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Phytochemical profiling of *Ailanthus excelsa* leaf extracts using GC-MS

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Abstract
Ailanthus excelsa Roxb belongs to Simaroubaceae. It is commonly called as "Tree of Heaven". The GC-MS analysis of methanolic and ethanolic leaf extracts revealed 47 compounds. Among them seven compounds *viz.*, 1-Dodecano, 12-Tridecen-2-one, 1-Dococsanol, Benzyldiethyl-(2, 6-xylylcarbamoylmethyl)-ammonium benzoate, Dibutyl phthalate, Heneicosane and Hexadecanoic acid-methyl ester posses biopesticidal property. Hence, there is a scope of utilizing *A. excelsa* as botanical pesticide in the management of insect pests.

Keywords: *Ailanthus excelsa*, chemical profiling, GC-MS, biopesticide

Introduction
Ailanthus excelsa Roxb is a multipurpose, deciduous fast growing tree species belongs to Simaroubaceae. The generic name *Ailanthus* is derived from ailanthus meaning 'tree of heaven', while, in Latin *excelsa* means 'tall' [5]. It thrives well in arid and semi-arid region. Due to multivarious utility *viz.*, leaves as fodder and stem in the production of match wood, box plank, packing cases, paper, toys, plywood veneers [13], and pencil [11]. The leaves are having considerable amount of proteins and rich with various secondary metabolites. The phytochemicals are non-nutritive plant chemicals possess various protective and preventive properties [10].
Nowadays there is a huge demand to find an alternative to chemical pesticides. Interest on manufacturing of botanical or plant based insecticides including essential oil and plant extracts is increasing due to environmental concern and problems experienced with insect resistance evolution to conventional chemical insecticides [9]. Plant based insecticides are gaining momentum, especially in the field of organic agriculture due to the problems of chemical pesticide *viz*., residues in the crop produce, development of insecticide resistance in pests and resurgence of minor pests attaining the status of major pests.
The current investigation aims at characterizing the phytoactive compounds in ethanolic and methanolic crude extracts of *Ailanthus excelsa* leaves using Gas Chromatography and Mass Spectrometry (GC-MS). In present era the Gas Chromatography and Mass Spectrometric methods play vital role in screening and identification of various secondary metabolite profiling, in turn helps to evaluate the pharmaceutical and biopesticidal properties of various bioactive compounds.

Materials and Methods
Collection and processing of leaf sample
Leaves of *A. excelsa* were collected from the trees maintained in the Precision Silviculture farm of Department of Silviculture and Natural Resource Management, Forest College and Research Institute, Mettupalayam. The collected leaf samples were washed thoroughly with tap water and shade dried at room temperature (25±2 ºC) for a period of 10 to 15 days until the leaves were completely dried. The dried samples were powdered using mixer grinder, sieved to fine powder and stored in air tight glass containers for further analysis.

Preparation of crude extract
Five grams of powdered leaf sample was packed in a filter paper and made into 20 cm × 4.5 cm size cylindrical thimbles. The sample filled thimbles were kept in the cylindrical sample holder present in the soxhlet apparatus and filled with organic solvents such as ethanol and methanol, individually. Plant samples were extracted with the said organic solvents. Organic solvents when mixed with plant sample produced coloured solution. Extraction was continued till the coloured solvent became transparent. During the extraction process, temperature of soxhlet apparatus set up was maintained at 79 ºC for ethanol and 65 ºC for methanol extracts.

The extraction process has taken approximately 6-8 hours for each sample. Both the extracts were concentrated under room temperature and dried crude extracts were subjected to further analysis.

