ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Suppl. 3, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF METHANOLIC LEAF EXTRACT OF *CASSIA ANGUSTIFOLIA* VAHL.

SHAHINA PARVEEN*, ANWAR SHAHZAD, ANAMICA UPADHYAY, VIKAS YADAV

Department of Botany, Plant Biotechnology Laboratory, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Email: shahina.sony3@gmail.com

Received: 05 August 2016, Revised and Accepted: 30 August 2016

ABSTRACT

Objective: The leaves of *Cassia angustifolia* Vahl. are employed for the treatment of several human diseases. Therefore, the present study was undertaken to determine the phytocomponents present in the methanolic extract of the leaves by gas chromatography-mass spectrometry (GC-MS)

Methods: The collected leaf samples were dried and extracted in methanol. Screening of the extract was done by GC-MS which is an important technique for the separation and identification of different phytochemicals.

Results: The methanolic extract of the leaves of *C. angustifolia* revealed the presence of 45 different phytochemicals. The prevailing compounds were 1 butanol, 3 methyl acetate (area % 7.47), 6, 6-dideutero-nonen-1-Ol-3 (area % 10.45), pentadecanoic acid (area % 9.22), and squalene (area % 12.30). Vitamin E (area % 3.85) has also been found in the leaf extract. Some of the compounds possess biological activities.

Conclusions: It can be concluded from the present study that some of the identified phytochemicals could be responsible for the medicinal value or biological activity of the plant leaves.

Keywords: Senna, Leaf extract, Methanol, Gas chromatography-mass spectrometry, Phytochemicals, Vitamin E.

 DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9s3.14512

INTRODUCTION

Each and every plant contains a certain specific type of chemical substances/compounds, which are produced during the normal growth and development of the plant body. These compounds are generally referred to as "phytochemicals" or sometimes also known as "secondary metabolites." These phytochemicals attribute to the medicinal value of the plant, and that's why, plants are being used to cure several diseases all over the world since time immemorial. Screening of active compounds present in the plant leads to the invention of new drugs which are used to cure various serious diseases such as cancer and Alzheimer's disease [1]. Medicinal plants are directly analyzed by gas chromatography-mass spectrometry (GC-MS) for their existing phytochemicals. GC-MS is an advanced technology to determine the presence of phytochemicals in plant tissues, which is extensively being utilized to screen the phytochemicals in medicinal plant species. Using GC-MS, it is now possible to identify volatile compounds with ease.

Cassia angustifolia Vahl. commonly known as "senna" is one of the most important medicinal shrubs belonging to family Fabaceae (Sub family: Caesalpinioideae). It is a native of Saudi Arabia and has been naturalized in India, Pakistan, and Egypt. The plant is a small shrub with usually 5-8 leaflets, lanceolate, glabrous, and axillary erect [2]. Senna is widely used in the Ayurvedic and Unani system of medicine. Leaves and pods are used as a febrifuge in splenic enlargements, curing various diseases such as anemia, typhoid, jaundice, and cholera as well as reported to be an excellent blood purifier. It has also been employed in the treatment of constipation, amoebic dysentery, as an anthelmintic and a mild liver stimulant. Plant contains rhein, chrysophanol, emodin, and aloe-emodin-a water-soluble glycoside, besides these, derivatives of anthraquinone glycosides are also present in the leaves and pods which are commonly referred as "sennosides" [2]. Considering the importance of *C. angustifolia*, this study was undertaken for the first time to analyze the bioactive compounds present in the methanolic extract of leaves of this plant.

METHODS

Collection of plant samples and extraction procedure

Leaves of *C. angustifolia* were collected from Botanical Garden of the Department of Botany, AMU, Aligarh. Only mature and healthy leaves were harvested and collected in the early morning, packed in polythene bags, and kept at room temperature for further processing. Leaves were washed with tap water and shade dried for 5-6 days. About 5 g of air dried leaves were grounded to fine powder in a mortar pestle. About 5 g of powdered leaves of senna were extracted with 100 ml methanol. It was left for 24 hrs so that terpenoids and other constituents if present will get dissolved. The methanolic extract was then filtered through syringe filter, and the residue was removed.

