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Chemical Characterization of Supercritical Fluid Extract (SFE) of *Ailanthus excelsa* and Estimation of Total Phenols and Flavonoids in SFE

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ailanthus excelsa which is usually called as tree of heaven is known to possess anti- fungal activity. The bio constituents such as flavonoids and phenols which are present in the leaves of *Ailanthus excelsa* is known to possess anti- bacterial, anti- fungal properties. This experiment was conducted

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to study the fungicidal activity of *Ailanthus excelsa* and its characeterization. Results revealed that the supercritical fluid extraction was effective at 60ºC of temperature and 225 bar pressure with solvent flow rate of 5 g/min and caron di- oxide flow rate at 20 g/min. The GC-MS analysis showed that forty different constituents were present in the *A. excelsa*. Among which, Ricinoleic acid, Diethyl phthalate, 1.63 Hexadecanoic acid, methyl ester and 9- octadecenoic acid (Z)-, methyl ester were found to impart anti- fungal activity. The total phenols and flavonoids was 19.65 mgGAEg-1 and 40.68 mgGAEg-1 respectively was found in the SFE extract of *A. excelsa.*

Keywords: Supercritical fluid extract; characterization; ailanthus excels; antifungal activities; anti-fungal properties; ayurvedic formulations.

1. INTRODUCTION

"Ailanthus excelsa Roxb. (Simaroubaceae) is commonly known as Mahanimba due to its resemblance with neem tree. It is used as a folk medicine for variety of purposes like asthma, cough, cancer *etc*.", [1]. "It consists of phytoconstituents such as quassinoids, alkaloids, protein flavonoids *etc.,* in different parts of the plants. It also has antifungal, antibacterial, antiviral, antimicrobial, antimalarial, antifertility, antitumour activities" [2]. The traditional claims, phytochemical investigations and pharmacological evaluations and some ayurvedic formulations provides the backbone to make this tree a tree of heaven. Quassinoids such as excelsin, glaucarubine, alianthinone glaucarubinone and glaucarubolone are found to show antifungal activity. The methanol extract of stem barks of *Alianthus excelsa* was partitioned with chloroform. The chloroform extract showed fungistatic and fungicidal activity against *Aspergillus niger*, *A. fumigatus*, *Penicillium frequentence, P. notatum* and *Botrytis cinerea*.

Ailanthus excelsa consists of protiens, phenols, flavonoids, alkaloids, triterpinoids and steroids in different parts of the plant [3]. The *Ailanthus excelsa* leaves contain proteins and bioactive compounds *viz*., phenols, flavonoids and antioxidants higher than other parts of the tree [4]. Leaves were also reported to have hepatoprotective effect and antidiabetic activity [5].

Drying of *Ailanthus excelsa* leaves is an important post harvest treatment to reduce the moisture content and to increase the shelf life. Since the bioactive components of *Ailanthus excelsa* are heat sensitive materials, drying under low temperature could be beneficial. Hence shade drying, tray drying and dehumidified air drying are beneficial [6].

There are different methods of extraction of bioactive components from different parts of the plant. Among all, the supercritical fluid extraction plays a very important role. Supercritical fluid is a fluid that exceeds its critical temperature and pressure where distinct liquid and gaseous phases do not exist while simultaneously possess properties of both phases. There are different applications pertaining to supercritical fluids such as supercritical fluid chromatography analysis. However, supercritical fluids are more commonly used for supercritical fluid extract (SFE) of phytochemical components from plant cell matrices through bulk supercritical solvent. SFE is favourable due to its unique extractive process that can operate in the absence of oxygen, contrary to conventional extraction techniques such as maceration and Soxhlet extraction, to avoid oxidation process. Separation of solutes from bulk solvent is normally achieved through depressurization process, where supercritical fluids are converted stepwise into gaseous phase. Hence the present research is planned to study the management of some major foliar fungal pathogens of sunflower by using SFE of *Ailanthus excelsa* both under *in vitro* and field conditions.