Gas Chromatograph and Mass Spectrometry (GC-MS) analysis

The phytochemical composition of ethanolic and methanolic extracts of *A. excelsa* leaves was analysed using Shimadzu Gas Chromatography and Mass Spectrometry (GC-MS) - QP 2020 with a SH-Rxi-5 Sil MS Cross Band (similar to 5% diphenyl 95% dimethy; polysiloxane) capillary mid-polar column (30m, ID: 0.25 mm and film thickness of 0.25 µm). Sample sizes of 1µl of each ethanolic and methanolic extracts were injected separately for analysis and Helium was used as a carrier gas at 1.2 mL/minute. The oven temperature was programmed from 80 ºC to 285 ºC (80 ºC for 5 min, 4 ºC rate 260 ºC, and 2 ºC rate 285 ºC hold for 10 minutes). The MS was set to scan from 45-650 Da. The MS also had inbuilt pre-filter which reduces the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum *viz.*, NIST4 and WILEY9 containing more than million references. The interpretation of mass spectrum of GC-MS was done by using the database of National Institute Standard and Technology (NIST4) and WILEY9 libraries. The relative percentage of extract constituent was expressed with peak area normalization.

Results and Discussion

Totally 47 compounds were identified in crude extracts of *A. excelsa* leaves as given in Table 01, Figure 01 and Figure 02. In ethanolic extract, 19 compounds were identified among them Tetradecane constitutes 14.47%, followed by Dibutyl phthalate (13.65%), Dodecane (12.96%), Nonadecane (12.51), Benzyldiethyl - (2,6-xylylcarbamoylmethyl) - ammonium benzoate (9.44%), Heneicosane (6.93%) and Octane 1,1'-oxybis (4.92%). The minor compounds are Octadecane (1.08%) and 4-Hydroxy-2,5-dimethyl-3-hexanone (1.04%).

With respect to methanolic extract, Dibutyl phthalate (25.68%), Mome inositol (8.51%), Cetene (6.82%), 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid (6.67%), 1-Nonadecene (5.82%), α.-D-Glucopyranoside, β.-D-fructofuranosyl (5.25%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]] (3.65%), 1-Docosanol (3.16%) and 1-Tetradecene (2.91%). The lowest peak area was recorded by N-[(+-)-3', 3'-Dimethyl-5-hydroxypyrrolidin-2-one with 0.67%.

Leaf extracts of *A. excelsa* had following compounds responsible for biopesticidal properties *viz*., 1-Dodecanol (insecticidal) [4], 12-Tridecen-2-one (pesticidal) [3], 1-Dococsanol (insecticidal) [15], Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate (pesticidal)[15], Dibutyl phthalate (scabicidal and repellent) [2], Heneicosane (pheromone) [7], Hexadecanoic acid-methyl ester (nematicidal and Pesticidal) [22] as shown in Figure 03.

The identified compounds were found to have more pharmaceutical importance and insecticidal activity. Among the two organic solvents used for extraction, methanolic extraction yielded noticeably higher amount of phytochemicals than ethanolic extraction. From the results it is suggested that solvent extraction with methanol will aid in development of methanol based biopesticidal formulations and the results are in agreement with the findings [17], who has reported that methanolic extracts possessed high biological activities like anti-oxidant, antifungal, antimicrobial, anti-inflammatory and pesticidal activities.

The results are similar with earlier findings of various leaf extracts in methanolic and ethanolic solvents. Parasuraman *et al.* (2009) [23] identified 17 compounds in methanolic extract of *Cleistanthus collinus*. Ravishanker and Ester (2017) [17] reported 21 compounds in methanolic extract of *Hydrophila auriculata*. Tyagi and Agarwal (2017) [20] reported several compounds in methanolic extract of *Pistia statoides* (8) and *Eichhornia crassipes* (12). Madkour *et al*. (2017) [12] extracted 27 compounds using methylene chloride and 46 compounds using n-hexane from the leaves of *Senna italica*. Elaiyaraja and Chandramohan (2016) [6] reported that *Indoneesiella echioides* yielded 9 compounds in ethanol, 10 in butanol, 7 in methanol, 5 in acetone, 9 in chloroform and 13 compounds in n-hexane solvent. Uraku (2016) [21] extracted *Spilanthes uliginosa* and reported 6 compounds. Amudha and Rani (2014) recorded 20 compounds in ethanolic extract of *Cadaba fructicosa*. Muthulakshmi *et al*. (2012) [1] reported 18 compounds in *Ferronia elephantum*. Sermakkani and Thangapani (2012) [19] recorded 17 compounds in *Cassia italica*. Gopalakrishnan and Udayakumar (2014) [8] documented 39 compounds from *Marsilea quadrifolia*. Rahman *et al*. (2014) [16] reported that methanolic extracts of *Jatropha curcas*, *Psidium gujava* and *Andrographis paniculata* yielded 9, 12 and 29 compounds, respectively.

