Research Article

Phytochemical, Fatty Acid Analysis of Ethanol Extract of *Ceiba* Seeds Using Gas Chromatography-Mass Spectrophotometry and Its Antimicrobial Potential

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ABSTRACT

Biodiversity brings vast profits to mankind from direct harvesting of plants for food, medicine, fuel, building material, and other uses to aesthetic, cultural, recreational and research values. Plants have been used to treat or prevent illness from ancient times. Hence, our present study was planned and *Ceiba* seeds were extracted with ethanol, hexane and phytochemicals, fatty acids were assessed through GC-MS. The compound observed in plenty was 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-, n-Hexadecanoic acid with respect to phytoconstituents. Hexadecanoic acid, methyl ester, 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester are the fatty acids that was higher in concentration when compared to other fatty acids.

Keywords: *Ceiba*, FAME, GC-MS, Phytoconstituents, Seeds.

INTRODUCTION

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic Phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies.[1] *Ceiba pentandra* L. belongs to the Malvaceae family.[2] It was native to Southeast Asia and cultivated in Southeast Asia, India, Sri Lanka and tropical America.[3,4] It was grown naturally in humid and sub humid tropical region. It is a deciduous tree, up to 30m tall; bark grey, covered with black, conical prickles. Leaves digitate, crowded at the ends of branches; leaflets 5-7, elliptic-lanceolate to ovate-lanceolate, glabrous, 5-23 x 1.5-9 cm, petioles long, petiolules small. Flowers bright red, crowded at the ends of branches, 8-14 cm across, appearing before the new leaves. Capsules woody, ellipsoid, 10-12cm long, 5-valved. Seed embedded in white, silky cotton, ovoid. *Ceiba pentandra* is generally drought-resistant tree and pods from these trees are leathery, ellipsoid and pendulous capsule.[5] *Ceiba pentandra* seeds occupy about 25–28% (w/w) of each fruit.[6] Emerging growth in population and huge growth of industries contributes to the depletion of fossil fuel. Hence, finding an alternate fuel is a must and methyl esters from vegetable oils and animal fats are alternative sources to fossil fuel.[7] Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. Since, seeds are the primary stage of plant life cycle, they have strong defense mechanism possibly due to the presence of phytoconstituents contributing to its antioxidant activity.[8] Hence, an attempt was initiated to study the phytoconstituents, fatty acids contained in it, and also its antimicrobial activity.

MATERIALS AND METHODS

Sample used for the study

The *Ceiba* seeds were purchased from local markets at Krishnagiri District, Tamil Nadu, India. The seeds purchased were cleaned, freed from debris and healthy seeds selected were powdered. The powdered seeds were transferred in to an air tight plastic container.

Extraction process

10gm of powdered samples were extracted with 30ml ethanol overnight and filtered. The extract was concentrated to 1ml by bubbling nitrogen in to the solution. 2µl of the ethanolic extract was employed for GC-MS analysis for the analysis of phytochemical compounds by GC-MS.[9] Further, the given samples were extracted with hexane and the extract was methylated and analyzed through GCMS for the identification of the fatty acid profile.

GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-5MS fused capillary column (30 x 0.25mm x 0.25µm df) composed of 5% Diphenyl / 95% Dimethyl poly siloxane. Helium (99.999%) was used as carrier gas at a constant flow of 1ml per min and injection volume of 2µl was adopted (split ratio of 10:1) The injected sample was detected by Turbo mass gold detector (Perkin Elmer) with the aid of Turbomass 5.2 software. During the 36 minute GC extraction process, the oven temperature was programmed from of 110°C with an increase of 10° C/min up to 200°C, then 5°C/min

up to 280°C (9 minutes hold). The injector temperature was set at 250°C (mass analyzer). Other parameters involved in the operation of Clarus 500MS, was also standardized (Inlet line temperature: 200° C; Source temperature: 200°C). Mass spectra were taken at 70eV and fragments from 45-450 Da. The MS detection was completed in 36 minutes. The detection employed the NIST (National Institute of Standards and Technology) - Year 2005 library.

Antimicrobial Assay

The antimicrobial activity was assessed by the method of Kirby-Bauer disk diffusion method.[10] Results of the zone of inhibition was observed and measured after 24hr period of incubation time.