GC-MS analysis and identification of components

The GC-MS analysis of the methanolic extract of the leaves of *C. angustifolia* was performed using a GC-MS of Hewlettt-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with Agilent 7890A/5975 C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d.) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 80°C (2 minutes) to 280°C at 1-40°C/minutes, detector temperature 250-280°C, and carrier gas He (0.9 ml/minutes). 2.0 µl of respective diluted samples was manually injected in the splitless mode, with split ratio and with mass scan of 50-600 amu. Total running time of GC-MS is 40 minutes, the relative percentage of each extract constituent was expressed as a percentage with peak area normalization.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standards and Technology (NIST) and Wiley library for mass spectra, having more than 62000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight (MW), and structure of the components of the test materials were ascertained.

RESULTS

In the present study, a total of 45 different phytocomponents have been found in the methanolic extract of the leaves of *C. angutifolia*. The identified compounds of senna, their retention indices (RT), molecular formulae, molecular structure, MW, and percentage composition (area %) are given in Table 1. The results showed the presence of five major components with maximum percentage (12.3%) of squalene, followed by 6, 6-dideutero-nonen-1ol-3 (10.45%), pentadecanoic acid (9.22%), 1-butanol, 3 methyl-, acetate (7.47%), and phytol (6.49%). The leaf extract also showed the presence of tocopherol (Vitamin E, 3.85% with RT of 30.4 minutes) and gamma-tocopherol (0.58% with RT of 28.6). The GC-MS chromatogram also shows 45 different peaks which confirm the presence of 45 compounds with their respective RT (Fig. 1). On comparison of the mass spectra of each phytochemical with the NIST and Wiley library, 45 phytoconstituents were characterized and identified. The individual fragmentation of five major components is illustrated in Fig. 2 (A-E). Some of the identified components possess biological activities which are listed in Table 2. These biological activities are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service USDA [3].

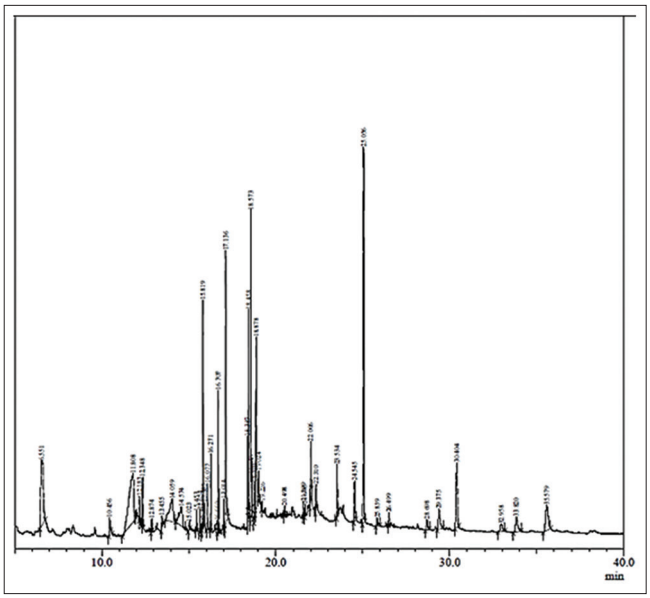


Fig. 1: Gas chromatography-mass spectrometry chromatogram of the methanolic leaf extract of Cassia angustifolia

DISCUSSION

GC-MS chromatogram of the methanolic extract of the leaves of *C. angustifolia* showed the presence of 45 different phytocomponents. From observations and comparison with the mass spectrum of each constituent with NIST and Wiley library, it was found that squalene, 6,6-Dideutero-Nonen-1-Ol-3, pentadecanoic acid, 1 butanol, 3 methyl acetate, and phytol are the major components (Fig. 2). Besides these compounds, vitamin E (3.85 %), gamma-tocopherol (0.58%), and several other antioxidants are also present.

