Int. J. Adv. Res. Biol. Sci. (2016). 3(2): 193-197

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-2-26

GC-MS and FT-IR Analyses of Ethylacetate Leaf extract of *Abutilon indicum* (L.) Sweet.

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Abstract

The present study was aimed at analysis of bioactive constituents of leaves from *Abutilon indicum.* The ethyl acetate extract of the leaves were subjected to Fourier transform infrared spectroscopy (FT-IR) and Gas chromatography- mass spectroscopic (GC-MS) analysis. GC-MS analysis of plant extract was performed using a Perkin-Elmer GC Clarus 500 system and Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) and IR spectrum was recorded in spectrophotometer (Thermo Scientific NICOLET-iS5). FT-IR analysis of peak values with various functional compounds such as alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids and aromatics. GC-MS analysis of compounds with totally, sixteen compounds major chemical compounds were identified, such as 4 hydroxyphenylacetic acid methyl ester, 5-Thio-D-glucose, 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole,E)-10-Heptadecen-8-ynoicacid methyl ester and Z-11-Hexadecenoic acid. FT-IR analysis of ethyl acetate extract of leaves of A. indicum was carried out and presented in Fig. 2. The compounds indicated shows that the band at 3308, 2962, 2066, 1657, 1541, 1456, 1384, 1308, 1383, 1170, 1124, 651, 584, 495, 475, 464 and 454 cm-1. From the present study, it is concluded that the phytochemicals was observed in ethyl acetate extract which reveals that *Abutilon indicum* is highly valuable in medicinal usage for the treatment of various human aliments.

Keywords: Phytochemical profile, FT-IR, GC-MS, *Abutilon indicum.*

Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Plants are part of life. Without them nobody can live. Mostly people believe in ayurvedic or unani medicines because they do not show any side effects compared to allopathic medicines. When a plant has antibiotic properties it is called medicinal plants. About 80% of the world population use traditional medicines, which are predominantly based on plant materials (WHO; 1993).

FT-IR is one of the widely used methods to identify the chemical constituents and has been used as requisite method to medicines in pharmacopeia of many countries (Liu et al., 2006). GC-MS analysis is a breakthrough in analysis of phyto constituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1ng (Liebler et al., 1996). Higher plants are sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on the green plants to represent a reservoir of effective chemotherapeutics, which are non-phytotoxic, more systemic and easily biodegradable (Kaushik *et al*., 2002; Chaman Lal *et al*., 2006). Plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are important source with a variety of structural arrangements and properties (De-Fatima *et al*., 2006).

Abutilon indicum (L.), a member of Malvaceae family commonly known as „Kanghi‟ in Hindi and „Atibala‟ in Sanskrit, is a perennial shrub and found as weed in almost all hot places of India. Plant has various biologically active secondary metabolites which conferred significant pharmacological and medicinal properties to this plant. Plant *A. indicum* is extensively utilized for the treatment of pharmaceutical disorders and ailments as it possesses wound healing, antioxidative, antitumor, antidiabetic, antifungal, antibacterial, larvicidal, hypoglycemic and hepatoprotective properties (Sharma *et al.,* 2013). The juice from the leaves is effectively utilized in ulcer, diabetes, diuretic infection and gingivitis (Chakraborthy, 2009; Krisanapun *et al.,* 2009; Dashputre and Naikwade, 2011). It has been reputed in Siddha system of medicine as a remedy to treat jaundice, piles, ulcer and leprosy (Yoganarsimham, 2000).

Therefore, the present research was conducted to investigate the phytochemical constituents of *A. indicum* using FT-IR and GC-MS.

Materials and Methods

Collection of Plant material and Extraction

The leaf of *Abutilon indicum* (L.) Sweet was collected from Kadavachery village (Lat, 11.24 ºN; Long, 79.44 ºE), Cuddalore District, Tamil Nadu, India during the month of December 2013. Herbarium was deposited in Department of Botany, Annamalai University (Voucher specimen No; AUBOT #325) and the different parts were washed with tap water, then surface sterilized with 10% sodium hypo-chloride solution to prevent contamination of any microbes. The samples were rinsed with distilled water and allowed to shade dried under room temperature followed by oven drying at 50ºC and then ground into powder using electric blender.

