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Research Article

IDENTIFICATION AND DETERMINATION OF BIOACTIVE PHYTOCHEMICAL CONSTITUENTS FROM THE HYDRO-ALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* WHOLE PLANT BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

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ABSTRACT

Objective: *Achyranthes aspera* Linn. commonly known as prickly chaff flower belonging to the family Amaranthaceae is said to show varied medicinal effects, including antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic, useful in pneumonia, edema, dropsy and piles, boils, eruptions of skin various other important medicinal properties. Hence, the present study deals with the determination of phytochemical constituents present in the hydro-alcoholic extract of *A. aspera* whole plant extract using the gas chromatography-mass spectrometry (GC-MS) technique.

Methods: The phytoconstituents present in the hydro-alcoholic extract of *A. aspera* whole plant was investigated extract using GC-MS technique, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library.

Results: GC-MS analysis of *A. aspera* whole plant extract revealed the existence of tetradecane (0.62%), benzaldehyde, 4-hydroxy-3,5-dimethoxy-(0.52%), 3-buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl) (2.65%), xanthoxylin (0.53%), phenol,4-(3-hydroxy-1-propenyl)-2-methoxy-(2.95%), patchouli alcohol (0.76%), dl-(2-fluorophenyl)-glycine (2.18%), flurenol butyl ester (0.91%), hexadecanoic acid, ethyl ester (3.28%), ethanone, 2-(benzoyloxy)-1-(1,1'-biphenyl)-4-yl- (0.93%), phytol (22.13%), 9,12-octadecadienoic acid (Z,Z)-(12.74%), 9,12-octadecadienoic acid (Z,Z)-,2,3-dihydroxypropyl ester (1.12%), squalene (0.55%), and lupeol (1.74%).

Conclusion: The present study revealed that *A. aspera* Linn. is an important plant with many therapeutically and pharmacologically active constituents justifying the use of this plant to treat many ailments in folk and herbal medicine.

Keywords: *Achyranthes aspera*, Gas chromatography-mass spectrometry technique, Herbal medicine, Lupeol, Phytol, Xanthophylin.

INTRODUCTION

After decades of serious problems with the modern medical practice, people have started looking at the ancient healing systems such as Ayurveda, Siddha, and Unani. This is because of the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs, especially in developing countries. The ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all the plant parts to be a potential source of medicinal substances [1]. There is a lack of knowledge of alternative medicines in the developed countries due to poor stringent quality control. There is a need for documentation of research work carried out on traditional medicines [2].The therapeutic properties of medicinal plants are mainly due to the existence of an assortment of complex chemical substances of diverse compositions which occur as secondary metabolites. The most significant of these bioactive constituents of plants are alkaloids, glycosides, tannins, proteins, phenolic compounds, and flavonoids [3].

A specific information about the lead chemical constituents present in the plants is attractive not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of new complex chemical substances and for discovering the actual significance of folkloric remedies. Hence, validation of herbal drugs now a day's plays a vital role in the standardization of herbal drugs and products. For the analysis of components existing in natural plants mass spectrometry (MS) coupled with gas chromatography (GC) was used. Recently, GC/MS have been used widely for the analysis of non-polar components and volatile essential oil, fatty acids, lipids, and alkaloids.

In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses. One of the many plants which are being evaluated for their therapeutic efficacies is *Achyranthes aspera* which is commonly known as Latjeera (Hindi) and Rough Chaff tree (English). It is an erect or procumbent, annual or perennial herb, 1-2 m in height, often with a woody base, commonly found as a weed of waysides, on roadsides [4-6].

A. aspera (Amaranthaceae) is a habitat of Asia, South America, and Africa. Fig. 1 shows the whole plant of *A. aspera.* The whole plant of *A. aspera* was used in traditional systems of medicines, whereas seeds, roots, and shoots are the most important parts which are used medicinally. In the traditional medicinal system, *A. aspera* is known for diuretic, hepatoprotective, and emmenagogue properties and used to cure several diseases *viz.*, malarial fever, dysentery, asthma, hypertension, and diabetics. Most recently, *A. aspera* is widely studied for its medicinal properties and reported to have immune stimulatory properties [7], wound healing activity [8], antioxidant activity, hemolyticactivity [9], anti-inflammatory [10], antibacterial activity [11], and antifungal activity [12]. The plant is used in the indigenous system of medicine as antiarthritic, antifertility, laxative, ecbolic, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anticoagulant, diuretic, and anti-tumor [13,14]. It is also useful to treat a cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles, and snake bites [15]. The juice of the plant is used in the treatment of boils, diarrhea, dysentery, hemorrhoids, rheumatic pains, itches, and skin eruptions [16]. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses.