RESULTS AND DISCUSSION

Gas chromatography and mass spectrometry is an effective combination for chemical analysis. The results of various phytochemicals observed are depicted in Table.1 and the chromatogram is shown in Figure 1.

Table 1: Bio-active compounds identified in *Ceiba* seeds [GC-MS study]

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	10.36	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.07
2	11.71	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.10
3	12.21	E-11-Hexadecenoic acid, ethyl ester	$C_{18}H_{34}O_2$	282	0.03
4	12.56	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.41
5	13.49	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.77
6	14.71	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	2.28
7	14.96	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.48
8	16.57	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	70.40
9	19.08	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	0.10
10	20.65	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	0.15
11	22.26	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	1.24
12	23.14	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.16
13	24.38	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356	0.12
14	25.62	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	0.17
15	26.42	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_2$	320	0.27
16	30.03	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.26

Parameters tested are not covered under the scope of NABL accreditation

Table 2: Fatty acids identified in *Ceiba* seeds [GC-MS study]

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	9.69	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	0.24
2	10.32	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.02
3	10.74	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	0.05
4	11.51	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268	0.52
5	11.82	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	40.43
6	12.54	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.91
7	13.91	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	34.67
8	14.09	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	8.59
9	15.10	10-Nonadecenoic acid, methyl ester	$C_{20}H_{38}O_2$	310	5.68
10	16.25	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240	0.68
11	16.48	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	2.29
12	16.62	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	2.48
13	17.81	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	1.11
14	19.34	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	1.34

