ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 6, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

GC-MS ANALYSIS OF THE METHANOL FRACTION OF *AILANTHUS EXCELSA* ROXB. FRUIT

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Received: 28 February 2022, Revised and Accepted: 15 April 2022

ABSTRACT

Objective: The objective of the study was to evaluate the phytochemical compounds present in the methanolic fruit extract of *Ailanthus excelsa*.

Methods: Phytochemical screening was carried out using the GC-MS instrument following the standard protocol.

Results: GC-MS studies revealed the presence of 65 compounds in fruit extract of *Ailanthus excelsa*. Among them, highest peak area (37.67%) was obtained for Mome Inositol (Six hydroxyl group polysaccharide) (Retention time 14.873).

Conclusion: This study identifies the presence of pharmacologically active compounds which can be constructive for the formulation of novel drugs.

Keywords: *Ailanthus excelsa*, GC-MS, Methanolic extracts, Retention time, Novel drugs.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i6.44551. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Medicinal plants are also called medicinal herbs which have been often used over the years for curing many diseases. There are 7, 50,000 plant species on earth according to statistical estimation, of which 1–10% are used as food and medicine [1,2]. The plants are sources of many efficient drugs and also will continue to be important for screening of new lead compounds of biological and pharmacological importance [3]. Medicinal plants exhibit numerous pharmacological properties such as antioxidants [4], anti-diabetes [5], antibacterial [6], antiviral [7], anticancer [8], and anti-ulcer activities [9]. Due to the presence of bioactive secondary metabolites in medicinal plants, several diseases are cured in mankind [10].

Ailanthus excelsa Roxb. (Family: Simaroubaceae) is a deciduous tree extensively distributed in Asia and North Australia. The plant is indigenous to China, and it is known as "tree of heaven" [11]. It is a fast growing, multipurpose tree that is well-adapted to arid and semiarid climates; furthermore, it requires minimal care and grows well in all kinds of soil. For these reasons, it has become the first choice in many agro- and social forestry programs [12]. The plant has various utilities, for example, leaves as fodder and stem in the production of match wood, box plank, packing cases, paper, toys, plywood veneers [13], and pencil [14]. It is extensively used as an avenue tree, in shelterbelts, in reforestation programs, for example, silkworm culture, biomass for fuel-wood, as a fodder source, and for medicinal purposes [15].

In the present scenario, understanding of the chemical constituents of plants with medicinal properties should not only to pave way for new drug discovery but also play a crucial role in identifying new source of economically viable plant metabolites [16]. Gas chromatography and mass spectrum are used in case of direct analysis of primary and secondary metabolites existing in traditional medicinal practices and medicinal plants [17,18].

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a well-suited technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [19]. Mass spectrometry in conjunction with gas chromatography is a powerful tool in biological and chemical studies [20].

In recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a reliable method for the analysis of plant metabolites [21,22]. These metabolites play a key role in health care systems [23]. In the present study, fruit samples of the plant were analyzed using GC-MS. This work will help to identify compounds, which can be utilized for therapeutic purposes.

METHODS

Collection of plant material

The fruit samples of *Ailanthus excelsa* Roxb. were *collected* from the University of Rajasthan campus, Jaipur in the month of February. Herbarium samples were deposited and authenticated in the Department of Botany, University of Rajasthan, and got a voucher specimen number RUBL211591.

Preparation of plant material

Fruits were rinsed with tap water, and dried under shade at room temperature and ground to fine powder by employing an electrical grinder and stored in air-tight containers.

Preparation of sample for GC-MS study

About 10 g of the dried powder was soaked in 100 ml methanol. It was left for 24 h so that non polar components, volatiles, and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No. 1 filter paper and the residue was removed. It was again filtered through sodium sulfate to remove the traces of moisture.

GC-MS analysis

The GC-MS analysis was conducted at AIRF (Advanced Instrumentation Research Facility), JNU, Delhi. GC-MS analysis was carried out on a GC-MS QP2010Ultra. The GC/MS instrument had Rtx-5MS column with a dimension of 30×0.25 mm×0.25 µmdf, composed of 5% Diphenyl, 95% Dimethyl poly siloxane, operated in electron impact mode at 70 eV. The carrier gas used was helium, which had a constant flow rate of 1.21 ml/min and with an injection volume of 1 µl was employed (split ratio of 10:1). The oven temperature was programmed initially

at 100°C for 2 min, then an increase to 250 at the rate of 10°C/min for 5 min and then programmed to increase to 280°C at the rate of 20°C/min for 21 min. The MS operating conditions were as follows interface Temp. 270.00°C, Ion Source Temp 230.00°C, Solvent Cut Time: 3.50 min, Scan Speed 3333, mass scan (m/z)-40-650, and threshold: 1000. GC-MS was analyzed using electron impact ionization at 70eV and data were evaluated using total ion count (TIC) for compound identification and quantification. The relative percentage amount of each compound present in the GC-MS spectrum was calculated by comparing the individual compounds average peak area to their total areas. Turbo mass 5.2 software was used to handle mass spectra and chromatograms.

