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GC-MS analysis and antibacterial activity of *Amherstia nobilis*. W leaf extract

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Abstract
Amherstia nobilis W is a plant of Burman origin also known as pride of Burma. It is the only plant found in the genus *Amherstia*. The present study carried out to analyse the unknown chemical compounds of the plant by GC-MS analysis of ethanolic leaf extract and the antibacterial effect of 70% ethanolic extract. GC-MS analysis revealed the presence of 27 chemical compounds. Some of the major compounds found are PHENOL, 2, 4-BIS (1, 1-Dimethylethyl), Eicosane, Tetradecanoic acid, 3-Heptadecanol, Neophytadiene, Phytol, acetate. Antibacterial activity was carried out using disc diffusion method in *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa, Escherichia coli.* Leaf extractshowed maximum zone of inhibition against *Staphylococcus aureus and Pseudomonas aeroginosa*.

Keywords: GC-MS analysis, antibacterial, *Amherstia nobilis*

1. Introduction
Pride of Burma also known as orchid tree is a tropical tree with beautiful flowers. It has only been collected from the wild, in the forests of Burma, leading to its common name Pride of Burma. In the early Nineteenth century Lady Sarah Amherst collected this plants in Asia and the genus named after this [1] plants are rich sources of secondary metabolic products having various therapeutic activities. GC-MS analysis of the plant extract can reveal the presence of the important phytoconstituents present, which may pay a path for finding new potent biomolecule of good therapeutic effect [2]. Present study carried out to analyze the unknown phytoconstituents of the leaf extract and to study the antimicrobial activity of the extract from the background of phytoconstituents found by GC-MS analysis. Bacterial infectious diseases are emerging drastically in the world and many of the bacterial strains became resistant to the antibiotics. Now it's the need to synthesize new antibiotics preferably from natural sources to resist such strains. [3] This study, carried out to analyse antibacterial activity of *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa, Escherichia coli. Staphylococcus aureus* and *Pseudomonas aeroginosa* by disc diffusion method.

2. Materials and Methods
2.1 Plant material
The plant specimens (Leaves) for the proposed study were collected from plants located at Mannuthy, Thrissur district. The collected plants were carefully examined and authenticated and herbarium was deposited at Kerala Forest Research Institute, Thrissur for future reference.

2.2 Preparation of extract
The leaf parts of *Amherstia nobilis* W was collected and dried under shade. These dried materials were mechanically powdered The shade dried coarse powder of the leaves of *Amherstia nobilis* (100g) was taken in conical flask and was macerated with 70% v/v ethanol (2000 ml) for 7 days with occasional shaking and filtered. Then the extract was distilled in vacuum in order to remove the solvent completely, dried in a desiccator [4].

2.3 Characterization of medicinal plant extract by GC-MS analysis
The Gas chromatography-Mass spectrometry (GC-MS) analysis of ethanol extract of *Amherstia nobilis* were performed using a GC-MS (Model; QP21S, Shimadzu, Tokyo, Japan) equipped with a Rxi-5Sil MS capillary column of 30 m length, 0.25mm diameter and 0.25µm film thickness. The column oven temperature was programmed to 80 °C. The temperature of the injector was fixed to 260°C.The column flow rate was maintained as 1.00ml/min. Splitless injection mode with a linear velocity of 36.8 cm/search is used. Total running time of GC-MS is 27min. The relative percentage of the each extract constituents were expressed as a

percentage with peak area normalization [5].

2.4 Antibacterial screening

Antimicrobial activity of plant extract was determined using a modified Kirby-Bauer disc diffusion method. Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria, 100 µl of microbial suspension was spread into the Nutrient agar plates. The extracts were tested using 5 mm sterilized filter paper discs. Discs were impregnated with 1 ml,4 ml (1000 µg and 4000µg concentrations) of the test samples (ethanol leaf extract), allowed to dry and placed onto inoculated plates (30 min incubation). The plates were allowed to stand at 4°C for 2 hours before incubation with the test microbial agents. Plates inoculated with *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and. *Pseudomonas aeruginosa* were incubated at 37°C for 24 hours, than the diameters of the inhibition zones were measured in millimetres. Each antimicrobial assay was performed in triplicate and mean values were reported. Standard antibiotics, ampicillin (25 µg/ disc), streptomycin (25 µg/disc), amoxicillin (25 µg/disc), served as positive controls for antimicrobial activity. Solvent control disc (70% v/v ethanol) was also placed in the test [6].