2. MATERIALS AND METHODS

2.1 Preparation of the Supercritical Fluid Extract of *Ailanthus Excels*

"The supercritical carbon dioxide extraction system (Waters Thar; SFE 500 system) was used for extraction of oil from leaves of *Ailanthus excelsa*, which included the accessories like 500 ml extraction vessel, high-pressure pump, automated back pressure regulator, water bath and pump unit. Circulated deionized water (at 5°C) was used for cooling different zones in the SC-CO_2 extraction system. The basic principle of supercritical fluid extraction (SFE) is that when the feed material comes in contact with a supercritical fluid, the volatile substances will get separated into the supercritical phase. After the dissolution of soluble material, the supercritical fluids containing the dissolved substances were

removed from the feed material. The extracted component is then completely separated from the supercritical fluid by means of a pressure change" [7].

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids.

Fresh *Ailanthus excelsa* leaves were collected and were dried in a shade drier for one day and the dried leaves were powdered in a grinder. This powder was further used for the SFE extract preparation.

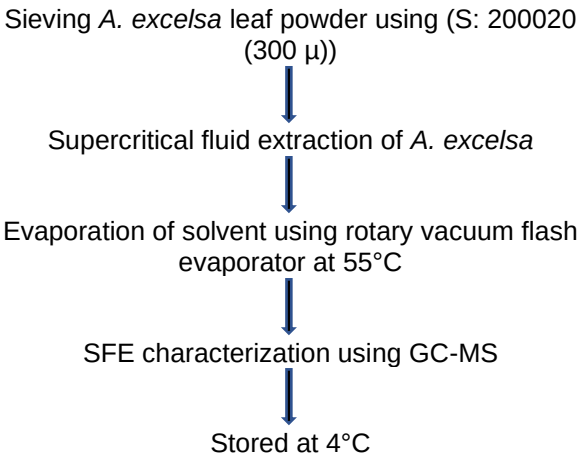
The supercritical fluid extraction was carried out at the processing and food engineering unit. SFE unit consists of CO_2 cylinder, Heat exchanger which maintains temperature, pressure cell to regulate pressure, a cell to contain the sample and another cell where the extract was collected. A modifier, which is ethanol was also be placed which further mixes with CO_2. The gas CO_2 was compressed at a certain temperature and pressure which is 60^0C temperature and 225 bar pressure. The CO_2 was mixed with ethanol and passes to the extraction vessel. *Ailanthus excelsa* was kept in muslin cloth bag and tied. That muslin cloth was placed in the extraction vessel [6].

The CO_2 and ethanol in fluid form, passed to extraction vessel. The bioactive components present in the sample were leached out and gets dissolved in CO_2. This was further passed through the condenser where the bioactive components in vapour form were condensed. Finally the extract prepared was collected at collecting cell and concentrated. Concentration of sample was done by the help of a Rotary flash evaporator which evaporates ethanol present in the sample leaving behind the bioactive component.

2.2 Procedure for the Preparation of Supercritical Fluid Extract from *Ailanthus Excels*

2.2.1 Supercritical fluid extraction of *A. excelsa* procedure

The collected leaves were shade dried in the department of Processing and Food Engineering Department, College of Agricultural Engineering, Raichur. Once the leaves were dried to the optimum moisture content, they were ground in to power king magic spice grinding machine to obtain fine powder. The powder was sieved using S:200020 (300 µ) sieve to get a fine powder. The extraction was carried out using fine powder.