METHODS

Collection of plant material

The fresh plant of *A. aspera* were collected during the month of January from VELS University campus, Pallavaram, Chennai and authenticated by Prof Jayaraman, Director of Plant Anatomy Research Centre (PARC), Reg.no: PRAC/2015/3095.

Processing of plant

A. aspera whole plant collected and washed thoroughly in distilled water and shade dried at room temperature as shown in Fig. 2 and pulverized to powder using a mechanical grinder. The required quantity of the whole plant powder of *A. aspera* was weighed transferred to a flask, treated with hydro alcohol (70% water + 30% ethanol) until the powder was fully immersed, incubated 72 hrs, and filtered through a filtered paper. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to phytochemical screening and GC-MS analysis.

GC-MS analysis

GC-MS analysis of the hydro alcohol extract of *A. aspera* was performed using a Perkin-Elmer GC Clarus 500 system and GC interfaced to an MS equipped with an Elite-I, fused silica capillary column (30 mm × 0.25 mm 1D × 1 µmdf, composed of 100% dimethyl poly siloxane. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/minute, and an injection volume of 2 µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45-450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas; software adopted to handle mass spectra, and chromatograms was a turbomass.

Identification of phytocomponents

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name; molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The lead phytocomponents present in the hydro alcohol extract of *A. aspera* whole plant was identified by GC-MS analysis. GC-MS chromatogram of hydro alcoholic extracts *A. aspera* whole plant was shown in Fig. 3. The phytocompound principles with their retention time, molecular formula, molecular weight, and concentration (%) in the hydro alcohol of parts of *A. aspera* were presented in Table 1. On comparison of the mass spectra of the constituents with the NIST library, the 15 phytocompounds were characterized and identified. The various phytochemicals which contribute to the medicinal activities of the plant were shown Table 2.

15 compounds were identified in the hydro alcohol extract of parts of *A. aspera*. The prevailing compounds were tetradecane (0.62%), benzaldehyde, 4-hydroxy-3, 5-dimethoxy-(0.52%), 3-Buten-2-one,4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-(2.65%), xanthoxylin (0.53%), phenol,4-(3-hydroxy-1-propenyl)-2-methoxy-(2.95%), patchouli alcohol (0.76%), dl-(2-Fluorophenyl)-glycine (2.18%), flurenol butyl ester (0.91%), hexadecanoic acid, ethyl ester (3.28%), ethanone, 2-(benzoyloxy)-1-[1,1'-biphenyl]-4-yl- (0.93%), phytol (22.13%), 9,12-octadecadienoic acid (Z,Z)- (12.74%), 9,12-octadecadienoic acid (Z,Z)-,2,3-dihydroxypropyl ester (1.12%), squalene (0.55%), and lupeol (1.74%). Fig. 4 shows the structure and chromatogram of identified phytochemical compositions. The mass of spectrum and structure of 15 compounds. Table 2 listed the major phytocompounds and its biological activities obtained through the GC-MS study of the all parts *A. aspera.*

Recent studies have shown that diets rich in phytochemicals can significantly reduce cancer risk by as much as 20% [17]. Lupeol and some have been shown to possess a range of folk and proven biological activities, and further a potential to be consumed as a dietary supplement to prevent cancer, coronary, and hepatic diseases [18]. Xanthoxylin induces melanogenesis via cAMP-mediated PKA activation [19]. Squalene, the main component of skin surface