Squalene, which constitutes about 12.3%, is a hydrocarbon which is used in cosmetics, as an immunologic adjuvant in vaccines and may be used as a chemopreventive substance that protects people from cancer [4,5]. However, pentadecanoic acid, which is 9.22% in the tested extract, is a saturated fatty acid and is rare in nature but found at the level of 1.2% in the milk of cows [6]. Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin

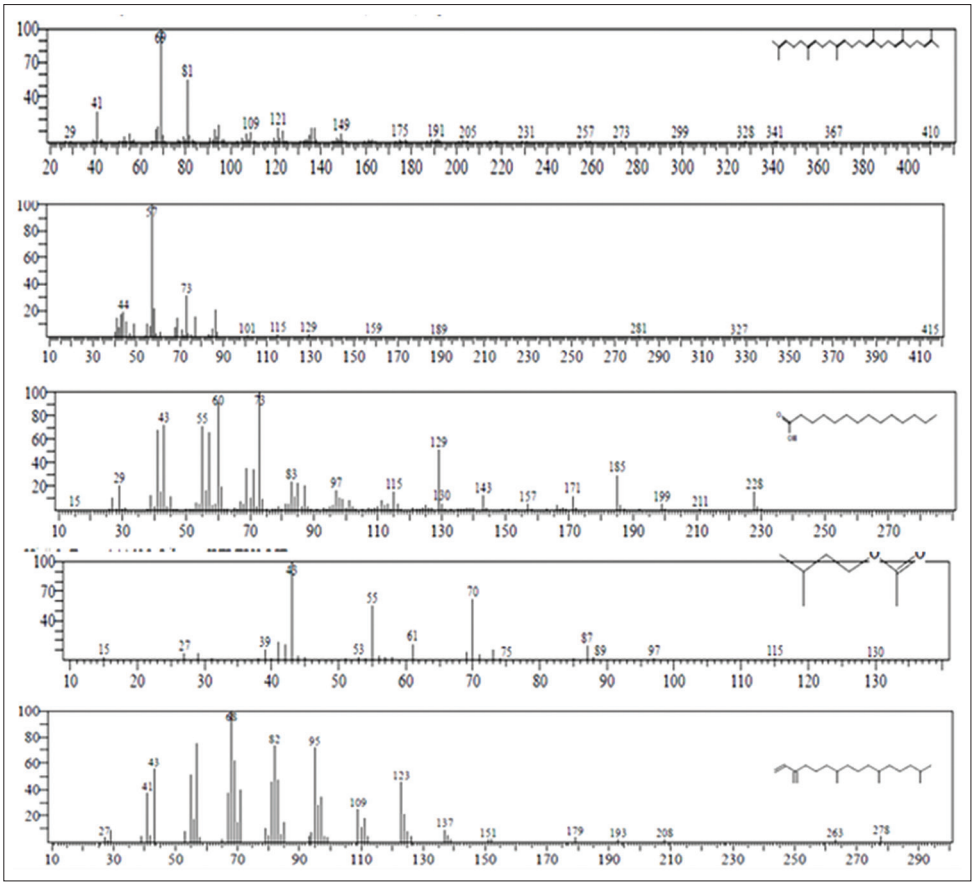


Fig. 2: Mass spectrum of (A) squalene (area % 12.30), (B) 6,6-dideutero-nonen-1-Ol-3 (area % 10.45), (C) pentadecanoic acid (area % 9.22), (D) 1 butanol, 3 methyl acetate (area % 7.47), and (E) phytol (6.49%)

Table 1: Phytocomponents detected in the methanolic leaf extract of *Cassia angustifolia*