2.2.2 Process flow chart for supercritical fluid extraction of *Ailanthus excels*

Hundred grams of *Ailanthus excelsa* leaf powder was placed into the extractor vessel. Liquid carbon dioxide and co-solvent ethanol were pumped into the extraction vessel after achieving the desired temperature. The flow rate of CO_2 and co-solvent was maintained at 20 g/min. and 5 g/min., respectively. Static extraction process was performed for 30 min after the desired pressure and temperature were attained. The dynamic extraction time was started by opening the exit valve for the SC-CO_2 extraction system. The static extraction time allowed the sample to soak in the CO_2 and co-solvent in order to equilibrate the mixture at desired pressure and temperature. The static extraction time of 30 min was observed to achieve the supercritical condition for every run. During the dynamic extraction time 120 min., CO_2 carrying the crude extract flowed out of the extraction vessel. Finally, the extract prepared was collected at collecting cell and concentrated. Concentration of sample was done by the help of a rotary flash evaporator which evaporates ethanol present in the sample leaving behind the bio active components [6,8].

2.2.3 Characterisation of supercritical fluid extract of *Ailanthus excelsa* by GC-MS analysis

GC-MS analysis of supercritical fluid extract of *Ailanthus excelsa* was done in the Centre for nanotechnology, College of Agricultural Engineering, Raichur, Karnataka, so as to find

out the different bioactive compounds present in SFE of *Ailanthus excelsa.*

2.3 Estimation of Phenols and Flavonoids Content Present in *Ailanthus excelsa* leaves

2.3.1 Total phenolic content

"The phenolic content of crude extract was determined by Folin–Ciocalteu reaction. To 100 µL sample, 1900 µL of distilled water, 500 µL of Folin–Ciocalteu reagent and 1ml of 20 per cent of Na_2CO_3 were added. After mixing thoroughly, it was incubated at room temperature in dark condition for 20 min. The absorbance was measured at 730 nm using UV-Vis Spectrophotometer. The obtained triplicates were calculated by the linear equation of gallic acid standard curve and thus the result was expressed as $mgGAEg^{-1"}$ [5].

2.3.2 Total flavonoids

The total flavonoid content of crude extracts was determined by using quercetin as standard. In 500 µL sample, 4.3 mL of 80 per cent ethanol, 100 µL potassium acetate was added followed by 100 µL aluminium nitrate and incubated at room temperature for 40 min. The absorbance was measured at 415 mm [5] using UV-Vis spectrophotometer. The total flavonoid content was expressed in terms of Quercetin equivalents as mg

3. RESULTS AND DISCUSSION

3.1 Preparation of Supercritical Fluid Extract of *Ailanthus Excels*

Ailanthus excelsa is the plant which belongs to the family Simarubaceae. It is said to be originated in Western, Central and Southern India. But now it has spread to the areas of semi-arid and sub-tropical regions. The leaves of it are pinnate, greyish green, soft and velvety in its structure (Plate 1). The alcoholic extract of the leaves and bark of the plant is having anti - inflammatory, anti - malarial, anti - diabetic, anti - fungal, anti - bacterial properties [1].

"A. excelsa is one of the important ingredient in most of the ayurvedic preparations like, pusyanuga chura, a herbo – mineral Ayurvedic preparation of which *A. excelsa* is important constituent" [9].

The phytochemistry of *Ailanthus excelsa* includes tryptophan derived alkaloids (carbolines, canthinones), triterpene – derived quassinoids. The quassinoids are reported to have anti – viral, anti – bacterial, anti – fungal properties. The five major compounds which are identified from this plant includes excelsin 5, glaucarubine, ailanthinone, glaucarubinone and glaucarubolone [3].

The chloroform fraction of the ethanol extract of leaves of *A. excelsa* showed significant fungistatic and fungicidal activity against *Aspergillus niger*, *Penicillium notatum* and *Botrytis cinerea*. Among all the bioactive components which are obtained , Ricinolcic acid , Diethyl phthalate , 1.63 Hexadecanoic acid , methyl ester and 9-Octadecenoic acid (Z) , mehyl ester were found to import anti- fuingal activity .