One hundred grams of powdered material of leaf, bark and root samples were extracted in a Soxhlet apparatus for 8 hours with different solvents system like petroleum ether, chloroform, ethyl acetate and methanol (Vogel, 1978). The extracts were filtered, pooled and the solvents were evaporated with the help of rotary evaporator (Heidolph, Germany) under reduced pressure at 40 ºC and the crude extracts were kept at 4ºC in refrigerator for further analysis.

GC-MS Analysis

Gas chromatography (GC) analysis was carried out using Agilent 6890 N gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted with DB 5 MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The injector temperature was set at 280 °C and the oven temperature was initially at 45 °C then programmed to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. One microlitre of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of sample was calculated by the GC peak area. GC-mass spectrometry (GC-MS) analysis of sample was performed using Agilent gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 1000 amu. The extract was identified based on the comparison of their retention indices (RI), Retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data (Adams, 2009).

Fourier transform infra-red spectra

IR spectrum was recorded in spectrophotometer (Thermo Scientific NICOLET-iS5). The active principle was mixed with KBr and pellet technique was adopted to record the spectra.

Results and Discussion

The GC-MS characterization of ethyl acetate extract of leaves of *A. indicum* was identified and presented in Table 1and Fig.1. Totally, sixteen compounds major chemical compounds were identified, such as4 hydroxyphenylacetic acid methyl ester, 5-Thio-D-glucose, 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, E)-10-Heptadecen-8-ynoicacid methyl ester and Z-11-Hexadecenoic acid. (Kuo *et al.,* 2008) isolated two new compounds, abutilin A and (*R*)-*N*-(1'- methoxycarbonyl -2 '- phenylethyl) - 4-hydroxybenzamide, as well as 28 known compounds to possess decreasing peroxidative damage in liver through free radical scavenging activity due to its flavonoids.

Among the phytocompounds, 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, E)-10-Heptadecen-8-ynoicacid methyl ester are best known for their antioxidant properties, which has led to their evaluation

in a number of diseases associated with reactive oxygen species (ROS) such as cancer, cardiovascular and neurodegenerative diseases (Ajayi *et al.*, 2011).

The activities of some phyto-components with compound nature of flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecaonoic acid), unsaturated fatty acid and octadecatrienoic acid might cause as antimicrobial (Kumar *et al.*, 2010). The compounds may be highly responsible for their antimicrobial and antioxidant activities.

FT-IR analysis of ethyl acetate extract of leaves of *A. indicum* was carried out and presented in Fig. 2. The compounds indicated shows that the band at 3308, 2962, 2066, 1657, 1541, 1456, 1384, 1308, 1383, 1170, 1124, 651, 584, 495, 475, 464 and 454 cm^{-1}. The broad band at 3308 cm^{-1} amizone to OH stretching in alcohol and phenol group, 2962 cm^{-1} to 2066 cm^{-1} attributed to C-H stretching vibration in alkanes group, the peaks around 1657 cm^{-1} are due to the amide I and II region that are characteristic of protein and enzyme, 1541 cm^{-1} secondary amide C=O stretching amides groups, 1456 cm^{-1} CH_2 bending vibration alkanes group, 1384 cm^{-1} C–H stretching alkanes group, 1170 to 1124 cm^{-1} skeletal vibrations C-H stretching isopropyl group, and 651 cm^{-1} C-H out of plane bending alkenes group. FT-IR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation (Coats et al., 2000). Ragavendran et al. (2011) screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata*. Thangarajan Starlin et al. (2012), while analyzing the ethanolic extracts of Ichnocarpus frutescens, by FT-IR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens. Parag A Pednekar and Bhanu Raman (2013) analyzed the methanolic leaf extract of Ampelocissus latifolia by FT-IR and reported that the transition metal carbonyl compounds and aliphatic fluoro compounds were only present in the extract.