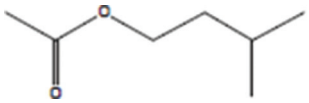
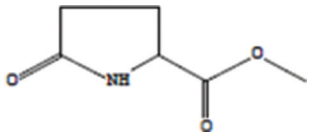
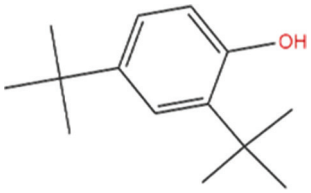
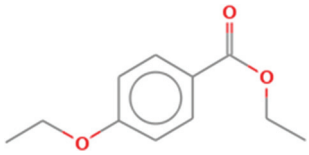
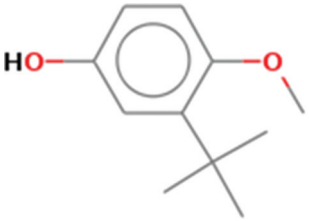
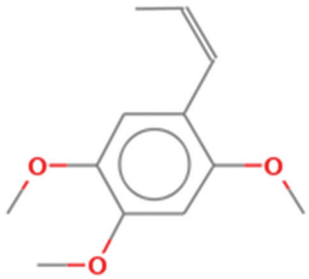
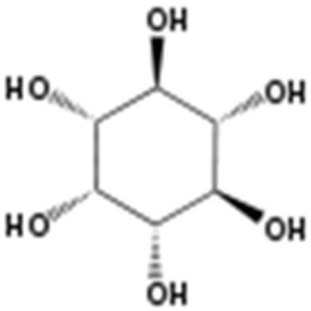
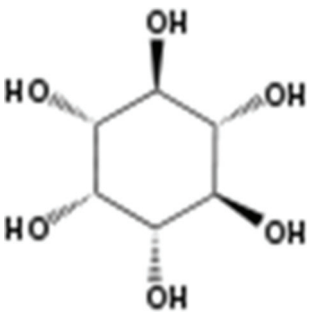
Name of the compounds	RT	Molecular formula	Molecular structure	MW	Area %
1-butanol, 3-methyl-, acetate	6.551	$C_7H_{14}O_2$		130	7.47
5-oxo-pyrrolidine-2-carboxylic acid methyl	10.456	$C_6H_9NO_3$		143	0.84
6,6-dideutero-Nonen-1-Ol-3	11.808	$C_9H_{16}D_2O$		144	10.45
Phenol, 2,4-bis (1,1-dimethylethyl)-	12.183	$C_{14}H_{22}O$		206	0.57
Benzoic acid, 4-ethoxy-, ethyl ester	12.348	$C_{11}H_{14}O_3$		194	0.99
Phenol, (1,1-dimethylethyl)-4-methoxy-	12.874	$C_{11}H_{16}O_2$		180	0.31
benzene, 1,2,4-trimethoxy-5-(1-propenyl)-, (Z)-	13.455	$C_{12}H_{16}O_3$		208	0.25
Mome inositol	14.059	$C_7H_{14}O_6$		194	3.93
Mome Inositol	14.574	$C_7H_{14}O_6$		194	2.77

Table 1: (*Continued*)