3. Results and Discussion

3.1 GC-MS Analysis

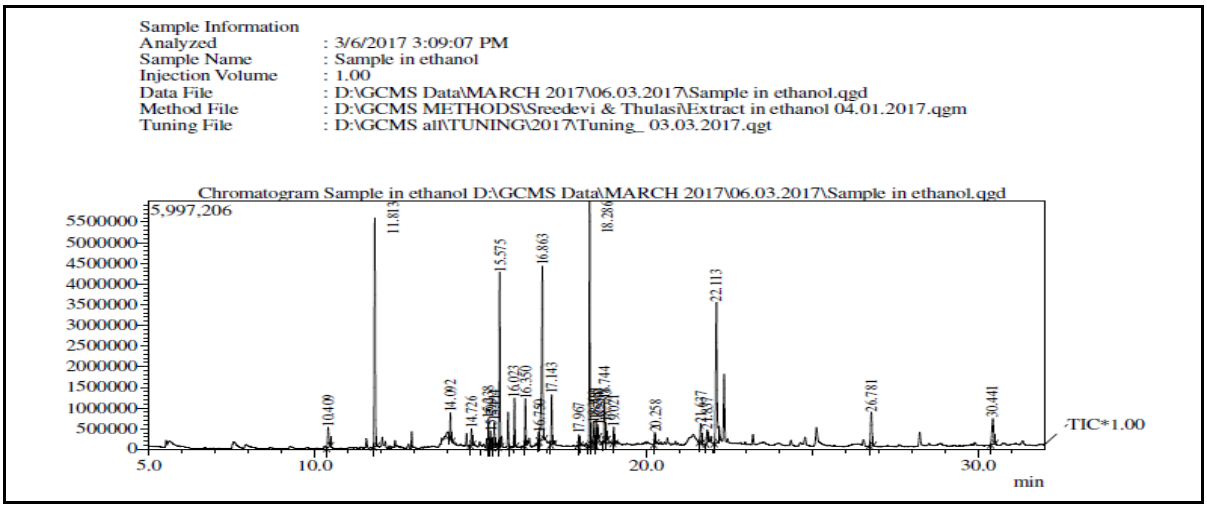

Fig 1: GC-MS Chromatogram of Ethanolic leaf extract of *Amherstia nobilis* W

Table 1: Phytocompounds identified in Ethanolic leaf extract of *Amherstianobilis* and their therapeutic uses

Sl. No	Retention time	Name of the compound	Molecular formula	MW	Peak area	Therapeutic use
1	10.409	HEXADECANE	$C_{16}H_{34}$	226.448	1.89	
2	11.813	PHENOL, 2,4-BIS (1,1-DIMETHYLETHYL)-	$C_{14}H_{22}O$	206.329	15.6	Antibacterial, antiinflammatory, anti oxidant [7]
3	14.092	Eicosane	$C_{20}H_{42}$	282.556	1.24	Anti oxidant [8]
4	14.726	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.376	0.95	Fragrance ingredient, opacifying agent, surfactant, cleansner, emulsifierAntioxidant, Lubricant, Hypercholesterolemic, Cancer-preventive, Cosmetic[9]
5	15.23	3-Heptadecanol	$C_{17}H_{36}O$	256.474	1.59	Anti microbial [10]
6	15.299	2-Ethylhexyl salicylate	$C_{15}H_{22}O_3$	250.33	0.86	UV protectant [11]
7	15.414	Isopropyl myristate	$C_{17}H_{34}O_2$	270.457	1.26	Polar emollient [12]
8	15.575	Neophytadiene	$C_{20}H_{38}$	278.524	8.6	Anti bacterial [13]
9	16.023	Phytol, acetate	$C_{20}H_{42}O_2$	338.576	2.41	Anti microbial, anti-inflammatory, anticancer, Diuretic[14]
10	16.35	2-Bromotetradecane	$C_{14}H_{29}Br$	277.29	2.74	
11	16.75	4-Heptanol, 4-methyl-	$C_8H_{18}O$	***130.231***	0.47	
12	16.863	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.43	15.28	Anti oxidant,hypocholesterolemic, hemolytic, nematicides [15]
13	17.143	Hexadecanoic Acid, Ethyl Ester	$\boldsymbol{C}_{18}H_{36}O_2$	***284.484***	2.67	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, flavor, Hemolytic, Alphareductaseinhibitor [16]
14	17.967	Cyclohexanol, 1-butyl-	$\boldsymbol{C}_{10}H_{20}O$	156.269	0.61	
15	18.286	2-HEXADECEN-1-OL, 3,7,11, 15-TETRAMETHYL-, [R-[R*,R*-(E)]]- (T-PHYTOL)	$C_{20}H_{40}O$	296.539	13.61	Antimicrobial, Anticancer, Antiinflammatory, Diuretic,anti microbial [15]
16	18.393	OCTADECANE	$C_{18}H_{38}$	254.502	1.45	Anti fungal [17]
17	18.481	9,12-OCTADECADIENOIC ACID	$C_{18}H_{32}O_2$	280.452	1.42	Surfactant, antiinflammatory, Acne reductive [18]
18	18.534	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210.361	0.81	