Fig. 1. Phytocompounds identified from ethyl acetate extract of *Abutilon indicum* leaves by GC-MS analysis

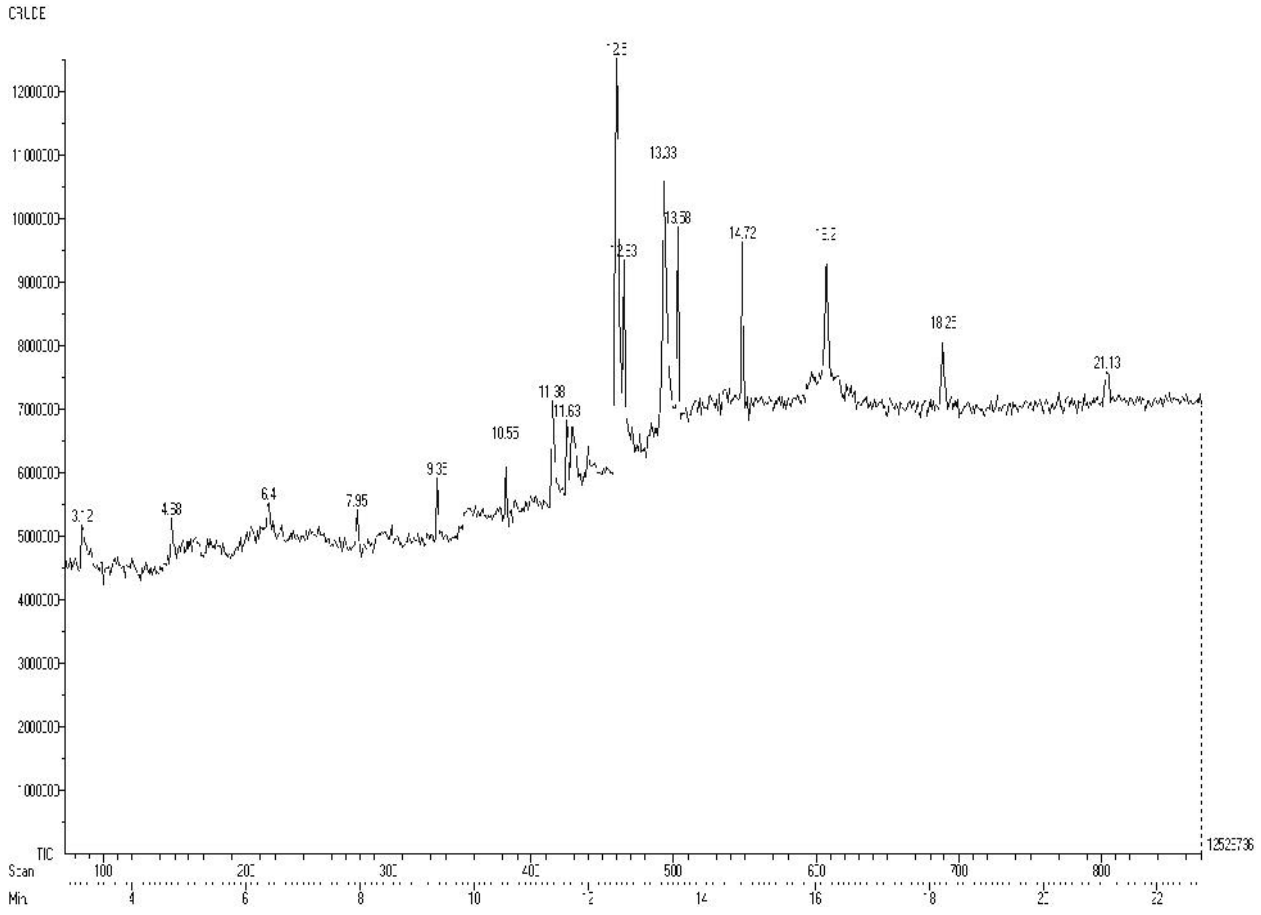
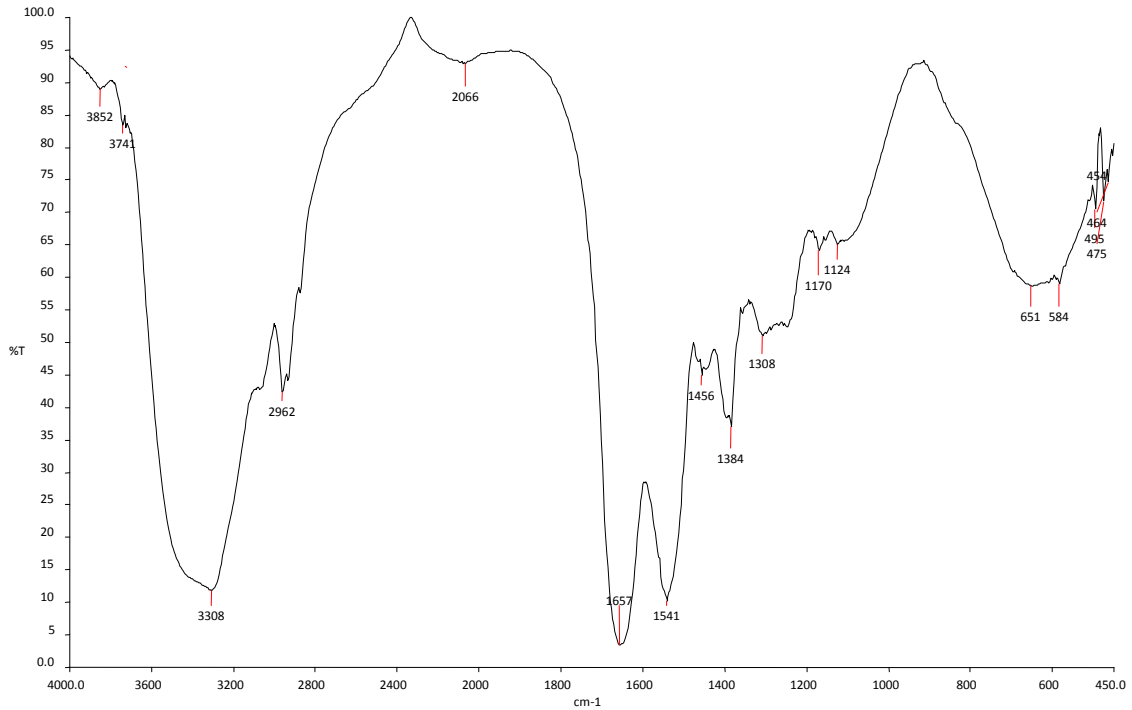