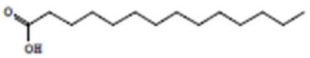
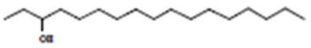
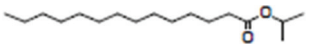
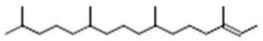
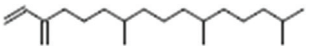
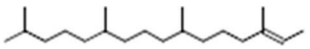
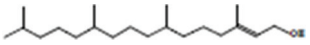
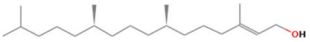
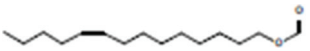
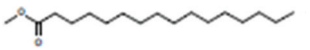
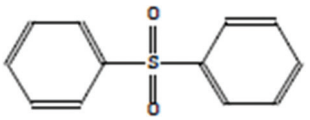
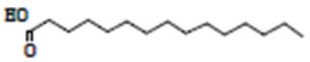
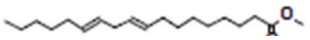
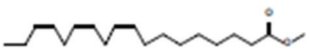
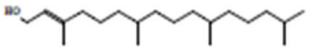
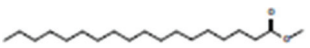
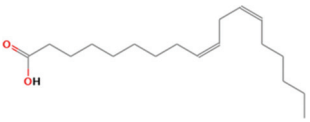
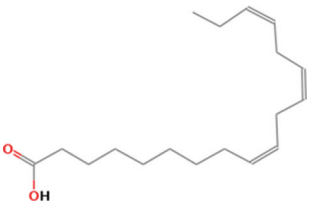
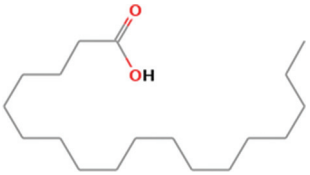
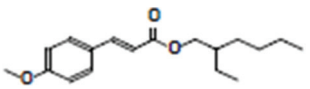
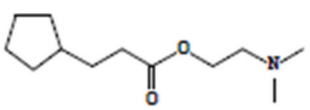
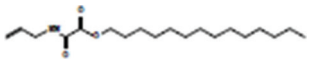
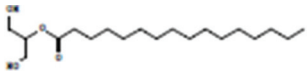
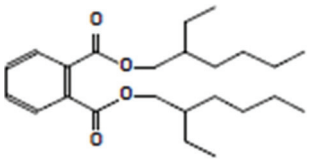
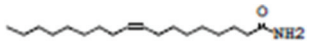
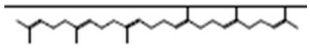
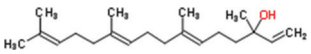
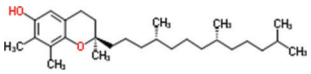
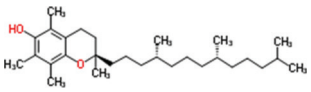
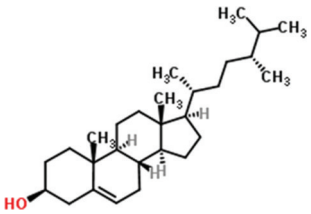
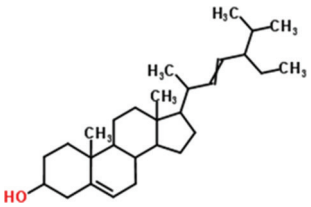
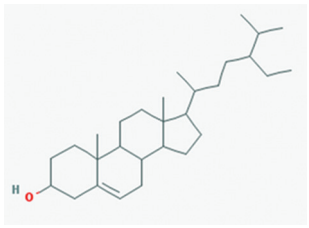
Name of the compounds	RT	Molecular formula	Molecular structure	MW	Area %
Tetradecanoic acid	15.023	$C_{14}H_{28}O_2$		228	0.18
3-Heptadecanol	15.453	$C_{17}H_{36}O$		256	0.36
Isopropyl tetradecanoate	15.654	$C_{17}H_{34}O_2$		270	0.38
(2e)-3,7,11,15-tetramethyl-2-hexadecene	15.761	$C_{20}H_{40}$		280	0.15
2,6,10-trimethyl, 14-ethylene-14-pentadecne	15.819	$C_{20}H_{38}$		278	4.24
(2e)-3,7,11,15-tetramethyl-2-hexadecene	15.885	$C_{20}H_{40}$		280	0.36
3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	16.077	$C_{20}H_{40}O$		296	0.99
2-Hexadecen-1-Ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]-	16.271	$C_{20}H_{40}O$		296	1.42
Z-9-Tetradecen-1-Ol formate	16.660	$C_{15}H_{28}O_2$		240	0.13
Hexadecanoic acid, methyl ester	16.707	$C_{17}H_{34}O_2$		270	2.46
Benzene, 1,1'-sulfonylbis-	17.014	$C_{12}H_{10}O_2S$		218	0.61
Pentadecanoic acid	17.136	$C_{15}H_{30}O_2$		242	9.22
9,12-Octadecadienoic acid (Z, Z)-, Methyl Ester	18.387	$C_{19}H_{34}O_2$		294	1.42
9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	18.458	$C_{19}H_{32}O_2$		292	5.32
Phytol	18.573	$C_{20}H_{40}O$		296	6.49
Methyl stearate	18.650	$C_{19}H_{38}O_2$		298	0.63
9,12-Octadecadienoic Acid (Z, Z)-	18.797	$C_{18}H_{32}O_2$		280	1.17
9,12,15-octadecatrienoic acid, (Z, Z, Z)-	18.878	$C_{18}H_{30}O_2$		278	6.32
Octadecanoic acid	19.024	$C_{18}H_{36}O_2$		284	0.79
9,12-Octadecadienoic acid (Z, Z)-, methyl ester	19.226	$C_{19}H_{34}O_2$		294	0.20
2-Ethylhexyl (2e)-3-(4-methoxyphenyl)-2-propenoate	20.498	$C_{18}H_{26}O_3$		290	0.17
3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	21.599	$C_{12}H_{23}NO_2$		213	0.27