Fig. 1: The whole plant of *Achyranthes aspera*

Fig. 2: The dried *Achyranthes aspera* whole plant

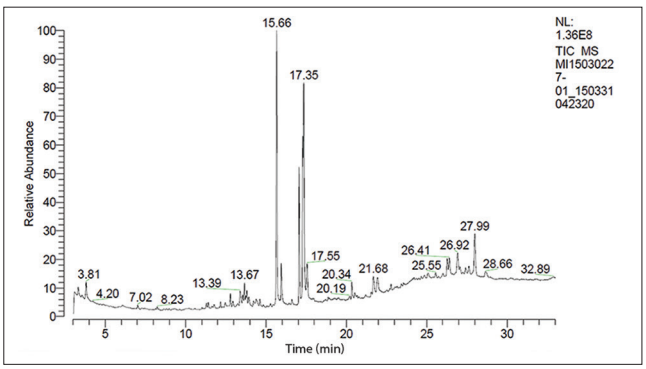

Fig. 3: Typical chromatogram of *Achyranthes aspera* whole plant hydro alcoholic extract, RT: 3.00-33.00 SM: 9G

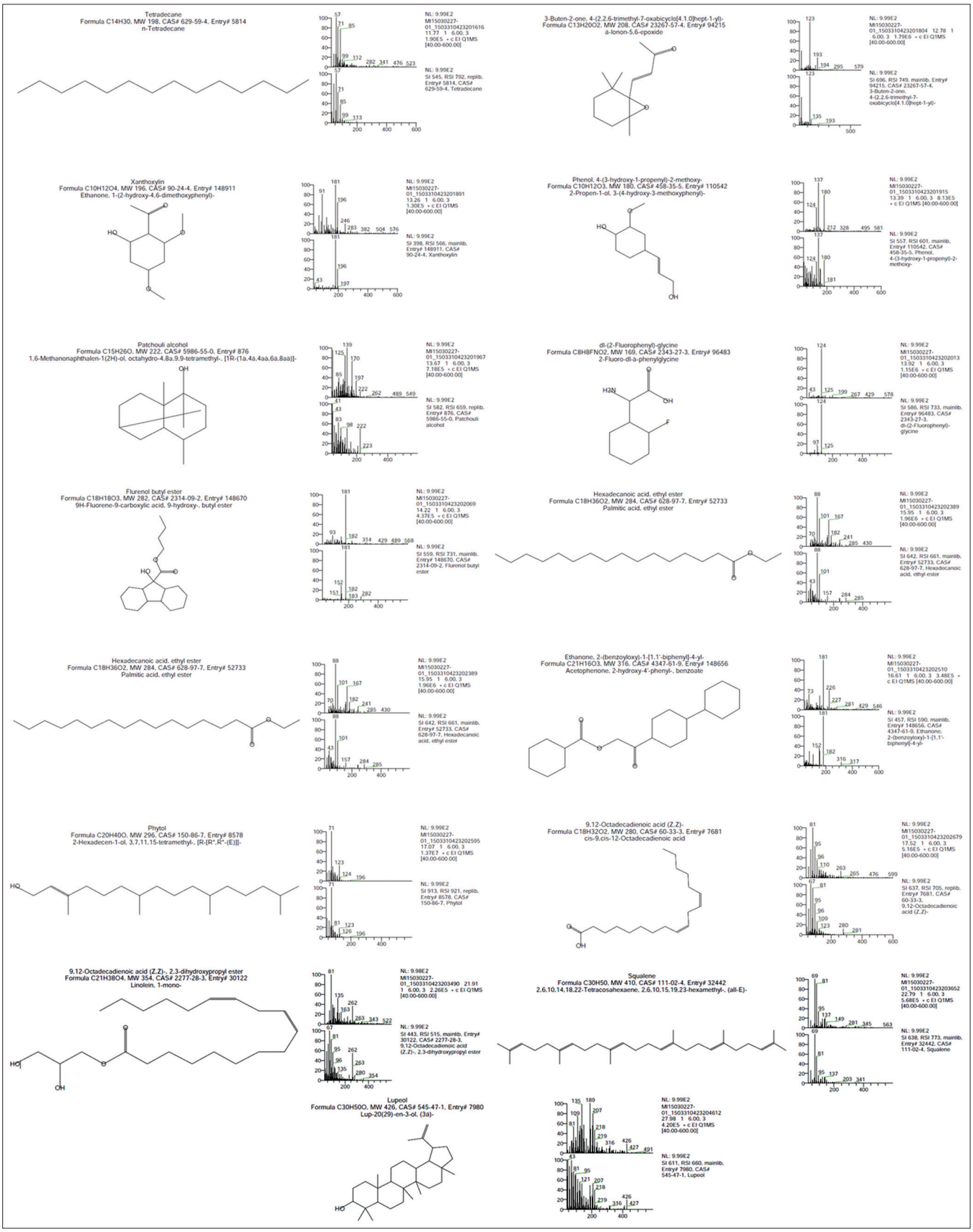

Fig. 4: (a-p) The structure and chromatogram of identified phytochemical compositions

polyunsaturated lipids, shows some advantages for the skin as an emollient and antioxidant, and for hydration and its antitumor activities [20].

Thus, the identification of lead phytocompounds from the hydro alcohol extract of *A. aspera* whole plant by GC-MS might have some ecological significance.

Table 1: The phytochemical composition of hydroalcoholic extract of *Achyranthes aspera* whole plant

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1.	11.77	Tetradecane	$C_{14}H_3O$	198	0.62
2.	12.46	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$	182	0.52
3.	12.78	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0] Hept-1-yl)-	$C_{13}H_{20}O_2$	208	2.65
4.	13.26	Xanthoxylin	$C_{10}H_{12}O_4$	196	0.53
5.	13.39	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	$C_{10}H_{12}O_3$	180	2.95
6.	13.67	Patchouli alcohol	$C_{15}H_{26}O$	222	0.76
7.	13.92	dl-(2-Fluorophenyl)-glycine	$C_8H_8FNO_2$	169	2.18
8.	14.22	Flurenol butyl ester	$C_{18}H_{18}O_3$	282	0.91
9.	15.95	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3.28
10.	16.61	Ethanone, 2-(benzoyloxy)-1-[1,1'biphenyl]-4-yl-	$C_{21}H_{16}O_3$	316	0.93
11.	17.07	Phytol	$C_{20}H_{40}O$	296	22.13
12.	17.52	9,12-Octadecadienoicacid (Z, Z)	$C_{18}H_{32}O_2$	280	2.16
13.	21.91	9,12-Octadecadienoic acid (Z, Z)-,2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354	1.12
14.	22.79	Squalene	$C_{30}H_{50}$	410	0.55
