Research Article

GC-MS Analysis of Phytochemicals, Fatty acid Profile, Antimicrobial Activity of Gossypium Seeds

Marimuthu Krishnaveni*, Ravi Dhanalakshmi, Nagaraj Nandhini
*Assistant Professor, Department of Biochemistry, Periyar University, Salem, Tamilnadu, India.
M.Phil Students, Department of Biochemistry, Periyar University, Salem, Tamilnadu, India.
***Corresponding author's E-mail:** logasarvesh@gmail.com,

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ABSTRACT

A major portion of cotton production is occupying textile industry. The seeds of cotton were selected for the present study to know its phytochemical components, fatty acids present and also its antimicrobial activity. According to our GC-MS results obtained for phytochemical analysis, 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- shows highest peak area percent and it is a unsaturated in nature. n-Hexadecanoic acid otherwise called palmitic acid is showing higher peak area percent and then followed by linoleic acid ethyl ester, 12-Methyl-E,E-2,13-octadecadien-1-ol and also found to contain ç-Sitosterol, ç-Tocopherol, Vitamin E in trace amount. Likewise, among the fatty acids studied 9,12-Octadecadienoic acid (Z,Z)-, Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid (Z,Z)-methyl ester was found to be high. Traces of margaric acid methyl ester were also observed along with other methyl esters. It was found to be active in killing bacteria than fungi.

Keywords: Antimicrobial activity, Fatty acid profile, GC-MS, Gossypium, Phytochemicals.

INTRODUCTION

Cotton is currently the leading plant fibre crop worldwide and is grown commercially in the temperate and tropical regions of more than 50 countries.[1] It is estimated that cotton is cultivated on approximately 2.4% of the worlds arable land.[2] The genus Gossypium was named by Linneaus in the middle of the 18th century. It is in the family Malvaceae, Order Malvales and Tribe Gossypieae. *Gossypium barbadense* L was named after its assumed habitat of Barbadas and a cotton variety of India. The common name cotton comes from the Arabic 'quotn' and generally refers to species that produces spinnable fibres (lint) on their seed coat.[3] The oldest known words for cotton are Karparsai, in the language Sanskrit and Karapas used in early Bible manuscripts.[4] Hybrid cotton consisting of either intra specific or inter specific hybrids between G.hirsutum and G. barbadense is widely grown in some countries including India and China. In India seeds of hybrid cotton are commercially produced by hand emasculation and pollination, or hand pollination of male sterile lines. Cotton is primarily grown as a fiber crop. It is harvested as seed cotton which is then ginned to separate the seed and lint. The long lint fibers are further processed by spinning to produce yarn that is knitted or woven in to fabrics. Cotton fabrics, used in clothing, upholstery, towels and other household products are made from cotton lint. Approximately five to seven days after a flower appears it usually dries and falls from the plants exposing the developing cotton fruit or boll.[5] The cotton seed are large, covered with thick fibers and enclosed in a tough ball that retains most of the seeds on the plant.[6] Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance.[7] The suitability of oil for a particular purpose, however, is determined by its characteristics and fatty acid composition. The world production of fatty acids (FAs) from the hydrolysis of natural fats and oils totals about 4 million metric tons per year. FAs are utilized in a wide variety of end-use industries that include food, medicine, rubber, plastics, detergents, and cosmetics.[8] Fats and oils make up the greatest proportion of raw materials in the chemical industry.[9] In this study, we analyzed through GC-MS the phytochemicals, fatty acid profile, antimicrobial activity of Gossypium seeds in order to find the phytochemicals, fatty acids present. The seeds were purchased from local market at Krishnagiri, Krishnagiri District, Tamil Nadu, India.

MATERIALS METHODS

Sample used for the study

The seeds were purchased from local markets at Krishnagiri, Krishnagiri District, Tamil Nadu, India. The seeds purchased were cleaned, freed from debris and then grounded to obtain powder. The powdered seeds were transferred in to an air tight plastic container and used for analysis.

