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Phytochemical Screening and GC MS analysis of *Achyranthes aspera* Linn.

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Abstract
The present study was carried out to determine the chemical components of *Achyranthus aspera* leaves using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of methanolic extract of *Achyranthus aspera* leaves revealed the existence of Linoleic acid (12%), Palmitic acid(8.67%), 2-Furaldehyde,5-(Hydroxymethyl) (7.13%) and 3,5-Dihydroxy-6-Methyl-2,3- Dihydro-4H-Pyran-4-one (2.64%). Qualitative phytochemical screening of the methanolic extracts of the leaves revealed the presence of many compounds such as phenlic compounds, flavonoids, tannins, alkaloids, terpenoids, steroids and saponins. The results of this study offer a platform of using *Achyranhus aspera l*eaves as herbal alternative for various ailments.

Keywords: secondary metabolites, solvents, herbal medicine

Introduction
The genus *Achyranthes* belongs to the family *Amaranthaceae*. It is perennial stiff erect herb, growing up to 1000 m height. Stems are square, leaves elliptic, ovate or broadly rhombate. The plant is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America (Hariharan and Rangaswami, 1970). It is used in the treatment of fever, especially malaria fever, dysentery, asthma, hypertension and diabetes (Girach and Khan, 1992; Tang and Eisenbrand, 1992). Traditionally, the plant is used in the treatment of asthma and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc. Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints. The flowering spikes or seeds, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles, used in night blindness and cutaneous diseases (Nadkarni, 2009). The plant possesses various medicinal properties, the aim of the present study was to identify the phytocompounds in the ethanol extract of leaves of *A.aspera* by qualitative phytochemical screening and to identify the compounds present in the ethanol extract of the leaves by Gas Chromatography – Mass Spectrum (GC-MS) analysis.

Materials and methods
Collection of plant materials
The fresh leaves of *Achyranthes aspera* were collected from the local areas. The collected plant material was authenticated by Dr. V. Nandagopalan, Associate Professor, PG & Research Department of Botany, National College, Tiruchirappalli, Tamilnadu. The voucher specimen has been deposited at the department of Microbiology, Kamaraj College, Tuticorin, Tamilnadu. The collected samples were air dried for 7 days at room temperature (25°C). The dried samples were ground into fine powder and kept away from heat, moisture, and sunlight.

Preparation of extracts
500g dry powder of *Achyranthes aspera* was sequentially extracted with hexane, toluene, tetrahydrofuran, methanol and water using the Soxhlet apparatus on the water bath for 12 h each.
Each of the mixtures was carefully filtered using filter paper (Whatmann No. 1) and concentrated using a rotary evaporator. The extracts were stored in sterile bottles at -18 º C kept as aliquots until further evaluation.

Phytochemical screening
Phytochemical screening of plant extracts was carried out qualitatively for the presence of carbohydrates, terpenoids, tannins, flavonoids, phenolic compounds, saponins, phlobactanin, quinones and alkaloids (Ferranti *et al*., 1998).

GC MS Analysis
GC-MS technique was used in this study to identify the components present in the extract. GC-MS technique was carried out at The *Cashew Export Promotion Council* (*CEPC*), Kollam, Kerala. GC-MS analysis was carried out on a GC Clarus 500 Varian, USA system comprising a AOC-20I auto sampler and gas chromatograph interfaced to a mass spectrophotometer instrument employing the following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm I.D ×1 µ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µ l was employed (split ratio of10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10

°C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time is 46min.

Result and Discussion

Medicinal plants are rich source of secondary metabolites such as alkaloids, phenol, cardiac glycosides, flavonoids, tannins and terpenoids determined by gas chromatography and mass spectrum. The indigenous medicinal plants *Achyranthes aspera* were collected from Veli hills, Kumaracoil, Kanyakumari District, Tamilnadu. The phytochemical analysis of the whole plant of *Achyranthes aspera* showed the presence of carbohydrates in all the four extracts except hexane. Flavonoids and alkaloids were present in tetrahydrofuran, methanol and aqueous extracts. Terpenoids were found in toluene, tetrahydrofuran and methanol extracts. Saponins were found in methanol and aqueous extracts. Phenolic compounds and tannins were present only in methanol. Quinones were present in toluene and aqueous extracts (Table 1).

