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Phytochemical and GC-MS analysis of ethyl acetate extract of *Ailanthus triphysa* leaves.

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Abstract

Introduction: This study is to analyse the phytochemical present in ultrasonicated extract of *Ailanthus triphysa* leaves and to determine the bioactive component using Gas Chromatography Mass Spectroscopy. **Methods:** The leaf of *Ailanthus triphysa* was extracted using ethyl acetate by ultra sonication method for phytochemical analysis. Gas chromatography Mass spectroscopy determines bioactive compounds from ethyl acetate extract. **Results:** The phytochemical analysis revealed the presence of alkaloids, flavonoid, steroids and terpenoids. The GC-MS analysis led to the identification of 10 compounds from the ultrasonicated ethyl acetate extract of *Ailanthus triphysa*. The compounds are pentadecanoic acid, 14-methyl-, methyl ester, heptacosanoic acid, methyl ester, 9-octadeenoic acid (z)-, methyl ester, pentadecanoic acid and squalene, 2-isoprppyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate, dodecanal, iodobis (n, n-di-isobutyldithiocarbamato) arsine and 2,4-dimethyl-7-oxo-4,7-dihydro-triazolo (3,2-c) triazine. Further studies are needed to determine its therapeutic values for the development of new drugs.

Keywords: *Ailanthus triphysa*, phytochemicals, Gas chromatography.

INTRODUCTION

Medicinal plants used for ancient medicines which contain variety of substance used to treat infectious and chronic diseases. Several biological activities are existing in medicinal plant such as antifungal, antibacterial, antioxidant, anti-inflammatory, anticancer, antidiabetic and wound healing activity [1]. Organic product, such as crude extract, plant extract and pure extract provides unlimited possibilities for discovering of new drug. World health organization (WHO) reported that over 80% of humans used ancient drug as treatment such as herbs medicine [2]. Accordingly, to WHO, 20,000 of medicinal plant are available in 91 countries. Plant are used as curative agents in both organic(Unani, Ayurveda) and organized (tribal, folk, native) form [3]. Plants has been recognized has therapeutic agent due to presence of nutritional (Vitamins and minerals), and non-nutritional (fibres, active compound including the saponins, plant sterols, curcumins, lignans, sulphides, polyphenolics, coumarins, flavonoids, terpenoids**)** [4]. *Ailanthus triphy*sa (also called as *Ailanthus malabarica*) which has an antipyretic property and also used to treat dyspeptic. It belongs to the family of Simaroubaceae and it is commonly known as halmaddi in India, originated from Asia and Australia. Flowers are creamy green in colour and mainly Flowering in February to march in India, fruits are reddish-brown, flat, seed compressed [5].The woods contain various quassinoids, beta-carboline and alkaloids, it has been used for treatment of various diseases like, bronchitis, dysentery dyspepsia; bark is used in constipation typhoid; root bark is used for cobra poisoning and it is also used in asthma. The plant, leaves, roots, gum and bark are used as medicine in India. When the bark is cut, the sticky resin is discharge, it become fragile and drying and they are used for medicinal purpose. This plant is aromatic in nature, so the timber is used to manufacturing of matchboxes and incense this plant is the highest priority needs of security matches industry and real wood utilized for manufacturing of splints [6]. The objective of our study is to determine the chemical composition of ethyl extract of *Ailanthus triphysa* leaves by GC-MS and phytochemical.

Phytochemical analysis:

Phytochemical are chemical compounds made by plants which assist them to thrive and protect them from competitor, pathogen or predators some phytochemical is used as poisons and some are used as drugs. The analysis of phytochemicals is a very important step as it reveals the presence of new drugs or medicinal compound which is very important at present time since many disease-causing microorganisms are becoming resistance to drugs available at the movement. Phenolics are the biggest group of phytochemicals have been said to represent the greater part of the antioxidant activity. These classes, (for example, alkaloids, flavonoids, phenols, saponins, steroids, sugars and tannins) of compound are known to have healing action against a few pathogens and in this way could recommend the utilization generally for the treatment of different diseases [7].Column chromatography is used to separate mixture of chemical substance into its individual compounds. It is a laboratory method widely used for purification or separation of chemical compound mixture. Chromatography consists of two phases, mobile phase which is liquid and stationary phaseconsist of solid. The mixture of compound moves along with mobile phase through stationery phase and separates depending on the different degree of adhesion of each component in the compound mixture [8].Thin layer chromatography is a "solid-liquid adsorption" chromatography. In this technique stationary phase is a solid substance (silica gel, alumina cellulose),which is coated on a glass plates and mobile phase consist of liquid substance It is advanced method of paper chromatography, when sample has been loaded on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn by the plate through capillary action. Thus, the separation of sample has been achieved. The upward travelling rate depends upon the polarity of a solid phase, materials and the solvent [9].