Identification of components

The identification of the components in the extracts was assigned by the comparison of their retention data and mass spectra fragmentation patterns with those stored in the computer library and also with published literatures. NIST08.LIB [24], WILEY8.LIB [25] library sources were used for matching the identified components from the plant material.

RESULTS AND DISCUSSION

GC-MS is the best technique to identify the bioactive components of long chain hydrocarbons, alcohols, and acids used in the analysis of the herbal medicines, there are more significant advantages of GC-MS [26].

GC-MS spectra of fruit extract revealed the peaks that indicated the occurrence of different constituents (Fig. 1). The spectral fingerprint of compounds identified using the data library and molecular weight, the compound names are listed in Table 1. The most prevalent constituent was found to be Mome Inositol (37.67%). The other major compounds are 1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid (28.43%), n-Hexadecanoic acid (6.82%), Heptadecene-(8)-Carbonic acid-(1) (2.71%), 4H-Pyran-4-one-,2,3,-dihydro-3,5-dihydroxy-6-methyl-(2.25%), 1,2,3-Propanetriol (2.01%), Stigmast-5-En-3-Ol, (3. Beta)-(1.59%), 5-Hydroxymethylfurfural (1.48%), Linoleic acid (1.25%), 4-Ethyl-3-methyl-4-pentene-2-one (1.23%), N-2 (Nitrophenyl) pentofuranosylamine (1.09%), Octadecanoic acid (1.03%), Lupeol (0.98%), etc.

Previously, it has been reported that the methanolic extracts of plants possess several biological activities such as anti-oxidant, antifungal, antimicrobial, anti-inflammatory, and pesticidal activities [27].

Most of these constituents have been found to show interesting biological activity against certain illnesses. The major constituent, Mome inositol, a polysaccharide, is reported as anti-alopecic, anti-cirrhotic, anti-neuropathic, cholesterolytic, lipotropic, and a sweetener [28].

4H-Pyran-4-one-,2,3,-dihydro-3,5-dihydroxy-6-methyl-, a flavonoid, reported to have mutagen antimicrobial, anti-inflammatory, and antioxidant capacity [29-31].