The leaves which were collected from the tree *A. excelsa* were dried under shade drying at the temperature of 30 ± 1 ^oC the moisture content of the leaves decreased with increase in drying time. But after certain period the moisture content of the leaves remained constant due to complete evaporation of water with respect to time. The supporting results were seen in the studies carried out by Ali *et al*. [10]

Milling process of the dried leaves of *A. excelsa* was carried out by normal pulverizer. The average powder outlet temperature was 35 ± 1 ^oC from the normal pulverizer. The variation in temperature of the outlet powder was mainly due to the water circulated through it. The *A. excelsa* leaf powder was passed through 25 mm sieve to obtain uniform sized particles [11,12,6].

3.2 Supercritical Fluid Extraction

The supercritical fluid extraction from *A. excelsa* was carried out using supercritical carbon dioxide and ethanol as a solvent at the flow rate of about 20 g/min and 5 g/min, respectively at the temperature of 60^oC. The procedure was followed as described in material and methods.

The procedure was in confirmation with the experimental results obtained by Roopa Bai [6]. The extraction efficiency obtained from *A. excelsa* leaf powder at different SFE temperature (40, 50 and 60^oC) and pressure (125, 175 and 225 bar) combinations. It is observed that, the extraction efficiency varied in the range of 53.49 to 65.01 per cent at 40^oC, 67.67 to 79.41 per cent at 50^oC and 85.67 to 91.43 per cent at 60^oC for different pressures. Among the treatment combinations, the extraction efficiency of 91.4 (%) was the highest at 225 bar and 60°C,

whereas the lowest of 53.49(%) was recorded at 125 bar and 40°C. The extraction yield obtained from Soxhlet extraction (control) of *A. excelsa* extract was found to be 49.17(%) lower compared to SFE extract. The SFE extract of *A. excelsa* leaves were found to have more antifungal and antibacterial activity when compared with solvent extraction [8].

3.3 Gc-Ms Analysis of Supercritical Fluid Extract

The different constituents which are present in the *A. excelsa* are the major reasons for anti-fungal activity which is of our interest. In order to know the constituents which are present in *A. excelsa*, GC-MS analysis was carried out in the Centre for Nanotechnology, College of Agricultural Engineering, University of Agricultural Sciences, Raichur. The procedure was followed as mentioned in material and methods. As per this study, alkaloids, quassinoids, flavonoids and other bioactive compounds were obtained which formed 40 different types of biomolecules. Among all the bioactive components which are obtained, Ricinoleic acid, Diethyl phthalate, 1.63 Hexadecanoic acid, methyl ester and 9-Octadecenoic acid (Z)-, methyl ester were found to impart anti- fungal activity. The bioactive compounds which were obtained are mentioned in the Table- 1 & Fig.1. GCMS real time analysis Software for analysis of bio molecules by using standard data program of NIST (National Institite of Standard and Technology, USA) for Mass Spectrophotometer (Shimadzu, QP, 2020).

The mode of action of Diethyl phthalate which is present in leaf extract is, it recognizes four major ROS comprising of superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen and will dehydrates the cell [13].

3.4 Estimation of Phenols and Flavonoids in *Ailanthus Excelsa* Leaves

For the estimation of phenols and flavonoids, the method which was suggested by Deepika *et al* [5] was followed and it was found that 19.65 $mgGAEg^{-1}$ and 40.68 $mgGAEg^{-1}$ of phenols and flavonoids were present in SFE of *A. excelsa*.

The similar results were obtained during the investigations by Kumari and Sharma [4] where they found the phenolic and flavonoid content of about 36.04 $mgGAEg^{-1}$ and 21.5 $mgQEg^{-1}$, respectively.

The results were also in confirmation with the experiment which was carried out by Soumya [14]. The results from the experiment revealed that, the phenolic and flavonoid content of about 18.75 $mgGAEg^{-1}$ and 42.86 $mgGAEg^{-1}$, respectively.