Table 1: Preliminary phytochemical screening of *Achyranthes aspera*

Tests	Hexane	Toluene	Tetrahydrofuran	Methanol	Aqueous
Alkaloids	-	-	+	+	+
Carbohydrates	-	+	+	+	+
Saponins	-	-	-	+	+
Phenolic compounds	-	-	-	+	-
Flavonoids	-	-	+	+	+
Terpenoids	-	+	+	+	-
Tannins	-	-	-	+	-
Quinones	-	+	-	-	+

(+ present; - absent)

Eighteen phytochemical compounds were identified the methanol extract of *Achyranthes aspera* using GC MS analysis (Fig 1). The Retention time, Peak area, IUPAC name, Chemical formula, Molecular weight and Chemical structure were present in table 2. GC/MS analysis of ethanolic extract of *Achyranthus aspera* leaves revealed the existence of Linoleic acid (12%), Palmitic acid(8.67%), 2-Furaldehyde,5-(Hydroxymethyl) (7.13%) and 3,5- Dihydroxy-6-Methyl-2,3-Dihydro-4H-Pyran-4-one (2.64%). As per the literature available, eleven among eighteen compounds were identified in the *Achyranthes aspera* and were found to possess various biological activities (Table 3).

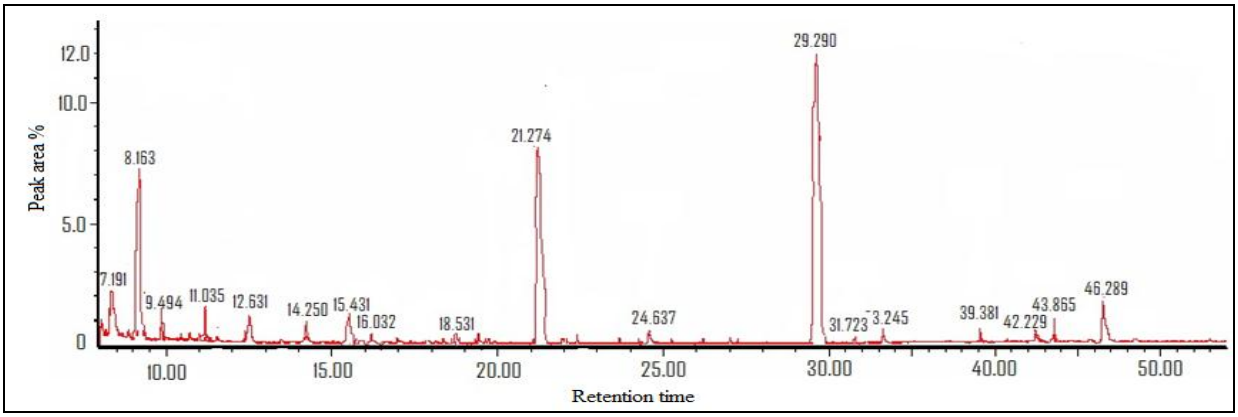
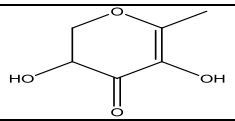
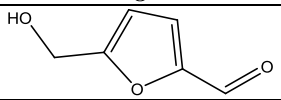
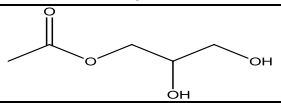


Fig 1: GC MS analysis of methanol Extract of *Achyranthes aspera*

Table 2: Compounds identified in the GC MS analysis of *Achyranthes aspera*

S. No.	RT	Peak Area	Name of the Compound	Chemical Formula	Mol. Wt	Chemical Structure
1	7.191	2.64%	3,5- Dihydroxy-6-Methyl-2,3- Dihydro-4H-Pyran-4-one	$C_6H_8O_4$	144	
2	8.613	7.13%	2-Furaldehyde,5-(Hydroxymethyl)	$C_6H_6O_3$	126	
3	9.494	1.85%	Glycerine monoacetate	$C_5H_{10}O_4$	134	