19	18.744	Octadecanoic acid	$C_{18}H_{34}O_2$	282.468	2.64	Surfactant and emulsifying agent for fragrance, Anti inflammatory, hypocholestrolemic, cancer preventive, hepato protective, Anti histaminic, Anti arthritic [8, 19]
20	18.783	Cyclopropane, 1-(1-hydroxy-1-heptyl) -2-methylene-3-pentyl-	$C_{16}H_{30}$	238.409	0.97	
21	19.021	OCTADECANOIC ACID, ETHYL ESTER	$C_{20}H_{40}O_2$	312.538	0.9	
22	20.258	TETRAPENTACONTANE	$C_{54}H_{11}O$	759.474	0.66	
23	21.637	1,3,5-Trisilacyclohexane	$C_{17}H_{29}N_3Si_4$	387.78	1.3	
24	21.837	d-Ribose, 2-deoxy-bis (thioheptyl)-dithioacetal	$C_{19}H_{40}O_3S_2$	380.646	1.08	
25	22.113	Hexadecanoic acid, 2-hydroxy -1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5026	12.64	Hemolytic, pesticide, Anti inflammatory, Flavour [18]
26	26.781	Squalene	$C_{30}H_{50}$	410.73	3.37	Anti oxidant, reduce cholesterol, Adjuent therapy of cancer, anti ageing, analgesic, anti diabetic, Anti dermatetic, anti leukemic, anti tumour, hepatoprotective, anti ulcerogenic, vasodilator, antispasmodic, anti bronchitic, anti coronary [20]
27	30.441	beta.-Tocopherol	$C_{28}H_{48}O_2$	416.69	2.98	Anti oxidant [21]

3.2 Antibacterial assay

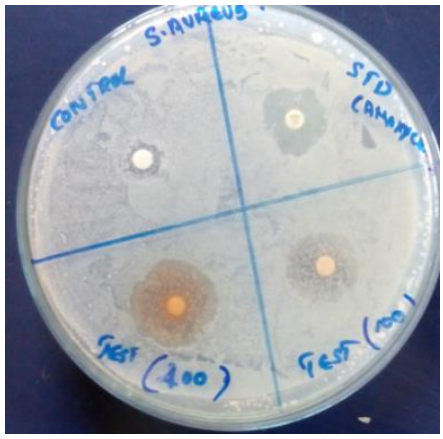

Fig 2: Zone of inhibition of ethanolic extracts against *Staphylococcus aureus*

Organism : *Staphylococcus aureus*
T1 : 1000µg/ml T2 : 4000µg/ml
+ : Ampicillin - : Control

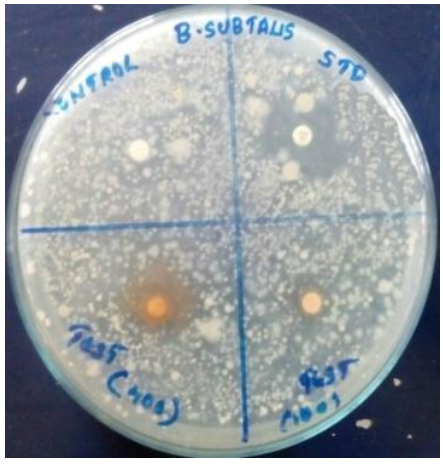

Fig 3: Zone of inhibition of ethanolic extracts against *Bacillus subtilis*

Organism : *Bacillus subtilis*
T1 : 1000µg/ml T2 : 4000µg/ml
+ : Amoxicillin - : Control

