of *Sesbania grandiflora.* Sein *et al*., 2019 [20] reported the presence of alkaloids, steroids, terpenoids and tannins in methanol extract of *Sesbania grandiflora.* Semwal *et al*., 2018 [21] also reported the presence of steroid, saponins, flavonoids, tannins and phenol in ethyl acetate extract of seed of *Sesbania grandiflora.*

In the present study, the GCMS results of petroleum ether and ethyl acetate extract showed the presence of several phytochemicals and is relevant to the current scenario of ayurvedic medicine. About twenty compounds were observed in extract of petroleum ether and about thirty compounds were observed in extract of ethyl acetate (Table 3-4 & Fig. 4-5).

Earlier reports indicate the presence of secondary metabolites in *Sesbania grandiflora.* Hussain & Kumaresan 2014 [22] reported the presence of 3,4,5-Trimethoxyphenol, erucic acid, Phytofluene, 2-Furancarboxy aldehyde, Nonanoic acids, Acrylonitrile, 4-Methyloxazole, 1-Propanol 2-methyl, 3-Hexen-2-one 3,4-Dimethyl, 6-Octadecenoic acid methyl ester, 3,5-Di-t-butyl phenol, Urea, Palmticacid, 9-Hexadecenol, Dioctyl ester, Vitamin E acetate and Malonic acid ethyl 3-

hexyl ester in leaf extract of *Sesbania grandiflora*. Shareef *et al*., 2012 [23] reported the presence of Tocopherol, Campesterol, Stigmasterol, Beta sitosterol and Avenasterol in seed oil of *Sesbania grandiflora*. In which the three compounds namely Gamma-Tocopherol, Camesterol and Stigmasterol also observed in present study (Table 3).

Mustafa *et al*., 2010 [24] studied total phenolic profiling of methanolic extract of *Sesbania grandiflora* leaves and showed high phenolic contents of quercetin and kaempferol. Recently, the isoflavonoids, isovesitol, medicarpin, together with the lupine triterpene betulinic acid were isolated from roots of *Sesbania grandiflora* by Hasan *et al*., 2012 [25]. Sein *et al*., 2019 [20] reported the presence of 3,4,5-Trimethoxyphenol, 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.

Conclusion

The plant *Sesbania grandiflora* belongs to the family Fabaceae is a well-recognized medicinal plant in numerous countries like India, Srilanka and Southeast Asia. The Plant possess numerous medicinal properties and it is effective against various diseases, tumours, swelling, antiulcer, rheumatism, gout, leprosy, inflammation, bronchitis, nasal catarrh, sore throat anaemia, small pox sores, bruises, dysentery, fevers, headache etc.

The preliminary study focused on the antibacterial and phytochemical analysis of seeds of the plant and this is the first report on seeds of *Sesbania grandiflora*. For this, the seeds were dried and extracted in petroleum ether, ethyl acetate, ethanol, methanol and water. The antibacterial activity was evaluated using different pathogenic bacterial strains *viz*., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. The antibacterial activity was calculated using found to be maximum on ethyl acetate extract (3.1 cm) and lowest on water extract (1.25cm). Based on this data the extracts were subjected to qualitative phytochemical analysis. The secondary metabolites such as alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, quinones, steroids, and proteins were found to be present in different extracts. The presence of compounds showed by qualitative analysis was further confirmed by GC-MS. The GC-MS results revealed the presence of secondary metabolites such as Oxirane tetramethy, Cyclopentane-1-ethyl-2-methyl-cis-, 2-Decenal (E), 2,4-Decadienal, (E, E), 2,4-Decadienal, (E, E), Eicosane, Myristic acid glycidyl ester, 9,12-Octadecadienoyl chloride, (z, z), 9-Octadecenoic acid (z)-Oxiranylmethyl ester, 9,12-Octadecadien-1-ol, (z, z), Glutraic acid Di(cis-non-3-enyl) ester, Bis (2-ethylhexyl) phthalate, Decyl Sulfide, Ethanol 2-(9,12-Octadecadienyloxy), Gamma-Tocopherol, Campesterol, Stigmasterol, 16-Hentriacontanone, 2-Hexanol 2-methyl, Cyclopentane 1-Ethyl-1-Methyl,2-Octenal (E), 1-Octen-3-ol, 3,5-Octadien-2-ol, 5-Tridecene (z), 2-Decenal (E), 2,4-Decadienal, Tetracontane 3,5, 24-Trimethyl, Beta-Citronellol Chlorodifluroacetate, Bis (Cyclohex-3-enylmethyl) amine, Ethyl 14-methyl-hexadecanote, Linoleic acid ethyl ester, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester, 6,9-Octadecadienoic acid methyl ester, Oleonyl chloride, 9,12-Octadecadienoyl chloride (z, z), 9-Octadecenoic acid(z), Undec-10ynoic acid but-3-yn—2-yl ester, Bis (2-ethylhexyl) phthalate, n-Propyl 9, 12-Octadecadienoate, cis-13,16-Docasadienoic acid, 3-Trifluoro methyl benzoic acid octadecyl ester and Beta-Amyrone. These compounds belong to fatty acids, hydrocarbons, alkanes, ester, dialkyl ethers, vitamin, steroids, alcohol, aldehydes, monocarboxylic acid, aldehydes, poly unsaturated fatty aldehydes, alkanes, terpenoids and amines.

The results clearly revealed, that the plant is highly medicinal and is effective against various pathogenic bacteria, so that we can effectively use to treat various pathogenic diseases. The results also suggested that the seeds are a rich source of valuable primary and secondary metabolites exhibiting antimicrobial activities. But further studies are recommended for the purification and characterization of these compounds to demonstrate the exact medicinal properties.

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