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GC-MS analysis of bioactive compounds on hexane leaf extract of *Anogeissus latifolia* (ROXB. EX DC.) WALL. EX BEDD)

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Abstract
Plants are important source of secondary metabolites they contain many bioactive constituents with interesting biological activities. *Anogeissus latifolia* is an important multipurpose tree species belongs to Combretaceae family. *Anogeissus latifolia* is a high medicinal value tree species as it is used to cure various diseases like cardiac disorder, vomiting, cold, diarrhea, dysentery, snake and scorpion bite, fever, skin diseases, diabetes, anemia, piles, stomach ache, anemia and urinary discharge. The aim of the study was to determine the phytochemicals presence in the plant extract of *Anogeissus latifolia* and analyze the extract using GC-MS analysis. The GC-MS analysis shows different peaks with low and high molecular weight determining the presence of 40 phytochemical compounds. These 40 compounds are understood to have different therapeutic activities *viz*., Antioxidant, anticancer, nematicide, pesticide, antiandrogenic, plasticizer, local anesthetic and a drug allergen, etc., The findings of this study suggest the use of leaf extract of *Anogeissus latifolia* for treatments of various ailments, since it contain various compounds which have various medicinal properties.

Keywords: *Anogeissus latifolia*, GC-MS, phytochemical, Therapeutic activities

Introduction
Anogeissus latifolia belonging to the family Combretaceae is an important multipurpose tree species. The tree is commonly called as axle wood or ghatti gum. It is a medium to large sized tree up to 36 m height with straight and cylindrical bole. A large tree found abundantly in the deciduous forests of India and Sri Lanka. It is one of the useful trees as medicinally important, timber, fuel, production of agriculture implements and the leaves and bark are being used for tanning. The plant is used traditionally as medicine to treat various human ailments and conditions such as cardiac disorder, vomiting, whooping cough, cold, diarrhea, dysentery, snake and scorpion bite, fever, skin diseases, diabetes, anemia, piles, fistula, stomach ache, sexual debility, anemia, and urinary discharge. *A. latifolia* is one of the ingredients in several Ayurvedic formulations. The stem bark of *A. latifolia* is one of the ingredients of an Ayurvedic formulation Ayaskrti. The important analgesic prototypes (Salicylic acid and morphine) were originally derived from plant sources and traditionally used as pain killers. Most of the pharmaceutical industries depend on these plants for the secondary metabolites for the development of health care products. The secondary metabolites are the phytochemical constituents present in the crude extracts of the plants (Gomathi *et al*., 2015) [1]. In India, there is an increasing demand for natural products from plant sources due to their medicinal properties and safety issues. According to World Health Organization, about 80% of the population follows plant-based traditional medicines for primary healthcare (WHO, 1993) [2]. Traditional systems of medicines are prepared from a single plant species or combinations of several plants species. The bioactive component of the plant may be derived from any parts of the plant like leaves, roots, bark, flowers, fruits, and seeds (Gordon & David, 2003) [3]. Plant-based medicines that are derived from crude leaf extracts contain different phytochemicals. These phytochemicals are the bioactive principles having a unique and complex structure to treat various ailments. Screening of plants by chromatographic methods provides information on its pharmacological activities which help to select the plant of medicinal property (Juszczak *et al*., 2019) [4]. Gas chromatography-mass spectrometry (GC–MS) is the accurate technique employed for the detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants (Satapute *et al*., 2019) [5].

In this paper, the GC-MS analysis of leaf extract has been studied as part of the exploration for bioactive compounds.

Materials and Methods

Fresh leaves of *Anogeissus latifolia* were collected from Nilgiris of Tamil Nadu. The *Anogeissus latifolia* leaves were washed thoroughly with distilled water and shade dried for 5 days at room temperature. The dried leaves were finely ground using an electric grinder and stored in air-tight containers for further use.

Soxhlet extraction method was used to extract the *Anogeissus latifolia* leaves and the extraction process was performed using solvent *viz*., hexane. The extraction process was done for 6 hours at constant temperature of 60 °C. Repeated extraction was done with the same solvent until a clear colorless solvent was obtained. Obtained extract was evaporated to dryness and stored at 4 °C in an airtight container for further use.

The chemical composition of the leaf extract was analysed using GC – MS instrument (Perkin Elmer Clarus SQ8C make) and the column DB 5 - MS capillary standard non - polar column (30 m, ID: 0.25 mm and film thickness of 0.25 µm). One micro litre of hexane extract was injected for analysis and Helium was used as a carrier gas at 1 mL/ min. The instrument was set as follows, Injector port temperature set to 250 °C, source kept at 220 °C. The oven temperature was programmed from 70 °C to 260 °C at the 5 °C/ min rate. The MS was set to scan from 50 - 500 Da. The MS also had inbuilt pre - filter which reduced the neutral particles. The data system had two inbuilt libraries for searching and matching the spectrum, NIST4 and WILEY9 containing more than five million references.