Table 1: (*Continued*)

Name of the compounds	RT	Molecular formula	Molecular structure	MW	Area %
Oxalic acid, monoamide, N-allyl-, tetradecyl ester	21.690	$C_{19}H_{35}NO_3$		325	0.24
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	22.006	$C_{19}H_{38}O_4$		330	1.49
1,2-Benzenedicarboxylic acid	22.310	$C_{24}H_{38}O_4$		390	0.48
Pentacosane	23.534	$C_{25}H_{52}$		352	1.24
9-octadecenamide	24.545	$C_{18}H_{35}NO$		281	1.41
Squalene	25.056	$C_{30}H_{50}$		410	12.30
Tetracontane	25.839	$C_{40}H_{82}$		562	0.41
Neryl linalool isomer	26.499	$C_{20}H_{34}O$		290	0.45
Gamma-tocopherol	28.698	$C_{28}H_{48}O_2$		416	0.58
1-Heptacosanol	29.375	$C_{27}H_{56}O$		396	1.59
Vitamin E	30.404	$C_{29}H_{50}O2$		430	3.85
Ergost-5-En-3-Ol, (3.Beta.,24r)-	32.958	$C_{28}H_{48}O$		400	0.69
Stigmasta-5,22-Dien-3-Ol	33.820	$C_{29}H_{48}O$		412	1.67
Stigmast-5-En-3-Ol, (3.Beta.)-	35.579	$C_{29}H_{50}O$		414	2.74

RT: Retention time; MW: Molecular weight, *C. angustifolia: Cassia angustifolia*

E [7] and vitamin K [8]. It is used in shampoos, cosmetics, toilet soaps, fragrance industry, household cleanser, and detergents [9]. Some of the antioxidants found to be present in senna leaf extract are tetradecanoic acid (synonym: Myristic acid), hexadecanoic acid, heptadecanoic acid, squalene (synonym: 2,6,10,14,18,22-tetracosahexaene), vitamin E (synonym: D-alpha-tocopherol), gamma-tocopherol (synonym: 7,8-dimethyltocol), campesterol (synonym: Ergost-5-en-3.beta.-ol), and β-stigmasterol (synonym: Stigmast-5-En-3-Ol, [3.Beta.]). In our study, the leaf extract of senna contains 2.74% β-sitosterol. However, Rastogi and Mehoratra [10] reported that the plant contains only 0.33% β-sitosterol. Some of the components such as hexadecanoic acid methyl ester, phytol, 9,12-octadecanoic acid (Z,Z)-, 9,12,15-ooctadecatrienoic acid (Z,Z,Z)-, octadecanoic acid, and squalene have also been reported in the methanolic leaf extract of *Cassia alata* [11]. The methanolic leaf extract of *Lannea kerstingii* and *Nauclea diderrichii* also reported to contain hexadecanoic acid methyl ester and pentacosane [12].