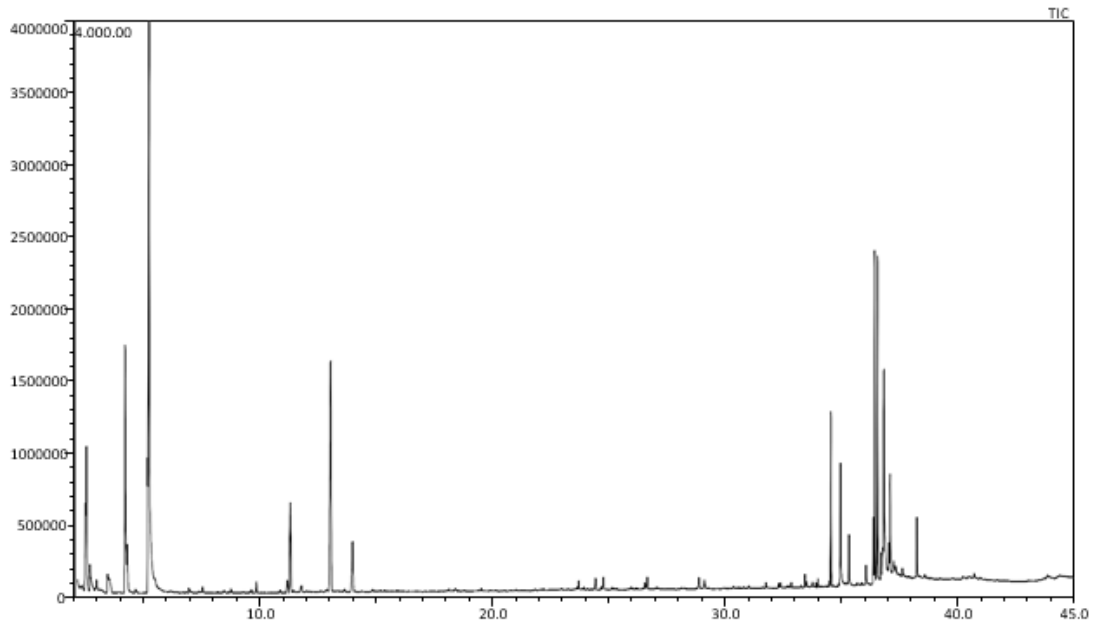


Fig. 1. Mass spectrum of the SC-CO_2 extract of the *Ailanthus excelsa* as determined by GC-MS analysis