Result and Discussion

GC-MS is the best technique to identify the bioactive constituents such as long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino, and nitro compounds present in plant species. Hence, gas chromatography (GC) and mass spectroscopy (MS) associated with particular detection techniques have become sophisticated means for analysis of various compounds of medicinal importance. The GC-MS chromatogram of the hexane leaf extract of *Anogeissus latifolia* showed the peaks indicating the presence of 40 bioactive compounds in Fig. 1. The compounds with their molecular formula, molecular weight and match were presented in Table 1.

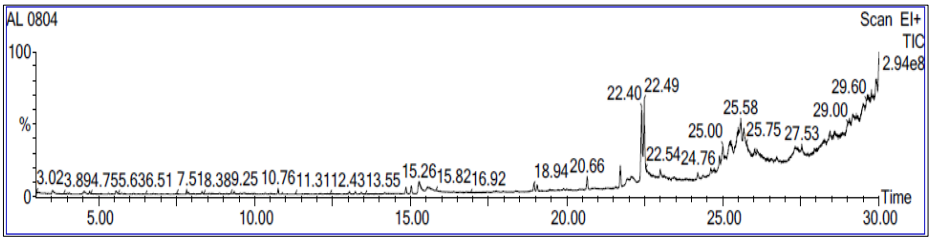

Fig 1: GC-MS spectrum of hexane extract of *Anogeissus latifolia*

The major bioactive compounds identified were Phthalic acid, hex-3-yl isobutyl ester, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Dibutyl phthalate, Milnacipran, 9,12-Octadecadienoic acid (Z,Z)-, 10-Octadecenoic acid, methyl ester, trans-13-Octadecenoic acid, Heptaethylene glycol monododecyl ether, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester. The name of the major compounds and their bioactivity were highlighted in Table 2.

Table 1: Phytochemical profiling of hexane extract of *Anogeissus latifolia* leaf sample

Sl. No	Chemical Component	Match	Molecular formula	Molecular Weight (g/mol)
1.	N-Methyl-2-[1,1-dicyano-2-(4-methoxyphenyl)-vinylimino]pyrrolidin	391	$C_{16}H_{16}N_4O$	280.32
2.	N-[3,5-Dinitropyridin-2-yl]proline	393	$C_{10}H_{10}N_4O_6$	282.21
3.	Dihydroxyacetone	653	$C_3H_6O_3$	90.08
4.	Methane, nitro-	780	CH_3NO_2	61.04
5.	1,3-Benzenediol, 4-propyl-	818	$C_9H_{12}O_2$	152.19
6.	1,3,5-Benzenetriol	752	$C_6H_6O_3$	126.11
7.	6-Oxododecanedioic acid	388	$C_{12}H_{20}O_5$	244.28
8.	Phthalic acid, hex-3-yl isobutyl ester	853	$C_{18}H_{26}O_4$	306.4
9.	Hexadecanoic acid, methyl ester	817	$C_{17}H_{34}O_2$	270.5
10.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	647	$C_{19}H_{38}O_4$	330.5
11.	Glycerol 1-palmitate	511	$C_{19}H_{38}O_4$	330.5
12.	n-Hexadecanoic acid	874	$C_{16}H_{32}O_2$	256.42
13.	Dibutyl phthalate	931	$C_{16}H_{22}O_4$	278.34
14.	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9-(acetyloxy)-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9a-trihydroxy-3-(hydroxymethyl)-1,1,6,8- tetramethyl-, [1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-	423		
15.	17-Pentatriacontene	580	$C_{35}H_{70}$	490.9
16.	(1S,14S)-Bicyclo[12.10.0]-3,6,9,12,15,18,21,24-octaoxatetracosane	492	$C_{16}H_{30}O_8$	350.4
17.	Milnacipran	806	$C_{15}H_{22}N_2O$	246.35
18.	Delphinidin 3-O-glucoside cation	916	$C_{21}H_{21}O_{12}^+$	465.4
19.	Octaethylene glycol monododecyl ether	457	$C_{28}H_{58}O_9$	538.8
20.	10,13-Octadecadienoic acid, methyl ester	629	$C_{19}H_{34}O_2$	294.5