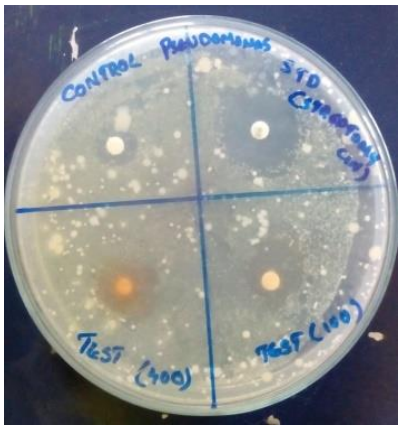

Fig 4: Zone of inhibition of ethanolic extracts against *Pseudomonas aeroginosa*

Organism : *Pseudomonas aeroginosa*
T1 : 1000µg/ml T2 : 4000µg/ml
+ : Streptomycin - : Control

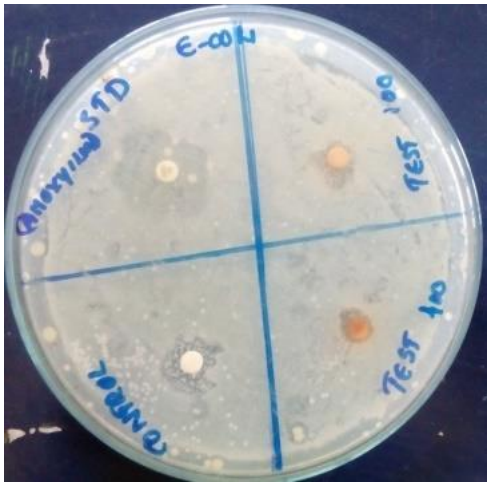

Fig 5: Zone of inhibition of ethanolic extracts against *Escherichia coli*

Organism : *Escherichia coli*
T1 : 1000µg/ml T2 : 4000µg/ml
+ : Amoxicillin - : Control

Table 2: Antibacterial activity of the ethanolic extract of *Amherstia nobilis*

Organism	Spectrum	Antibiotic used	Diameter of zone of inhibition (mm)			
			Standard (25 µg/disc)	Control	1000µg/ml	4000µg/ml
Staphylococcus aureus	Gram (+)	Ampicillin	19	9	21	29
Bacillus subtilis		Amoxicillin	29	9	11	21
Pseudomonas aeroginosa	Gram (-)	Streptomycin	30	16	19	23
Escherichia coli		Amoxicillin	24	11	12	13

4. Discussion

Plants have provided mankind with herbal remedies for several diseases for many centuries. The therapeutic potentials of plant and animal origin crude drugs are being used from the ancient times by the simple process without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. GC-MS analysis of ethanolic extract revealed the presence of many therapeutically important compounds. Some of the useful compounds found from the extracts include PHENOL, 2,4-BIS (1,1-DIMETHYLETHYL), Eicosane, Tetradecanoic acid, 3-Heptadecanol, Neophytadiene, Phytol, acetate. Antibacterial activity at different doses was done by disc diffusion method. Concentration was in the range of 1000 and 4000 µg/disc. Activity was dependent on the dose of the test material. As the concentration increased the inhibition zone was also increased. Against ethanol leaf extract, *Staphylococcus aureus* and *pseudomonas aeruginosa* showed a maximum inhibition zone where as *Escherichia coli* showed a lesser inhibition zone.

5. Conclusion

The present study was carried out to analyze the phytoconstituents present in the leaf extracts of Amherstia nobilis. GC-MS analysis of the ethanolic leaf extract revealed the presence of 27 phytoconstituents of important pharmaceutical use. compouns like PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-, 3-Heptadecanol, Neophytadiene, 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(E)]]- (T-PHYTOL) are present in the leaf extract which has antimicrobial activity. Antibacterial studies were carried out showed that the ethanolic extract possessed a significant antibacterial activity aginst gram negative *Escherichia coli* and *Pseudomonas aeroginosa*, gram positive *Staphylococcus aureus* and *Bacillus subtilis*. The results were compared with the standard drugs Amoxicillin, Ampicillin and streptomycin. *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed maximum zone of inhibition. The antimicrobial activity of the leaf extract may be due to the presence of the above mentioned phytoconstituents. Isolation of the phytoconstituents and further studies including spectral characterization of the isolated molecule can yield promising drugs for future.

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