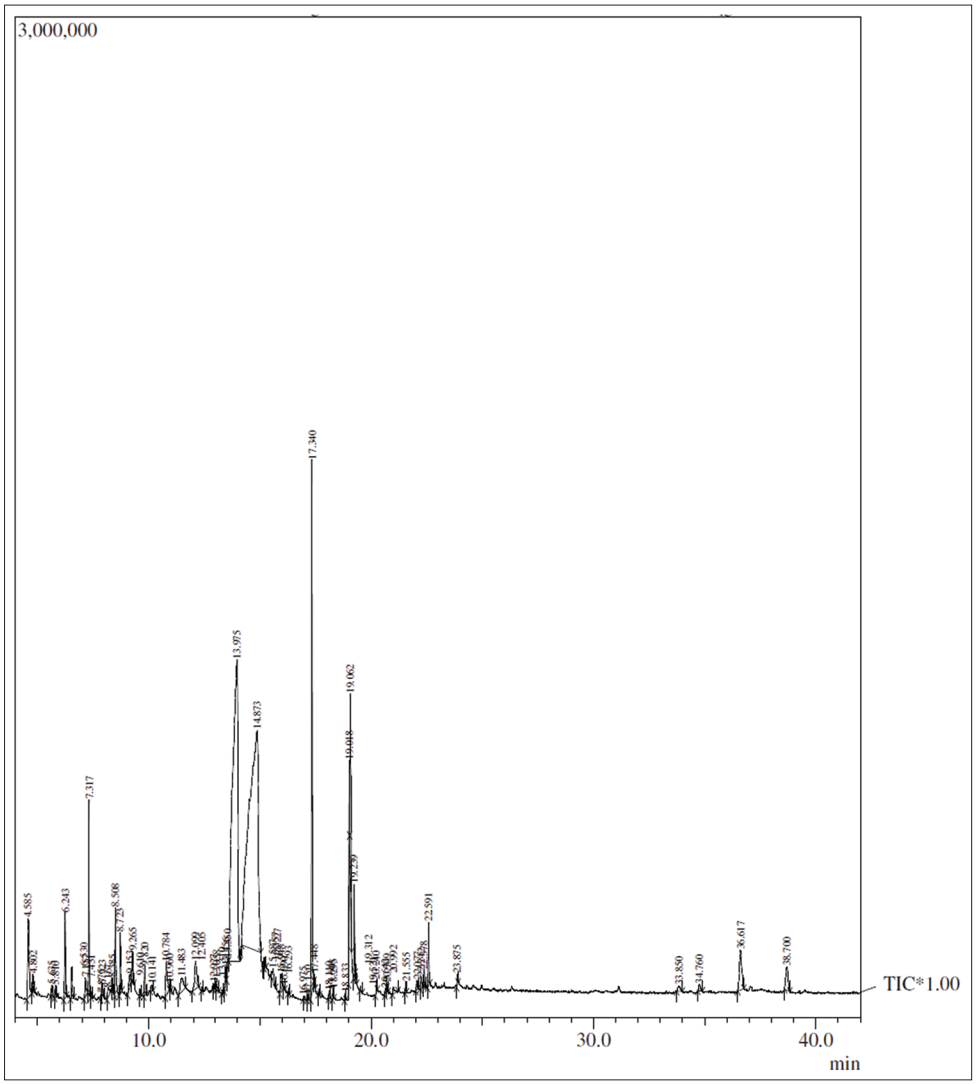

Fig. 1: GC-MS Chromatogram of methanolic fruit extract of *A. excelsa*

Table 1: GC-MS analysis of methanol extract of *A. excelsa* fruit

S. No.	R. time	Area%	Compound name	Mol. formula	Mol. wt.
1	4.585	2.01	1,2,3-Propanetriol	$C_3H_8O_3$	92
2	4.802	0.25	2,4-Dihydroxy-2,5-dimethyl-3 (2H)-furan-3-one	$C_6H_8O_4$	144
3	5.635	0.22	2,4-Dimethyl-3-Pentanone	$C_7H_{14}O$	114
4	5.810	0.19	4-Oxo-Pentanoic acid	$C_5H_8O_3$	116
5	6.243	1.23	4-Ethyl-3-methyl-4-penten-2-one	$C_8H_{14}O$	126
6	6.530	0.49	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130
7	7.157	0.44	2-Acetyl-2-Hydroxy-.Gamma.-Butyrolactone	$C_6H_8O_4$	144
8	7.317	2.25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
9	7.431	0.08	1-Aminoguanidine bicarbonate	$C_2H_8N_4O_3$	136
10	7.875	0.04	Isobutyl formate	$C_5H_{10}O_2$	102
11	7.923	0.15	2-Methyl-2H-Pyran-3,4,5 (6H)-Trione	$C_6H_6O_4$	142
12	8.167	0.29	Pyrocatechol	$C_6H_6O_2$	110
13	8.385	0.13	Coumaran	C_8H_8O	120
14	8.508	1.48	5-hydroxymethylfurfural	$C_6H_6O_3$	126
15	8.723	0.77	1,2,3-Propanetriol, monoacetate	$C_5H_{10}O_4$	134
16	9.153	0.41	2,3-Dihydroxypropanal	$C_3H_6O_3$	90
17	9.265	0.59	6-oxoheptanoic acid	$C_7H_{12}O_3$	144
18	9.610	0.09	3-Heptanol	$C_7H_{16}O$	116
19	9.820	0.47	Cis-Dimethyl morpholine	$C_6H_{13}NO$	115
20	10.141	0.14	Triacetin	$C_9H_{14}O_6$	218
21	10.784	0.93	1,2,3-Benzenetriol	$C_6H_6O_3$	126
22	10.960	0.07	2-Butene-1,4-diol, trimethlsilyl ether	$C_7H_{16}O_2Si$	160
23	11.483	0.76	Guanosine	$C_{10}H_{13}N_5O_5$	283
24	12.099	1.09	N-2(Nitrophenyl) pentofuranosylamine	$C_{11}H_{14}N_2O_6$	270
25	12.405	0.03	1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	206
26	12.927	0.06	Dodecanoic acid	$C_{12}H_{24}O_2$	200
27	13.058	0.17	Vannilic acid	$C_8H_8O_4$	168
28	13.310	0.03	2,4,6-Trimethyl-4-heptanol	$C_{10}H_{22}O$	158
29	13.456	0.30	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
30	13.550	0.18	2-Ethyltetrahydrothiophene	$C_6H_{12}S$	116
31	13.975	28.43	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192
32	14.873	37.67	Mome Inositol	$C_7H_{14}O_6$	194
33	15.157	0.08	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180
34	15.227	0.11	Decanoic acid	$C_{10}H_{20}O_2$	172
35	15.587	0.43	7-Methoxy-3,7-Dimethyloctanal	$C_{11}H_{22}O_2$	186
36	15.948	0.39	Shikimic acid	$C_7H_{10}O_5$	174
37	16.090	0.03	2-Isopentylbicyclo (2.2.1) heptanes	$C_{12}H_{22}$	166
38	16.293	0.07	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
39	16.975	0.03	Hexanoic acid, Methyl ester	$C_7H_{14}O_2$	130
40	17.150	0.08	3-Methyl-1-hepatnol	$C_8H_{18}O$	130
41	17.340	6.82	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
42	17.448	0.08	Dibutyl phthalate	$C_{16}H_{22}O_4$	278
43	17.646	0.06	Hexadecanoic acid, Ethyl ester	$C_{18}H_{36}O_2$	284
44	18.110	0.12	1-Hexyl-3-methylcyclopentane	$C_{12}H_{24}$	168
45	18.295	0.11	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
46	18.833	0.08	Sulfurous acid, octadecyl 2-pentyl ester	$CH_{48}O_3S$	404
47	19.018	1.25	Linoleic acid	$C_{18}H_{32}O_2$	280
48	19.062	2.71	Heptadecene-(8)-Carbonic acid-(1)	$C_{18}H_{34}O_2$	282
49	19.239	1.03	Octadecanoic acid	$C_{18}H_{36}O_2$	284
50	19.312	0.10	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282
51	19.521	0.07	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
52	20.240	0.19	Methyl 2-O-benzyl-d-arabinofuranoside	$C_{13}H_{18}O_5$	254
53	20.642	0.06	Bicyclo (5.1.0) Octan-3-Ol	$C_8H_{14}O$	126
54	20.709	0.04	Pentadecanal-	$C_{15}H_{30}O$	226
55	20.992	0.17	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
56	21.555	0.11	Undecanal	$C_{11}H_{22}O$	170
57	22.077	0.16	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296
58	22.242	0.19	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{18}H_{36}O_4$	316
59	22.378	0.17	Palmitaldehyde	$C_{16}H_{32}O$	240
60	22.591	0.72	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390
61	23.875	0.12	Nonacxosane	$C_{29}H_{60}$	408
62	33.850	0.22	22,23-Dibromostigmasterol acetate	$C_{31}H_{50}Br_2O_2$	612
63	34.760	0.21	22,23-Dibromostigmasterol acetate	$C_{31}H_{50}Br_2O_2$	612
64	36.617	1.59	Stigmast-5-En-3-Ol, (3.Beta.)-	$C_{29}H_{50}O$	414
65	38.700	0.98	Lupeol	$C_{30}H_{50}O$	426