Table.1. GC-MS profiling of *A. excelsa* leaf extracts in ethanol

Sl. No.	R. T	I. T	F. T	Area	Area(%)	Height	Height(%)	A/H	Name	FWHM
1	2.458	2.395	2.470	1320787	2.33	605966	2.62	2.18	1-Propanol, 2-methyl-	0.033
2	2.498	2.470	2.605	2447758	4.31	998285	4.32	2.45	1-Propanol, 2-methyl-	0.046
3	2.648	2.625	2.760	526141	0.93	177725	0.77	2.96	Ethane, 1-ethoxy-1-methoxy-	0.037
4	2.922	2.890	2.960	92237	0.16	55498	0.24	1.66	1-Butanol	0.026
5	3.402	3.375	3.430	147474	0.26	80124	0.35	1.84	Methyl methacrylate	0.027
6	4.156	4.110	4.225	4533294	7.98	1712672	7.41	2.65	1-Butanol, 3-methyl-	0.036
7	4.247	4.225	4.335	819306	1.44	325700	1.41	2.52	1-Butanol, 2-methyl-	0.044
8	5.125	5.110	5.140	1270691	2.24	929415	4.02	1.37	Formamide, N, N-dimethyl-	0.023
9	5.181	5.140	5.415	13346747	23.50	3963837	17.15	3.37	Formamide, N, N-dimethyl-	0.046
10	9.800	9.755	9.855	191812	0.34	77000	0.33	2.49	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-en	0.039
11	11.133	11.075	11.185	301778	0.53	85942	0.37	3.51	Bicyclo [3.1.0] hexane, 4-methylene-1	0.058
12	11.263	11.185	11.325	2100371	3.70	620897	2.69	3.38	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methyl	0.055
13	12.998	12.900	13.070	5538557	9.75	1596573	6.91	3.47	**Ricinoleic acid**	0.055
14	13.944	13.855	14.000	1156008	2.04	345464	1.49	3.35	gamma. - Terpinene	0.050
15	23.665	23.625	23.710	124216	0.22	58849	0.25	2.11	1-Tetradecene	0.034
16	24.381	24.340	24.435	174677	0.31	71966	0.31	2.43	Caryophyllene	0.039
17	24.718	24.675	24.765	196192	0.35	82346	0.36	2.38	Cis – alpha - Bergamotene	0.038
18	26.490	26.465	26.575	105461	0.19	33785	0.15	3.12	alpha -Farnesene	0.043
19	26.607	26.575	26.660	203880	0.36	80764	0.35	2.52	beta - Bisabolene	0.042
20	28.829	28.785	28.895	201101	0.35	73480	0.32	2.74	**Diethyl Phthalate**	0.043
21	29.069	29.035	29.130	129944	0.23	51106	0.22	2.54	1-Heptadecene	0.041
22	33.395	33.360	33.425	143345	0.25	86503	0.37	1.66	Neophytadiene	0.026
23	33.951	33.920	33.990	95904	0.17	57709	0.25	1.66	Neophytadiene	0.026
24	34.433	34.460	34.460	63190	0.11	39061	0.17	1.62	1.62 Methyl hexadec-9-enoate	0.026
25	34.502	34.860	34.550	1968319	3.47	1209944	5.23	1.63	**1.63 Hexadecanoic acid, methyl ester**	0.025
26	34.916	35.240	35.020	1909180	3.36	837821	3.62	2.28	n-Hexadecanoic acid	0.032
27	35.279	35.975	35.355	599192	1.05	341518	1.48	1.75	Hexadecanoic acid, ethyl ester	0.025
28	36.015	36.290	36.090	280786	0.49	130024	0.56	2.16	1,3-Dicyclohexylurea	0.030
29	36.330	36.355	36.355	760447	1.34	455940	1.97	1.67	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	0.026
30	36.391	36.435	36.435	3877400	6.83	2296640	9.94	1.69	9,12,15-Octadecatrienoic acid, methyl ester,	0.026
31	36.455	36.470	36.470	70433	0.12	44403	0.19	1.59	**9-Octadecenoic acid (Z)-, methyl ester**	0.027
32	36.507	36.595	36.595	3918273	6.9	2250100	9.73	1.74	Phytol	0.026
33	36.663	36.685	36.685	309844	0.55	186867	0.81	1.66	Methyl stearate	0.025
34	36.720	36.740	36.740	527829	0.93	215652	0.93	2.45		
35	36.788	36.925	36.925	4280463	7.54	1446798	6.26	2.96	9,12,15-Octadecatrienoic acid, (Z, Z, Z)	0.035
36	36.995	37.020	37.020	596704	1.05	231435	1.00	2.58	9,12-Octadecadienoic acid, ethyl ester	0.034
37	37.050	37.105	37.105	1241698	2.19	703940	3.05	1.76	9,12,15-Octadecatrienoic acid, ethyl ester,	0.026
38	37.199	37.255	37.255	384401	0.68	92304	0.40	4.16		
39	37.306	38.165	37.340	114717	0.2	49521	0.21	2.32	Octadecanoic acid, ethyl ester	0.026
40	38.202		38.275	729132	1.28	412159	1.78	1.77	9-Octadecenoic acid, 12-hydroxy-, methyl ester	0.026
				56799689	100	23115733	100			

Plate 1. *Ailanthus excelsa*

4. CONCLUSION

Among bioactive components present in supercritical fluid extraction of *A. excelsa* which are obtained, Ricinoleic acid, Diethyl phthalate, 1.63 Hexadecanoic acid, methyl ester and 9-Octadecenoic acid (Z)-,methyl ester were found to impart anti- fungal activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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