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Fathimath Shahla VP
Department of Pharmacognosy and Phytochemistry Research Laboratory Nehru College of Pharmacy Pampady, Thiruvilwamala, Thrissur, Kerala, India

Dr. Ragunathan Muthuswami
Department of Pharmacognosy and Phytochemistry Research Laboratory Nehru College of Pharmacy Pampady, Thiruvilwamala, Thrissur, Kerala, India

Corresponding Author:
Dr. Ragunathan Muthuswami
Department of Pharmacognosy and Phytochemistry Research Laboratory Nehru College of Pharmacy Pampady, Thiruvilwamala, Thrissur, Kerala, India

GC-MS analysis of ethanolic leaf extract of *Achyranthes aspera* Linn

Fathimath Shahla VP and Dr. Ragunathan Muthuswami

Abstract
The present study was carried out to determining the bioactive compounds present in the leaves of *Achyranthes aspera* Linn. by using GC-MS. Method employed was, the dried and pulverized plant materials were extracted with ethanol for 5 hours. The phytochemical constituents were analyzed by GC-MS. Then the identity and quantity of the 15 measured bioactive compounds were identified. Among these fifteen compounds detected from ethanolic extract, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (22.31%), Phytol, acetate (17.05%), 1-Hexadecyne (14.43%), 2-Decen-1-ol (4.06%), Decanoic acid (3.39%), 2-Nonen-1-ol,(E) (2.06%), and carboxylic acid and fatty acid derivate were identified as therapeutically active components. The GC-MS study helps to determining the formula and structure of phytoconstituents which can be used as drugs, and then these results may leads to development of a new drug formulation.

Keywords: Amaranthaceae, folk remedy, Hexadecyne, Kadaladi, secondary metabolites

Introduction
Achyranthes aspera Linn. (Fig.1) is a species of plant belongs to the family Amaranthaceae. The plant is distributed as weed throughout India, tropical Asia and other parts of the world. In eastern Africa, it is found in areas where the mean annual rainfall is within the range 700 - 1,300mm. Grows best in a fertile soil.it is an erect, sometimes sprawling, long-lived herb which can grow up to 2 m tall, with stems becoming woody at the base. Its short-stalked leaves are opposite, simple and egg-shaped; they can be densely to sparsely hairy and are dark green above and paler below, with young leaves often silvery. The plant is locally known as "Kadaladi" in Malayalam. It is known as "Prickly chaff flower" in English and "Chirchita", "Onga", "Latjeera" or "Apamarga" in local language and dialects. Apamarg, literally means kept keeps away from the 'dosas'. Ayurveda, Yunani practitioners and use different parts of the plant to treat leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, renal and cardiac dropsy, kidney stone, diabetes, dermatological disorders, gynecological disorders, gonorrhea, malaria, pneumonia, fever, cough, pyorrhea, dysentery, rabies, hysteria, toothache etc. The plant is a popular folk remedy [1] in traditional system of medicine throughout the tropical Asian and African countries. Researchers found that plant comprised triterpenoid saponins [2], oleanolic acids [3] as the glycone, long chain alcohols, ecdysterones [4] etc. Literature survey shows that several therapeutic activities of *Achyranthes aspera* L including anti- microbial [5], anti –oxidant [6], anti –asthmatic [7], anti- inflammatory [8], anti- arthritic and wound healing studies [9] have been demonstrated using various plant extracts both in *in vitro* and *in vivo* studies. The current study aims to investigate the possible phytochemical components by first preparing the ethanolic leaf extract of *Achyranthe aspera* L and separation and identification of the chemical compounds by Chromatography – Mass Spectrum (GC-MS) analysis

Materials and Methods
Plant material collection and authentification
The plant *Achyranthes aspera* Linn. were collected from from Kerala Forest Research Institute at Peechi, Thrissur, Kerala, India. The specimens collected from nursery were identified with the standard literature and authenticated with valid voucher specimens. The plant materials were taxonomically identified by the Botanist, Dr. Udayan PS, Assistant professor and Head of PG Department of Botany and Research, Sree Krishna College Guruvayoor. The collected sample 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.

Extraction

The shade dried and coarsely powdered plant material was extracted with ethanol by using soxhlet apparatus for 5 hours. The extract was filtered through Whatman No. 1 filter paper and concentrated. The extract obtained was then subjected to GC-MS analysis.