21.	10-Octadecenoic acid, methyl ester	687	$C_{19}H_{36}O_3$	312.5
22.	Pregna-6,16-diene-11,20-diol, 3,9-epoxy-18-[N-methyl-N-[14-(2'- epoxyethyl)]amino]	463	$C_{25}H_{37}NO_5$	431.6
23.	(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	580		
24.	9,12-Octadecadienoic acid (Z,Z)-	717	$C_{18}H_{32}O_2$	280.4
25.	trans-13-Octadecenoic acid	637	$C_{18}H_{34}O_2$	282.5
26.	Heptaethylene glycol monododecyl ether	485	$C_{26}H_{54}O_8$	494.7
27.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	483	$C_{39}H_{76}O_5$	625
28.	Androst-4-en-11-ol-3,17-dione, 9-thiocyanato-	416	$C_{20}H_{25}NO_3S$	359.5
29.	Acetic acid, 17-(4-hydroxy-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14- pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17- tetradecahydrocyclopenta[a]phenanthryl ester	438	$C_{33}H_{56}O_4$	516.799
30.	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	501	$C_{34}H_{64}O_2$	504.9
31.	Heptaethylene glycol monododecyl ether	539	$C_{26}H_{54}O_8$	494.7
32.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	523	$C_{35}H_{68}O_5$	568.9
33.	Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3- (hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2- e]azulene-9,9a-diyl ester, [1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-	462		
34.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	531	$C_{35}H_{68}O_5$	568.9
35.	Docosanoic acid, 1,2,3-propanetriyl ester	462	$C_{69}H_{134}O_6$	1059.8
36.	5aH-3a,12-Methano-1H-cyclopropa[5',6']cyclodeca[1',2':1,5]cyclopenta[1,2- d][1,3]dioxol-13-one, 1a,2,3,9,12,12a-hexahydro-9-hydroxy-10- (hydroxymethyl)-1,1,3,5,7,7-hexamethyl-, [1aR-(1aà,3à,3aà,5aà,8aR*,9á, 12à,12aà)]-	470		
37.	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)- cyclohexanol	468	$C_{30}H_{52}O$	428.7
38.	15,17,19,21-Hexatriacontatetrayne	485	$C_{36}H_{58}$	490.8
39.	Hexa-t-butylselenatrisiletane	501	$C_{24}H_{54}SeSi_3$	505.9
40.	Phthalic acid, decyl isobutyl ester	536	$C_{22}H_{34}O_4$	362.5

Table 2: Major Phytochemicals identified in the hexane extract of *Anogeissus latifolia* leaf sample and their reported activity

Peak No	Compound Name	Nature of the component	Mol. formula	Mol. wt g/mol	Reported activity
1	Phthalic acid, hex-3-yl isobutyl ester	Aromatic compound	$C_{18}H_{26}O_4$	306.4	Antioxidant and anticancer
2	Hexadecanoic acid, methyl ester	Fatty acid methyl esters	$C_{17}H_{34}O_2$	270.5	Antioxidant, pesticide
3	n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256.42	Antioxidant, nematicide, pesticide, antiandrogenic
4	Dibutyl phthalate	Fatty acid	$C_{16}H_{22}O_4$	278.34	Plasticizer
5	Milnacipran	Serotonin	$C_{15}H_{22}N_2O$	246.35	Local anesthetic and a drug allergen.
6	9,12-Octadecadienoic acid (Z,Z)-	Lineolic acids	$C_{18}H_{32}O_2$	280.4	Biosynthesis of prostaglandins and cell membranes
7	10-Octadecenoic acid, methyl ester	Fatty acid	$C_{19}H_{36}O_3$	312.5	Antioxidant and anticancer
8	trans-13-Octadecenoic acid	Linoleic acid	$C_{18}H_{34}O_2$	282.5	Antioxidant
9	Heptaethylene glycol monododecyl ether	Steroids	$C_{26}H_{54}O_8$	494.7	Local anesthetic, treatment of esophageal and gastric varices and varicose veins
10	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Fatty acid ethyl ester	$C_{35}H_{68}O_5$	568.9	Anti microbial activity

GC-MS analysis of phytoconstituents in the hexane leaf extracts of *Anogeissus latifolia* highlights the pharmaceutical importance of the plant. The identified bioactive compounds occupy many biological properties. The present results are in agreement with the reports cited by earlier workers where compounds have been detected which are fatty acids such as hexadecenoic acid, terpenes and aromatic compounds like phthalic acid and which have antimicrobial activity (Aliyu and Sani, 2011) [7]. The compounds such as phthalic acid has antimicrobial activity. 9-Octadecanoic acid, ethyl ester has anti-inflamatory, anticancer, hypocholesterolemic and hexadecanoic acidmethyl ester have antioxidant, hypocholesterolemic, nematicide (Ahmad, 2014) [6]. Each compound identified was found to have a unique significant property to treat various diseases. Yet, isolation of unique phytocompound of pharmacological activity paves the way in the fled of drug discovery. The GCMS chromatogram of *Anogeissus latifolia* leaf extract with high percentage concentration of fatty acids and their derivatives which have been established by many scientific researches as potent antimicrobials may validate its use as a herbal treatment for many microbial diseases

Conclusion

GC-MS analysis of hexane leaf extract of *Anogeissus latifolia* revealed the presence of bioactive compounds like fatty acid, aromatic compounds, steroids, alkaloids, Serotonin and Linoleic acid. Among the identified compounds, Phthalic acid, hex-3-yl isobutyl ester, Hexadecanoic acid, n-Hexadecanoic acid, Dibutyl phthalate, Milnacipran, 9,12-Octadecadienoic acid (Z,Z) and 10-Octadecenoic acid, methyl ester are the major compounds that might contribute to biological activities such as antioxidant, anti-microbial, anti-cancer, anti-diabetic, pesticide and Plasticizer properties. Therefore, the present study results conclude that *Anogeissus latifolia* may serve as a potent source of medicinal compounds responsible for its pharmacological activities. However, further studies are essential to isolate, characterize, and purify the active components responsible for therapeutic activity.

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