Gas Chromatography Mass Spectroscopy

The Gas chromatography-Mass spectrometry (GC-MS) is an analytical method which combined the features of both gas chromatography and mass spectrometry. The GC-MS is an instrument used to separate chemical mixtures (GC component) and identifies the components at a molecular level (MS components). It is one of the most accurate tools to analyse the sample mixture. GC is a common type of chromatography in that a mixture will be separated into individual substance when it is heated and the heated gases are taken through column with an inert (such as helium, nitrogen). The separated substance moves out through column opening and they flow into MS. Mass spectrometry is an instrument used to identifies compound by the mass of sample molecule. It is considered as only definitive analytical detector [11].

MATERIALS AND METHODOLOGY:

Preparation of ultrasonicated extract of *Ailanthus triphysa* leaves:

The leaves of *Ailanthus triphysa* was collected from Bangalore Forest Department. The leaves were washed thoroughly, shade dried for 8 hours and grounded into fine powder. The powdered material was extracted by immersing it in ethyl acetate with ratio 1:10 for 12 hours. For breaking the heavier compounds, ultrasonication was done by using sound into the extracted sample. The melting point of the sample was noted and ultrasonicated by setting the pulse as 5:10 for 25 minutes.

Phytochemical analysis:

Phytochemical analysis were carried out onultrasonicated ethyl acetate extract using standard protocol described in . Various tests were performed like alkaloids, carbohydrate, glycosides, saponins, protein, phenol, terpenoids, flavanoids, tannins, steroids.

Phytoconstituents separation using chromatographic techniques:

The mobile phase was prepared by using different combination of hexane and ethyl acetate of the ratio (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10) with silica as a stationary phase. A cotton plug was tamped down in to the bottom of the column after which the column was filled with hexane and the silica gel was added using a beaker. The column was tapped as the silica gel was added. 10 ml of ultrasonicated extract was added carefully on to the glass column packed with silica gel and the column was run. Then 10 ml of eluted fraction was collected in the falcon tube by changing it simultaneously. Concentrations were eluted in the ratio of ethyl acetate: hexane ranging from 10:0 to 0:10. The collected purified fractions were subjected to rotary vapour from the column eluted fraction and they are subjected for GCMS analysis [10].

GC-MS analysis:

The ethyl acetate plant extract of *Ailanthus triphysa* were subjected to GCMS analysis which was prepared by ultrasonication method. GC-MS procedure was performed by utilizing GC Shimadzu QP2010 framework and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) outfitted with Elite-1 combined silica hair like segment. Helium gas (99.99%) was utilized as the bearer gas at a consistent stream rate of 1.51ml/min and an infusion volume of 2µl was utilized (part proportion: 20). Injector temperature was 200°C; Ion-source temperature 200°C. The stove temperature was modified from 70°C (Isothermal for 2 min.) with an expansion of 300°C for 10 min. Mass spectra were taken at 70eV; a sweep interim of 0.5 seconds with output scope of 40 - 1000 m/z. Add up to GC running time was 35 min [12].

Identification of bioactive compounds

The mass spectrum obtained from the GCMS was compared with the standard chart database of National Institute Standard and Technology (NIST).

RESULT AND DISCUSSION

Phytochemical analysis of Ultrasonicated extract of A*ilanthus triphysa* leaves.

The phytochemical analysis shows the presence of alkaloids, flavanoids, steroids and terpenoids in the ultrasonicated ethyl acetate extract. Then the sample was subjected to column chromatography in which all the fractions were dried and GCMS were also performed.