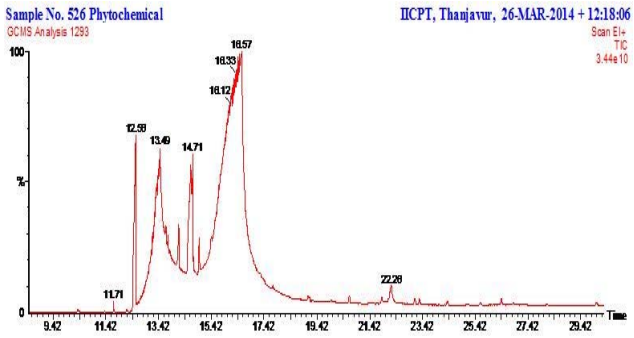

Figure 1: Chromatogram of phytochemicals in *Ceiba* seeds

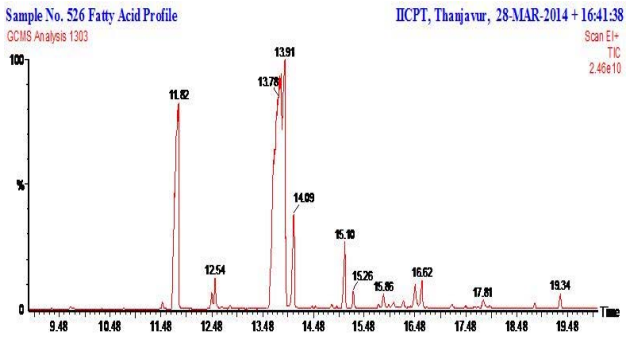

Figure 2: Chromatogram of fatty acids identified in *Ceiba* seeds

Phytochemical analysis

Sixteen phytochemicals were identified through GC-MS analysis. Compound 1 was identified as Tetradecanoic acid ($C_{14}H_{28}O_2$) having peak area 0.07%. Compound 2 identified was Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) with 0.10 peak area %. Compound 3 identified was E-11-Hexadecenoic acid, ethyl ester ($C_{18}H_{34}O_2$), have 0.03 as observed peak area %. Compound 4 identified was Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$) with 4.41% peak area. The compound 5 identified was n-Hexadecanoic acid ($C_{16}H_{32}O_2$), showing higher peak area percent of 19.77. Compound 6 identified was 9, 12- Octadecadienoic acid (Z, Z) ($C_{18}H_{32}O_2$) with peak area percent as 2.28. The seventh compound identified was Octadecanoic acid, ethyl ester ($C_{20}H_{40}O_2$) showing peak area % as 0.48. The eighth compound identified was 9, 12, 15,-Octadecatrienoic acid (Z, Z, Z,) ($C_{18}H_{30}O_2$) having peak area percent as 70.40. Compound nine identified was 12-Methyl -E, E-2, 13 -octadecadien-1-ol ($C_{19}H_{36}O$) showing 0.10 peak percent. Compound 10 identified was (E)-9-Octadecenoic acid ethyl ester ($C_{20}H_{38}O_2$) with 0.15 peak area %. Compound 11 identified was linoleic acid ethyl ester ($C_{20}H_{36}O_2$) having 1.24 as peak area %. Compound 12 identified was 8, 11, 14- Eicosatrienoic acid (Z, Z, Z) ($C_{20}H_{34}O_2$) having 0.16 as peak area %. Compound 13 identified was 9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxyl methyl) ethyl ester ($C_{21}H_{40}O_4$) showing 0.12 peak area %. Compound 14 identified was Z-(13, 14-Epoxy) tetradec-11-en-1-ol acetate ($C_{16}H_{28}O_3$) with 0.17 peak area %. Compound 15 identified was 8, 11, 14-Eicosatrienoic acid, methyl ester (Z,Z,Z) showing 0.27 peak area %. Compound 16 identified was 1-Heptatriacotanol ($C_{37}H_{76}O$) with 0.26 as peak area percent. Among the compounds identified, compound 8 showed highest peak area percent, followed by compound 5, 4 and compound 6. Very low peak area percent was observed with compound 3. The retention time/amount of time that a compound is retained in the GC column observed was from 10.36 to 30.03, which helps to differentiate various compounds. (Table 1, Figure 1) Each component ideally produces a specific spectral peak, measured from the baseline to the tip of the peak. The size of the peak obtained is proportional to the amount of substances in the specimen analyzed.

Fatty acid profile

The results of fatty acid profile of *Ceiba* seed is shown in Table.2 and Fig.2. Fourteen components were identified when fatty acid profile was assessed through GC-MS. The compounds identified was Decanoic acid, methyl ester ($C_{11}H_{22}O_2$) , Tetradecanoic acid, ethyl ester ($C_{16}H_{32}O_2$), Pentadecanoic acid, methyl ester ($C_{16}H_{32}O_2$), 7-Hexadecenoic acid, methyl ester, (Z)- ($C_{17}H_{32}O_2$), Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester ($C_{19}H_{34}O_2$), Octadecanoic acid, methyl ester ($C_{19}H_{38}O_2$), 10-Nonadecenoic acid, methyl ester ($C_{20}H_{38}O_2$), Z-8-Methyl-9-tetradecenoic acid ($C_{15}H_{28}O_2$), 11-Eicosenoic acid, methyl ester ($C_{21}H_{40}O_2$), Eicosanoic acid, methyl ester ($C_{21}H_{42}O_2$), 11,14-Eicosadienoic acid, methyl ester ($C_{21}H_{38}O_2$), Docosanoic acid, methyl ester ($C_{23}H_{46}O_2$). The highest peak area percent was observed with compound 5 and 7. Compound 8 showed peak area percent of 8.59 whereas it was 5.68 with compound 9. The peak area percent observed was 2.48, 2.29 for compound 12 and 11. Compound 6, 14 and 13 showed 1.91, 1.34, 1.11 as peak area percent. All the other compounds showed lesser peak area percent. (Table 2, Figure 2)

Antimicrobial activity

Table 3: Antimicrobial activity of *Ceiba* seeds

Microorganisms	Zone of Inhibition (mm)	
	50 µg	100 µg
Aspergillus niger	12	13
Aspergillus flavus	12	14
Escherichia Coli	14	17
Staphylococcus aureus	16	17

Parameters tested are not covered under the scope of NABL accreditation

The results of antimicrobial activity are shown in Table 3. Antimicrobial activity was performed against fungi and bacteria. The fungi selected were *Aspergillus niger, Aspergillus flavus* and bacteria such as *Escherichia coli, Staphylococcus aureus* was selected for the study. Among the antimicrobial activity tested, the powder was found to be active against bacteria on comparison with fungi. Both

50 and 100µg concentrations showed similar inhibitory activity. (Table 3)

CONCLUSION

Copious reports are there in literature regarding the presence of bioactive substances in plant seeds. Seeds from plants are important for their nutritional, industrial, medicinal values. Since seeds are the primary stage of plant life cycle, have strong defense mechanism possibly due to the presence of phytoconstituents contributing to antioxidant and antimicrobial activity.

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