Table 1. GC-MS analysis of ethyl acetate extract of leaves of *Abutilon indicum*

S. No.	RT	Name of the compounds	Peak area (%)
1	3.12	Triamcinolone Acetonide	2.40
2	4.68	Hydroxybenzoic acid ester	2.10
3	6.4	9,10-Anthracenedione,1,4 diamino-2-methoxy	1.20
4	7.95	10-Methoxydihydrocorynantheol;10-methoxycorynan-17-ol	1.80
5	9.35	Arabinitol pentaacetate	3.60
6	10.55	4-Methylcholestan-3-ol-, (3β,4α,5α)-; 4-α-Methyl-5-α-cholestan-3-β-ol	5.40
7	11.38	10-Hydroxy-2 decenoic acid methyl ester	6.10
8	11.68	[1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	3.60
9	12.50	4 hydroxyphenylacetic acid methyl ester	21.92
10	12.63	5-Thio-D-glucose	14.11
11	13.33	(E)-10-Heptadecen-8-ynoicacid methyl ester	7.80
12	13.58	5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole	9.60
13	14.72	Abutilin A	7.80
14	16.20	Z-11-Hexadecenoic acid	6.60
15	18.25	(R)-N-(1'-methoxycarbonyl-2'-phenylethyl)-4- hydroxybenzamide	4.50
16	21.13	3 hydroxy beta ionol	0.90
	Total compounds		100.0

Fig. 2. FT-IR spectrum of ethyl acetate extract of leaves of *Abutilon indicum*



Conclusion

The natural products proved less side effects and cure diseases effectively from ancient period. In this study, the crude extracts of *Abutilon indicum* and their compounds act as antimicrobials and it may be believed to be biodegradabe. In that way, medicinal plant, *Abutilon indicum* can be used as a potential source of natural antioxidant and antimicrobial agent. Finally it can be concluded that the phytochemicals was observed in ethyl acetate extract which reveals that *Abutilon indicum* is highly valuable in medicinal usage for the treatment of various human aliments.

Acknowledgments

The authors are thankful to Dr. K. Arumugam, Professor and Head, Department of Botany, Annamalai University for providing necessary laboratory facilities.

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How to cite this article:

D. Saranya and J. Sekar. (2016). GC-MS and FT-IR Analyses of Ethylacetate Leaf extract of *Abutilon indicum* (L.) Sweet. Int. J. Adv. Res. Biol. Sci. 3(2): 193-197.