Plants are rich sources of natural antioxidants [32]. Antioxidants acts against the oxidative stress in the animal body, that's why the defense mechanism gets weakened and causes oxidative damages to lipids, proteins, and DNA [33] and inhibits the initiation and propagation of reactive oxygen species (ROS) [34].

Hexadecanoic acid (fatty acid) reported to have antibacterial activity against *S. aureus* and *E. coli* [35]. Linoleic acid (Fatty acid) possesses the antibacterial activity against gram-positive (*B. cereus, B. pumilus*, *B. subtilis,* and *S. aureus*) bacteria [36]. Octadecanoic acid (Fatty acid) also has antimicrobial activity against *S. aureus* and *S. pyogenes*

[37]. Hexadecanoic acid, methyl ester belongs to the class of organic compounds known as fatty acid methyl esters, reported to have antibacterial and antifungal activities [38]. In another study, nematicidal and pesticidal activities of hexadecanoic acid, methyl ester have been reported [39]. Lupeol (phytosterol) has been reported to have anti-inflammatory and anticancer activities [40,41].

Hexadecanoic acid ethyl ester (fatty acid ester) acts as an antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, and 5-alpha reductase inhibitor [42].

Phytosterol derivative Stigmast-5-en-3-ol reduces blood level of glucose, possess anti-inflammatory, anti-pyretic, anti-arthritic, and anti-ulcer activity [43-46]. 5-Hydroxymethylfurfural (HMF) is a member of the class of furans. Oxidase activity of 5-hydroxymethylfurfural was identified in the bacterium *Cupriavidus basilensis* [47].

As a biofumigant, coumaran is reported to act against insect pests found in stored food grains [48].

CONCLUSION

The present investigation concludes that the stronger extraction capacity of methanol could have been produced a number of active constituents responsible for a lot of biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer.

ACKNOWLEDGMENT

The authors would like to acknowledge the Department of Botany, University of Rajasthan, Jaipur for providing basic facilities to conduct this research work.

AUTHOR'S CONTRIBUTIONS

Aruna kumari carried out the experimental work and manuscript writing. R. A. Sharma analysed the results.

CONFLICT OF INTEREST

This statement is to declare that the authors involved in this manuscript have no conflict of interest.

FUNDING

Nil.

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