GCMS analysis of ultrasonicated ethyl acetate extract of *Ailanthus triphysa*shows several peaks wih 10 bioactive compounds like pentadecanoic acid,14-methyl-, methyl ester, heptacosanoic acid, methyl ester, 9-octadeenoic acid (z)-, methyl ester, pentadecanoic acid squalene, 2-isoprppyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate, dodecanal, hexadecenal, iodobis (n, n-di-isobutyldithiocarbamato) arsine and 2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine. The activity of bioactive compounds were listed in Table 2.

Table 1. Phytochemical analysis done in ultrasonicated extract of*Ailanthus triphysa* leaves.

TEST	Ethyl acetate
Alkaloids	++
Flavonoids	++
Tannin	--
Carbohydrate	--
Steroids	++
Phenols	--
Terpenoids	++

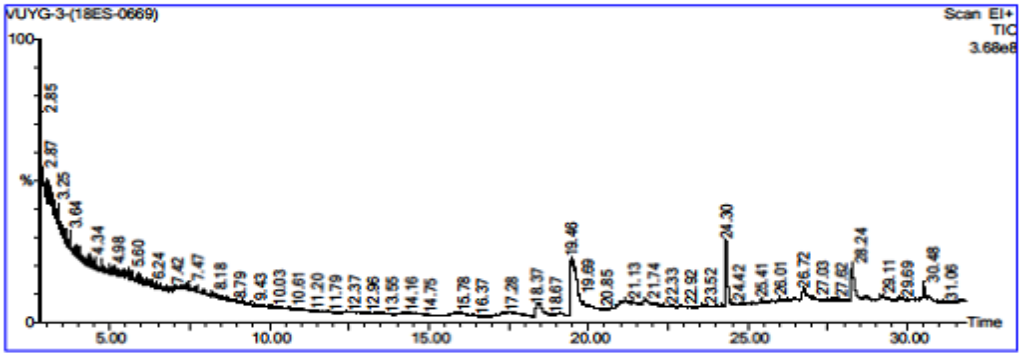

FIGURE 1: GC - MS of ultrasonicated *Ailanthus triphysa*

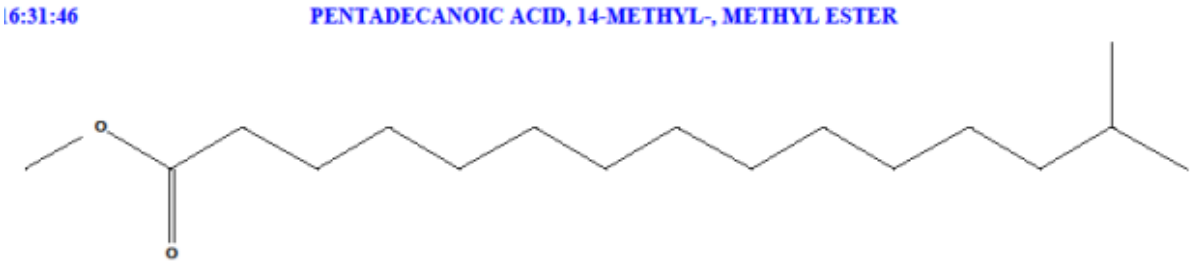

FIGURE 2:PENTADECANOIC ACID,14-METHYL-, METHYL ESTER.