Table 2: Biological properties of some of the phytocomponents identified in the methanolic extracts of *Cassia angustifolia* by GC-MS

Name of compounds	Biological properties*
2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-/squalene	Antibacterial, antioxidant, cancer-preventive, antitumor, immunostimulant, perfumery, pesticide, sunscreen
Pentadecanoic acid/myristic acid	Antioxidant, lubricant, hypercholesterolemic, cancer-preventive, cosmetic
3,7,11,15-tetramethyl-2-hexadecen-1-ol 2-pentadecanone, 6,10,14-trimethyl/phytol	Cancer preventive
Gamma-tocopherol	Anticancer, antioxidant, antitumor, anti-inflammatory, hypocholesterolemic, cardioprotective
Vitamin E	Antiaging, antialzheimeran, antidermatitic, antidiabetic, antioxidant, antitumor, cancer-preventive, hypocholesterolemic, immunostimulant
Ergost-5-en-3.beta.-ol/campesterol	Antioxidant, hypocholesterolemic
Stigmasta-5,22-dien-3.beta.-ol, (3.beta.,22E)-/stigmasterol	Antihepatotoxic, antiviral, antioxidant, cancerpreventive, hypocholesterolemic

*Source: Dr. Duke's phytochemical and ethnobotanical database www.ars-gov/cgi-bin/duke/[3]. *C. angustifolia: Cassia angustifolia*. GC-MS: Gas chromatography-mass spectrometry

CONCLUSION

Screening of leaf extract of *C. angustifolia* by GC-MS revealed the presence of 45 different phytochemicals. The study was undertaken for the first time, till date no information was available on GC-MS analysis of senna. The analysis showed the presence of highly valuable compound which could be used by various pharmaceutical or drug companies for the development of new medicines.

ACKNOWLEDGMENTS

Author, Dr. Shahina Parveen gratefully acknowledges the financial assistance provided by the Department of Science and Technology (DST) - Science and Engineering Research Board (SERB), Government of India, New Delhi, for the award of Young Scientist Project (Vide No. SB/YS/LS - 156/2013). Authors are also thankful to AIRF, JNU, New Delhi, for providing GC-MS facility.

REFERENCES

1. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. Phytother Res 2007;21(12):1142-5.
2. Anonymous. The Wealth of India, Raw Materials. Vol. 3. New Delhi: CSIR; 1992. p. 354-63.
3. Duke J. Phytochemical and Ethnobotanical Databases. U.S. Department of Agriculture, Agricultural Research Service; 1992-2016. Home Page. Available from: http://www.phytochem.nal.usda.gov/http://www.dx.doi.org/10.15482/USDA.ADC/1239279.
4. Smith TJ. Squalene: Potential chemopreventive agent. Expert Opin Investig Drugs 2000;9(8):1841-8.
5. Owen RW, Haubner R, Würtele G, Hull E, Spiegelhalder B, Bartsch H. Olives and olive oil in cancer prevention. Eur J Cancer Prev 2004;13(4):319-26.
6. Smedman AE, Gustafsson IB, Berglund LG, Vessby BO. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. Am J Clin Nutr 1999;69(1):22-9.
7. Thomas N. Synthesis of vitamin E. In: Gerald L, editor. Vitamin E. Vitamins & Hormones. Vol. 76. San Diego: Elsevier Academic Press Inc.; 2007. p. 155-202.
8. Alison D, Richard P, Mark H, Andrew A. The synthesis of naturally occurring vitamin K and vitamin K analogues. Curr Org Chem 2003;**7**:1625-34.
9. McGinty D, Letizia CS, Api AM. Fragrance material review on phytol. Food Chem Toxicol 2010;48 Suppl 3:S59-63.
10. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. 2. New Delhi India: Publications and Information Directorate; 1993. p. 179.
11. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian J Pharm Clin Res 2012;5(2):90-4.
12. Ibibia ET, Olabisi KN, Oluwagbemiga OS. Gas chromatography-mass spectrometric analysis of methanolic leaf extracts of *Lannea kerstingii* and *Nauclea diderrichii*, two medicinal plants used for the treatment of gastrointestinal tract infections. Asian J Pharm Clin Res 2016;9(4):179-82.