Table 1: GC-MS profiling of *Ailanthus excelsa* leaf extracts in methanol and ethanol

Sl. No.	Name of the compound	Peak area (%)		Biological activity
		Ethanol	Methanol	
1	(S)-(+)-2-Amino-3-methyl-1-butanol	-	1.00	Anti-bacterial
2	β-D-Glucopyranose, 1,6-anhydro-	-	0.93	-
3	1-Dodecanol	-	1.29	Insecticidal
4	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	-	6.67	
5	12-Tridecen-2-one	-	0.75	Pesticidal
6	1-Butanol, 3-methyl-, formate	-	2.17	Antiviral
7	1-Docosanol	-	3.16	Insecticidal
8	1-ethyl-2-methyl-cyclopentane	1.1	-	Antimicrobial
9	1-Hexacosanol	-	1.06	-
10	1-Nonadecene	-	5.82	Antimicrobial
11	1-Tetradecene	-	2.91	Antimicrobial
12	2,2,4-Trimethyl-3-pentanol	1.17	-	-
13	2,3-epoxy-5,8-hetadecadien-1-ol	-	1.01	-
14	2-Hexadecen-1-ol, 3,7,1,15-tetramethyl	2.73	1.42	-
15	2-methylene-3-buten-1-amine	-	0.92	-
16	3-Allyl-1,7,7-trimethylbicyclo[2.2.1]hept-2-en-2-yl Diethyl phosphate	-	0.79	-

17	3-Decanone, 2-methyl-	-	0.86	Antimicrobial
18	3-Methyl-3-nitro-2-butanol	-	1.17	-
19	3-methyl-5(4H)-isoxazolone	2.34	-	-
20	4-Hydroxy-2,5-dimethyl-3-hexanone	1.04	-	-
21	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	-	2.16	-
22	α-D-Glucopyranoside, β.-D-fructofuranosyl	-	5.25	Antibacterial
23	Benzyldiethyl - (2,6-xylylcarbamoylmethyl) - ammonium benzoate	9.44	-	Pesticidal
24	Butanoic acid, 3-hydroxy	-	1.44	Biodegradable plastic synthesis
25	Cetene	6.82	-	Antibacterial activity
26	Cyclohexane, (1-hexadecylheptadecyl)	-	1.91	-
27	Cyclohexane, octadecyl	-	0.91	-
28	Decane	1.66	-	
29	Dibutyl phthalate	13.65	25.68	Ectoparasiticide
30	Dichlorobenzoyl peroxide	-	0.68	Antibacterial and antifungal
31	Dodecane	12.96	-	Antibacterial
32	Heneicosane	6.93	-	Pheromone
33	Heptadecane	3.77	-	Anti-inflammatory
34	Hexacontanoic acid, 18-oxo-	-	0.74	Radical scavenging activity
35	Hexadecane	-	0.69	Antibacterial activity
36	Hexadecanoic acid, methyl ester	-	1.48	Antifungal, Nematicidal, Pesticidal
37	Hydroxyurea, N,N',O-trimethyl-	-	1.91	Anti-immunity
38	Methyl 2-Methyl-2-propenyl Ether	1.64	-	Antibacterial
39	Mome inositol	-	8.51	-
40	N-[(+-)-3',3'-Dimethyl-5-hydroxypyrrolidin-2-one	-	0.67	-
41	Nonadecane	12.51	-	Anti-fungal and Antimicrobial
42	Octadecane	1.08	-	Antibacterial
43	Octane, 1,1'-oxybis	4.92	-	Anti-static
44	Pentatriacontane	1.45	-	Antibacterial
45	Propane, 1,1,3-triethoxy-	2.87	-	Antimicrobial
46	Propane, 1,1-diethoxy-2-methyl-	2.19	-	Antimicrobial
47	Tetradecene	14.47	-	Antimicrobial