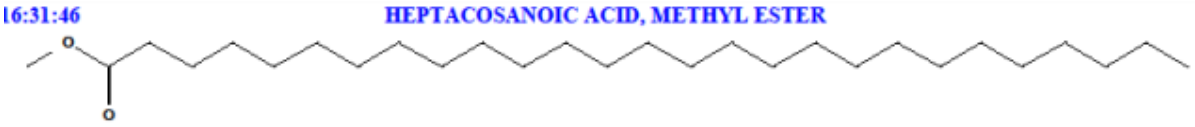

FIGURE 3:HEPTACOSANOIC ACID, METHYL ESTER

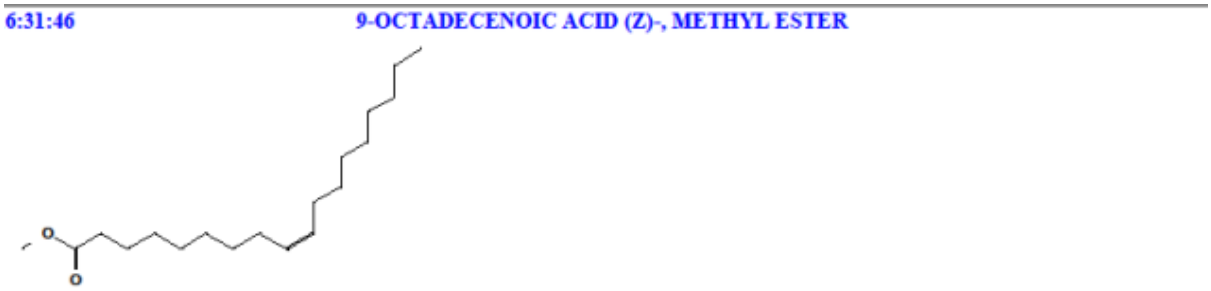

FIGURE 4:9-OCTADEENOIC ACID (Z)-, METHYL ESTER

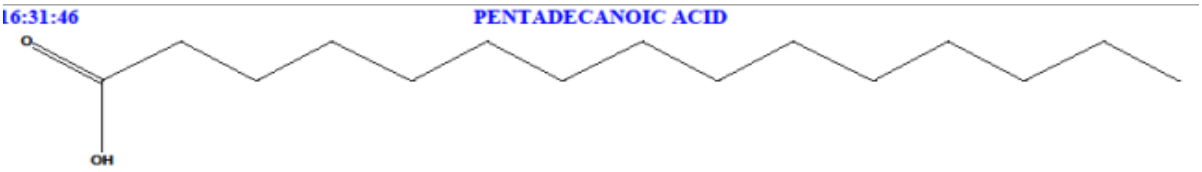

FIGURE 5:PENTADECANOIC ACID

FIGURE 6:HEXADECANAL

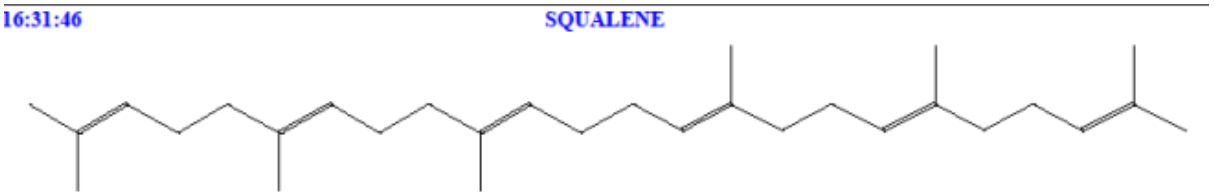

FIGURE 7:SQUALENE

FIGURE 8:DODECANAL

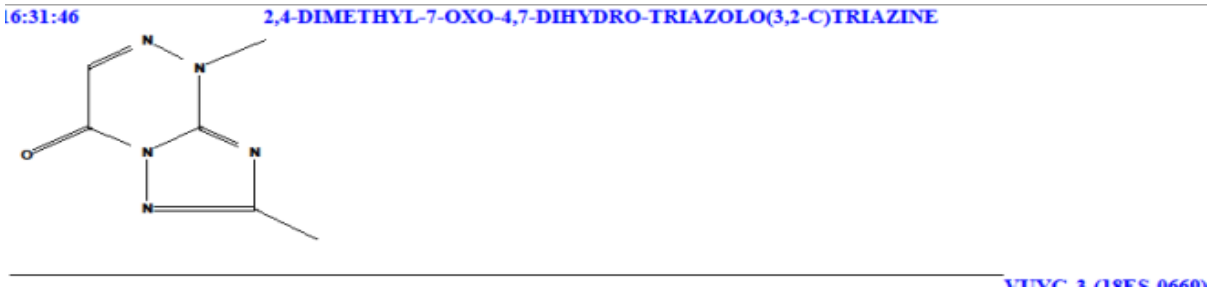

FIGURE 9: 2,4-DIMETHYL-7-OXO-4,7-DIHYDRO-TRIAZOLO(3,2-C) TRIAZINE.