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. 2 µl of the ethanolic extract was employed for GC-MS analysis for the identification of phytochemical compounds.[10] Further, the given samples were extracted with hexane and methylated, analyzed through GC-MS for 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 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.[11]

Table 1: Phytochemical Components identified in the Gossypium seeds by GC- MS

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	10.41	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.43
2	11.70	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.14
3	12.19	E-11-Hexadecenoic acid, ethyl ester	$C_{18}H_{34}O_2$	282	0.02
4	12.44	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.41
5	13.27	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.01
6	14.51	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.62
7	16.16	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	70.10
8	18.85	Hexadecanal, 2-methyl-	$C_{17}H_{34}O$	254	0.34
9	19.82	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	1.14
10	22.22	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	4.65
11	26.37	ç-Tocopherol	$C_{28}H_{48}O_2$	416	0.20
12	27.30	Vitamin E	$C_{29}H_{50}O_2$	430	0.26
13	30.04	ç-Sitosterol	$C_{29}H_{50}O$	414	0.67

*Parameters tested are not covered under the scope of NABL accreditation

Table 2: Fatty Acid Profile of Gossypium seeds analyzed by GC-MS

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	9.64	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	1.29
2	10.36	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.33
3	11.50	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268	0.90
4	11.87	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	29.70
5	12.50	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	8.33
6	12.83	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284	0.18
7	14.06	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	13.74
8	14.20	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	6.70
9	14.65	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	31.01
10	14.91	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	1.09
11	15.14	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	312	0.98
12	16.02	9-Octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	294	3.05
13	16.29	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	0.42
14	16.65	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	1.08
15	17.11	E-11-Hexadecenoic acid, ethyl ester	$C_{18}H_{34}O_2$	282	0.25
16	17.50	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368	0.18
17	19.34	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	0.77

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. Similarly, the fatty acid profile is depicted in Table 2 and Figure 2.

Phytochemical analysis

GC-MS is used both for the qualitative identification and for the quantitative measurement of individual components in complex mixtures. The heights of the peaks or the area under the peaks provide a quantitative measure of the amount of each component. The phytoconstituents present in the Gossypium seeds are depicted in Table 1. Compound 1 identified was Tetradecanoic acid $C_{14}H_{28}O_2$ (0.43%), compound 2 identified was Hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$ (0.14%), compound 3 identified was E-11-Hexadecenoic acid, ethyl ester $C_{18}H_{34}O_2$ (0.02%), compound 4 identified was Hexadecanoic acid ethyl ester, $C_{18}H_{36}O_2$ (0.41), compound 5 identified was $C_{16}H_{32}O_2$ (21.01%), compound 6 identified was 9,12- Octadecadienoic acid (Z,Z) $C_{18}H_{32}O_2$ (0.62), compound 7 identified was 9,12,15-Octadecatrienoic acid (Z,Z,Z) (70.10%), compound 8 identified was Hexadecanal 2 methyl $C_{17}H_{34}O$ (0.34%), compound 9 identified was 12-Methyl-E,E-2,13-octadecadien-1-ol $C_{19}H_{36}O$ (1.14%), compound 10 identified was linoleic acid ethyl ester $C_{20}H_{36}O_2$ (4.65%), compound 11 identified was ç-Tocopherol $C_{28}H_{48}O_2$ (0.20%), compound 12 identified was Vitamiin E $C_{29}H_{50}O_2$ (0.26%), compound 13 identified was ç-Sitosterol $C_{29}H_{50}O$ (0.67%). The retention time observed was 10.41 to 30.04 for different compounds with molecular weights ranging from 228 to 414. The highest peak area percent observed was with compound 7 and next fall's compound 5, 10 9. Trace amount of ç-Sitosterol, ç-Tocopherol, Vitamiin E were also observed (Table 1, Figure 1).