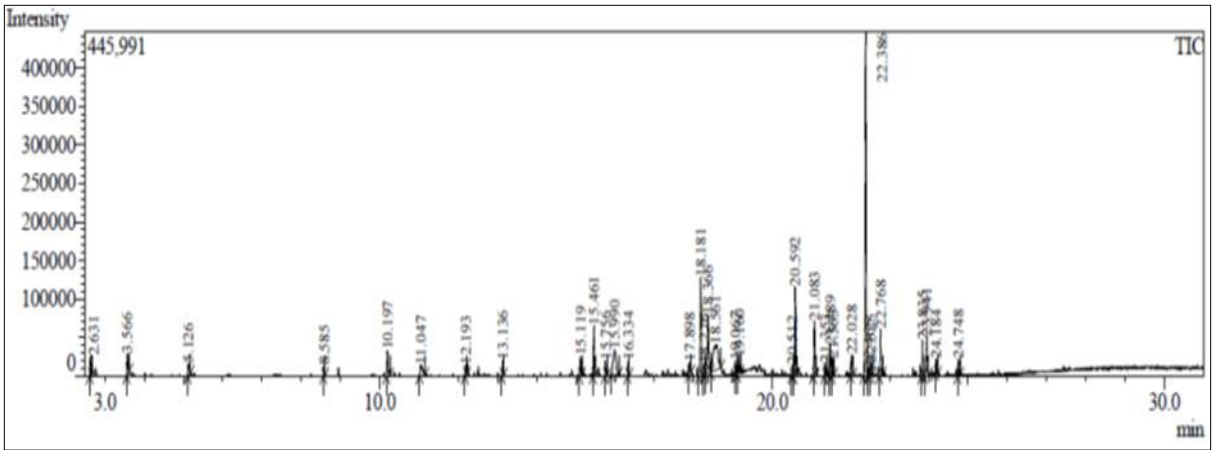

Fig 1: Gas-chromatograph of methanolic extract of *Ailanthus excelsa* leaves

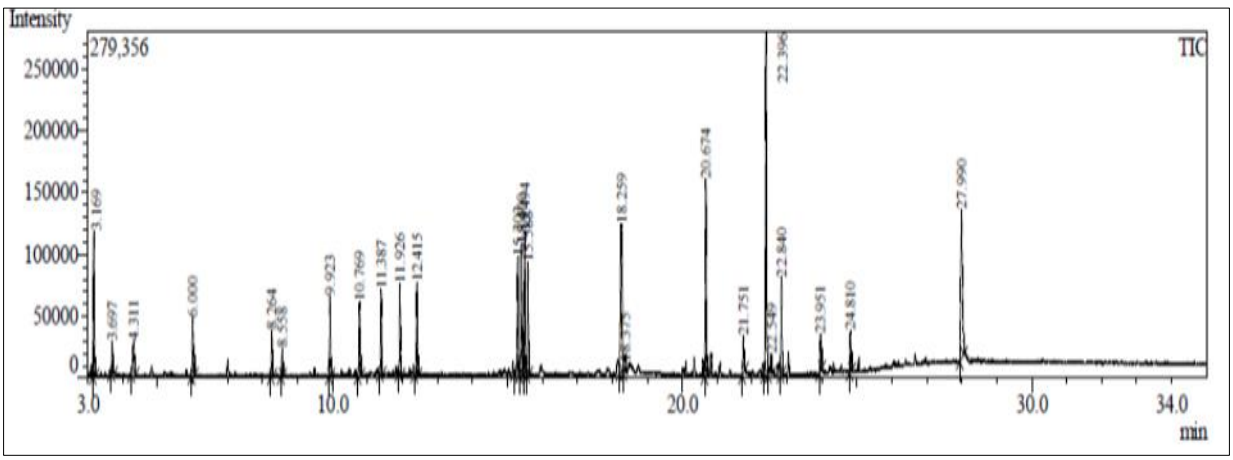

Fig 2: Gas-chromatograph of ethanolic extract of *Ailanthus excelsa* leaves

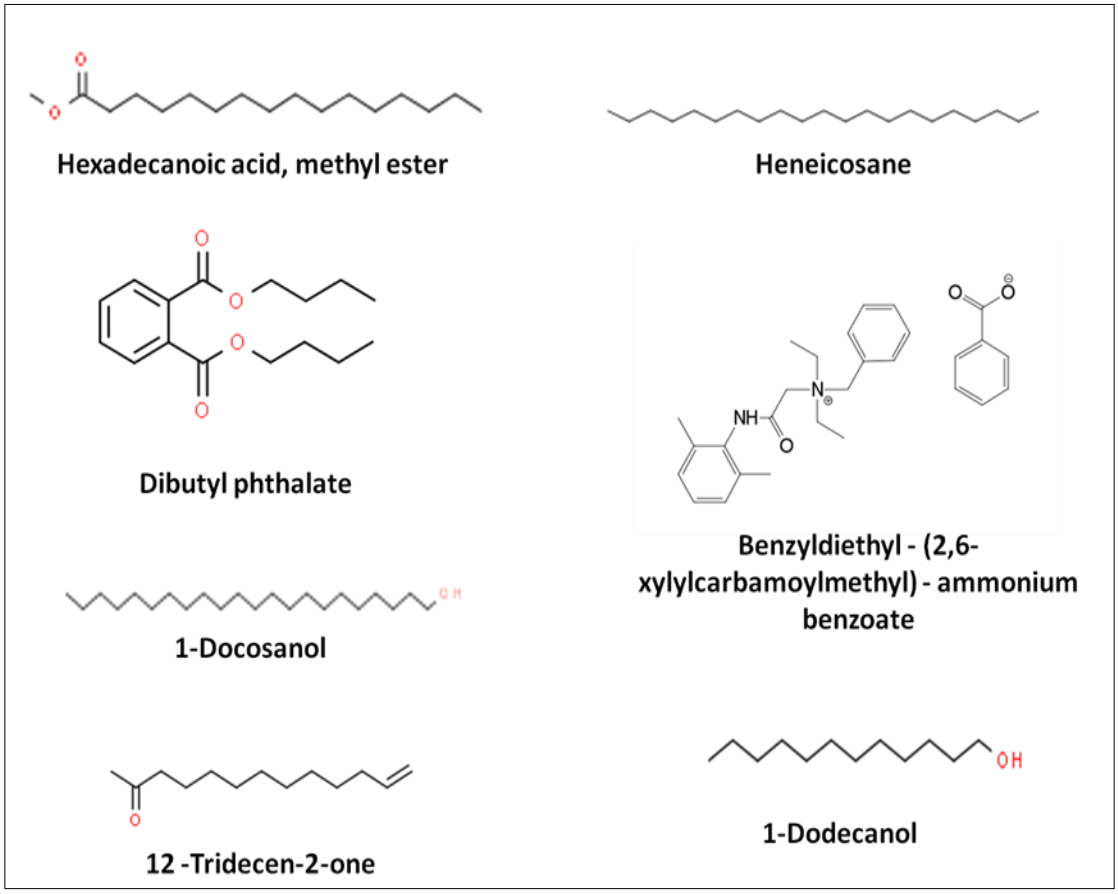

Fig 3: Chemical structure of compounds having biopesticidal properties

Conclusion

From the results of present investigation it is concluded that the stronger extraction capacity of methanol yielded higher number of bio-active constituents responsible for many biological activities *viz.*, biopesticidal, antimicrobial, antibacterial, antiviral, anti-inflammatory, scabicidal and more. Hence, this method of extraction can be utilized for the development of biopesticidal formulations, traditional medicines and novel drugs or formulations for the management of insect pests. Apart from this the technique of phytochemical fingerprinting with help of GC-MS aids in identifying false herbal products and to ascertain the quality of herbal medicines.

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