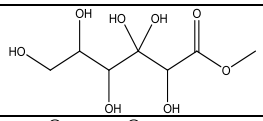
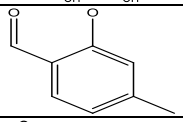
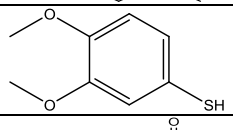
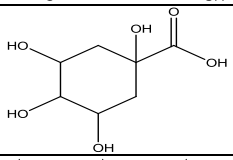
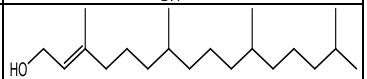
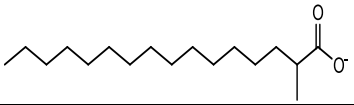
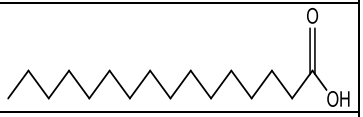
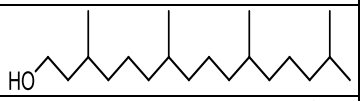
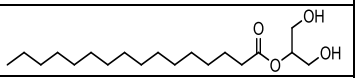
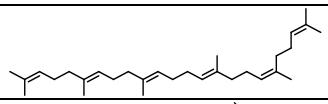
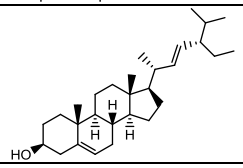
4	11.035	1.95%	Methyl beta hydroxyl hexonoate	$C_7H_{14}O_3$	146	
5	12.631	1.28%	2,4-Cresotaldehyde	$C_8H_8O_2$	136	
6	14.250	1.16%	3,4-Dimethoxythiophenol	$C_8H_{10}O_2S$	170	
7	15.431	1.74%	1,3,4,5-Tetrahydroxycyclohexane carboxylic acid	$C_7H_{12}O_6$	192	
8	16.032	0.47%	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	
9	18.531	0.48%	Methylhexadec-anoate	$C_{17}H_{34}O_2$	270	
10	21.274	8.67%	Palmitic acid	$C_{16}H_{32}O_2$	256	
11	24.637	0.67%	Phytol	$C_{20}H_{40}O$	296	
12	29.290	12%	Linoleic acid	$C_{18}H_{32}O_2$	280	
13	31.723	0.35%	Pentacosane	$C_{25}H_{52}$	352	
14	33.245	0.80%	Palmitin, 2-mono	$C_{19}H_{38}O_4$	330	
15	39.381	0.67%	Hexacosane	$C_{26}H_{54}$	366	
16	42.229	0.69%	n-Heneicosane	$C_{21}H_{44}$	296	
17	43.865	1.17%	Squalene	$C_{30}H_{50}$	410	
18	46.289	2.46%	Stigmasterol	$C_{29}H_{48}O$	412	

Table 3: Biological properties of the phytochemical compounds

S. No.	Compound Name	Nature of the Compound	Biological Activities
1	3,5- Dihydroxy-6-Methyl-2,3-Dihydro-4H-Pyran-4-one	Flavonoid	Antimicrobial, anti-inflammatory and antiproliferative activities
2	2-Furaldehyde,5-(Hydroxymethyl)	Sugars	anti-inflammatory, bacteriostatic
3	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene Alcohol	Antituberculosis , insecticidal, anti-inflammatory, antioxidant and antimicrobial activities
4	Palmitic acid	Ester	Antibacterial and antifungal activities
5	Phytol	Diterpene	Antimicrobial, anticancer and diuretic anti-inflammatory activities
6	Linoleic acid	Linoleic acid	Antibacterial and antifungal activities

7	Pentacosane	Aliphatic hydrocarbon	Antibacterial activity
8	Squalene	Triterpene	Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and pathology, and adjuvant activities.
9	Stigmasterol	Phytosterol	Anti-inflammatory, inhibit tumor promotion, anti-HIV reverse transcriptase, and anti-inflammatory activities.

In previous study it was reported that the components present in the leaves of *Shepherdia argentea* possess Pentacosane exhibiting antibacterial activity. Methanol extract of *Cassia italica* possess squalene which exhibit the antioxidant, immunostimulant, antibacterial and antitumor properties (Sermakkani and Thangapandian, 2012). The ethanol extract of the leaves of *Eugenia singampattiana* possess Squalene which exhibit antioxidant activity (Mary Jelastin Kala *et al*., 2011). In the present study also, compounds such as Pentacosane and Squalene found to possess various biological activities was detected in the GC MS analysis of *Achyranthes aspera*.

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