15.	27.98	Lupeol	$C_{30}H_{50}O$	426	1.74

Table 2: Bioactivity of phytocomponents identified in the hydro alcohol extracts of *Achyranthes aspera* by GC-MS analysis

S. No	RT	Name of the compound	Nature of compound	Activity
1.	11.77	Tetradecane	Alkanes	Not intended for therapeutic purpose
2.	12.46	Benzaldehyde, 4-hydroxy-3,5-dimethoxy	Aldehyde	Not intended for therapeutic purpose
3.	12.78	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0] Hept-1-yl)	Alkenes	Not intended for therapeutic purpose
4.	13.26	Xanthoxylin	Aromatic homomonocyclic compounds	Induces melanogenesis mainly via cAMP-mediated PKA activation
5.	13.39	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	Phenol	Not intended for therapeutic purpose
6.	15.95	Hexadecanoic acid, ethyl ester	Palmitic acid ethyl ester	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic, alphareductase inhibitor
7.	17.07	Phytol	Diterpene	Antimicrobial, anticancer, diuretic, anti-inflammatory
8.	17.52	9,12-Octadecadienoicacid (Z, Z)	Linoleic acid	Anti-inflammatory hypocholesterolemic cancer preventive hepatoprotective nematicide insectifuge, antihistaminic antieczemic antiacne, 5-alpha reductase inhibitor antiandrogenic antiarthritic anticoronary insectifuge
9.	22.79	Squalene	Vitamin E compound	Antiageing, Analgesic, antidiabetic, antiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
10.	22.98	Lupeol	Triterpene compound	Antimalarial, antioxidant, antiflue, antihyperglycemic, antitumor antiviral, pesticide, cytotoxic Anti-inflammatory

It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for therapeutic roles. Thus, this study explores the goodness of the *A. aspera* whole plant which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

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