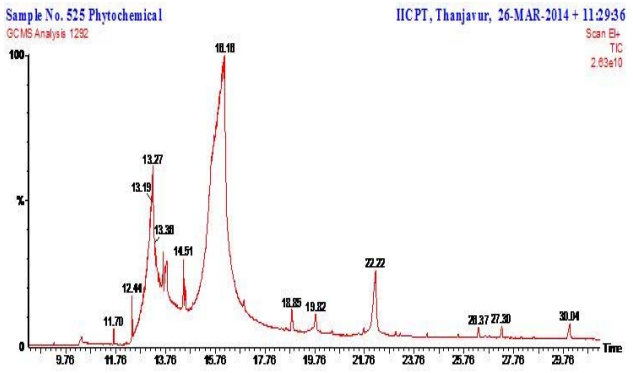

Figure 1: Chromatogram showing phytochemicals in Gossypium seeds

Fatty acid profile

The fatty acid profile of the sample is depicted in Table.2 and chromatogram in Figure 2. The compound 1 identified was Decanoic acid, methyl ester $C_{11}H_{22}O_2$ (1.29%), compound 2 identified was Tetradecanoic acid, ethyl ester $C_{16}H_{32}O_2$ (0.33%), compound 3 identified was 7- Hexadecenoic acid, methyl ester (Z) $C_{17}H_{32}O_2$ (0.90%), compound 4 identified was Hexadecanoic acid methyl ester $C_{17}H_{34}O_2$(29.70%), compound 5 identified was Hexadecanoic acid ethyl ester $C_{18}H_{36}O_2$ (8.33%), compound 6 identified was Heptadecanoic acid methyl ester $C_{18}H_{36}O_2$(0.18%), compound 7 identified was 9,12 Octadecanoic acid (Z,Z) methyl ester $C_{19}H_{34}O_2$(13.74%), compound 8 identified was Octadecanoic acid methyl ester $C_{19}H_{38}O_2$(6.70%), compound 9 identified was 9,12-Octadecadienoic acid (Z,Z) $C_{18}H_{32}O_2$(31.01%), compound 10 identified was Octadecanoic acid ethyl ester $C_{20}H_{40}O_2$(1.09%), compound 11 identified was Nonadecanoic acid methyl ester $C_{20}H_{40}O_2$ (0.98%), compound 12 identified was 9-Octadecynoic acid methyl ester $C_{19}H_{34}O_2$(3.05%), compound 13 identified was 11-Eicosenoic acid methyl ester $C_{21}H_{40}O_2$ (0.42%), compound 14 identified was Eicosanoic acid methyl ester $C_{21}H_{42}O_2$(1.08%), compound 15 identified was E-11-Hexadecenoic acid ethyl ester $C_{18}H_{34}O_2$ (0.25%), compound 16 identified was Docosanoic acid ethyl ester $C_{24}H_{48}O_2$(0.18%), compound 17 identified was Docosanoic acid methyl ester $C_{23}H_{46}O_2$(0.77%). The peak area observed is given in decreasing order: compound 9, compound 4, compound 7, compound 5, compound 8, compound 12, compound 1, compound 10, compound 14, compound 11, compound 3, compound 17, compound 13, compound 15, compound 2, compound 6 & 16. The molecular weight of the identified compounds ranges from 186 to 354. (Table 2, Figure 2). Heptadecanoic acid otherwise called margaric acid is a saturated fatty acid, amid palmitic acid and stearic acid, helps in reducing cardiovascular diseases. 9,12-Octadecadienoic acid (Z,Z) is otherwise called as omega 6 unsaturated fatty acid. Likewise, 7- Hexadecenoic acid, methyl ester (Z) is a mono unsaturated fatty acid. The amount of saturated fatty acid present is more when compared to unsaturated fatty acid. (Table 2)

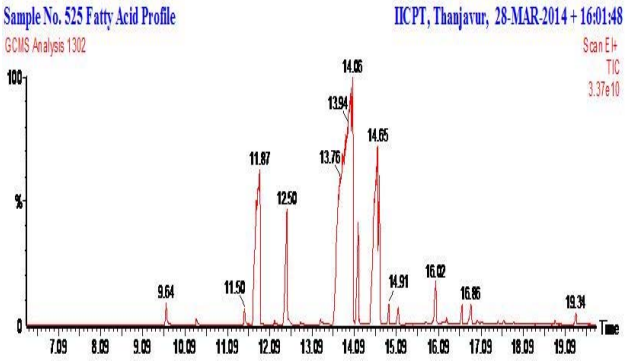

Figure 2: Chromatogram of Gossypium seeds showing fatty acid profile

Antimicrobial activity

Gossypium seed powder is able to kill *Aspergillus niger, Aspergillus flavus*. Likewise, it is also active against *Escherichia coli*, *Staphylococcus aureus*. The seed powder is found to be both antifungal and antibacterial in nature at 50, 100µg concentration. It was found to be more active in killing bacteria. (Table 3) The antimicrobial

activity exhibited with Gossypium seed powder is due to the presence of phytochemicals contained in it.

Table 3: Antimicrobial activity of Gossypium Seeds

Microorganisms	Zone of Inhibition (mm)	
	50 µg	100 µg
Aspergillus niger	11	16
Aspergillus flavus	12	13
Escherichia coli	13	20
Staphylococcus aureus	20	20

CONCLUSION

Phytochemical investigation provides an opportunity for taking up potential research, development for strengthening the existing pharmacological sciences besides, refining and utilizing traditional knowledge for the welfare of the society. Phytochemicals and fatty acids are the most important sources which is essential for the medicinal properties to be used as antimicrobial, antifungal agents. The phytochemical showing highest peak area was 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-linoleic acid, Hexadecanoic acid, methyl ester–Palmitic acid a saturated fatty acid, 9,12-Octadecadienoic acid (Z,Z)-methyl ester (linoleic acid methyl ester) are the fatty acids which are present in higher amount. Higher palmitic acid content can be used as good source of toiletry and laundry soap.

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