FIGURE 10:2-ISOPRPPYL-5-METHYLCYCLOHEXYL 3-(1-(4-CHLOROPHENYL)-3-OXOBUTYL)-COUMARIN-4-YL CARBONATE.

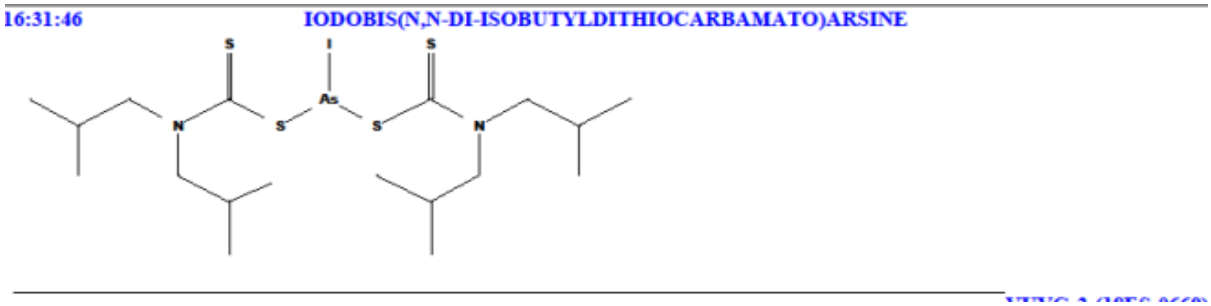

FIGURE 11: IODOBIS (N, N-DI-ISOBUTYLDITHIOCARBAMATO) ARSINE

TABLE 2. Phytocomponents identified in ethyl acetate leaves extract of *Ailanthus triphysa*.

S.No	Retention Time	Name of the compound	Molecular formula	Molecular weight	Peak area%	Biological activity
1	18.375	pentadecanoic acid,14-methyl-, methyl ester.	$C_{17}H_{34}O_2$	270	4.374	Antioxidant & antimicrobial.
2	18.465	heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	424	5.324	Antioxidant & biodiesel.
3	19.470	9-octadeenoic acid (z)-, methyl ester	$C_{19}H_{36}O_2$	296	46.930	Anti-inflammatory, antiandrogenic, and antimicrobialproperties
4	19.835	pentadecanoic acid	$C_{15}H_{30}O_2$	242	1.796	Anti-bacterial and antifungal.
5	21.361	hexadecanal	$C_{16}H_{32}O$	240	1.428	No activity
6	21.761	dodecanal	$C_{12}H_{24}O$	184	1.583	No activity
7	24.297	squalene	$C_{30}H_{50}$	410	14.71	Anticancer, antioxidant,drug carrier, detoxifier, skin hydrating
8	26.728	2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine.	$C_6H_7ON_5$	165	4.331	Un known
9	28.239	2-isoprppyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate.	$C_{30}H_{33}0_6Cl$	524	15.53	No activity
10	30.484	iodobis (n, n-di-isobutyldithiocarbamato) arsine	$C_6H_{12}O_2$	116	4.007	New compound

CONCLUSION:

The phytochemical analysis using ultrasonicated sample revealed the presence of alkaloids, flavonoids, tannin, steroids, terpenoids and saponin.The GCMS analysis led to the identification of 10 compounds from the ethyl acetate extract of *Ailanthus triphysa* leaves out of which 6 compounds such as pentadecanoic acid,14-methyl-, methyl ester, heptacosanoic acid, methyl ester, 9-octadeenoic acid (z)-, methyl ester, pentadecanoic acid and squalene have biological activity such as antioxidant, anticancer, antifungal, antimicrobial, anti-inflammatory, antiandrogenic etc and the compounds such as 2-isoprppyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate, dodecanal and hexadecenal does not have any biological activities but are used in fragrances. the remaining two compound iodobis (n, n-di-isobutyl dithio carbamato) arsine and 2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine activity is unknown. From the studies it has been cleared that the plant *Ailanthus triphysa* have many phytochemicals and active compounds which could be separated and used for further studies like antimicrobial, antifungal, antioxidant, anticancer to determine its therapeutic values for the development of new drugs.

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