GC -MS Analysis

Gas chromatography Mass spectroscopy analysis of ethanolic extract was performed using Shimadzu GC-MS Model number: QP2010S equipped with Column - ELITE-5MS (30 meter length, 0.25 mm ID, and 0.25 µm thicknesses). Electron ionization system was used; details of GC programme were given in Table 1. The oven temperature was programmed from 70.00 ^{0}C which is given in Table 2. Helium gas was used as the carrier gas. Details of GC-MS programme was given in Table 3. Programme specifications regarding Mass Spectra were depicted in Table 4. GCMS Software: GCMS Solutions, Libraries used: NIST 11& WILEY 8.

Table 1: GC programme (GC 2010)

GC-Parameters	Programme
Column temperature Injection temperature Injection mode Sampling time Flow control mode Pressure Total flow Column flow Linear velocity Purge velocity Split ratio	70.00 ^{0}C 260.00 ^{0}C Split less 2.00 min Linear velocity 61.5 pka 54.1mL/min 1.00mL/min 36.7 cm/sec 3.0mL/min 50.0

Table 2: oven temperature programme

Rate	Temperature (^{0}c)	Hold time(min)
- 10.00 5.00	70.0 200.0 280.0	2.00 5.00 15.00

Table 3: GC-MS programme (GCMS QP2010)

GC-MS Parameters	Programme
Ion source temperature Interface temperature Solvent cut time Detector gain mode Detector gain Threshold	200.00 ^{0}C 280.00 ^{0}C 6.50 min Relative 1.01 kV+0.00 kV 1000

Table 4: MS table

Mass spectroscopy parameters	Programme
Start time	6-7 min
End time	51.00 min
ACQ time	Scan
Event time	0.50 sec
Scan speed	1000
Start m/z	50.00
End m/z	500.00
Sample inlet unit	GC

Identification of compounds

The constituents in the extract were identified by comparing their relative retention time and confirmation was done by comparing the mass spectra with database from the Library of NIST 11 and Wiley8. GC-MS Chromatogram obtained was given in figure.2.

Results and Discussion

GC-MS analysis of ethanolic leaf extract of *Achyranthes aspera* L. were carried out and a group of 15 compounds were identified which are depicted in table.5. This includes several carboxylic acid, fatty acid derivatives and other biologically and pharmacologically active compounds. The compounds identified were Butanoic acid, 2-oxo- (0.97%), Phytol, acetate (17.05%), 1-Butanol, 3-Methyl- (1.74%), (+-)-Lavandulol acetate (0.97%), 6-Octen-1-ol, 3,7 Dimethyl-, Propanoate (3.01%), Decanoic acid (3.39%), 6-Methylfuro[2,3-C]pyrid-5-one (3.19%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (22.31%), 2-Decen-1-ol (4.06%), 3-Bromo-2-Methoxycyclohexanone (4.04%), 2-Nonen-1-ol (2.06%), Cyclohexane, 1-Methyl-2-Methylene-, 5-Deutero- (1.68%), 1-Hexadecyne (14.43%), 6-Octen-1-ol, 3,7 Dimethyl-, Propanoate (10.71%), 6-Octen-1-ol, 3,7 Dimethyl-, Propanoate (9.85%). Each of these constituents is responsible for various pharmacological and biological activities. Of these 15 compounds, diterpene primary alcohol derivatives such as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (22.31%), Phytol, acetate [10] (17.05%), were found as the first major compounds. They are having anti-inflammatory, anti-oxidant, anti-allergic, anti-tuberculosis, anti-microbial and anti-noiciceptive activities. 1-Hexadecyne [11] (14.43%) were found as the second major compound, this is a fatty acid derivatives having anti-microbial, anti-neoplastic and anti-fungal activity. 2-Decan-1-ol [12] (4.06%), were found to be third major therapeutically important compound, having strong nematicidal activity. Decanoic acid [13] (3.39%), mainly used as anti-bacterial, anti-fungal, anti-inflammatory and treatment of skin conditions. 2-Nonen-1-ol [14] (2.06%), showing the major anti-oxidant activity. Cyclohexane derivatives mainly used for the plant growth regulating activities. Butanoic acid, 2-oxo- (0.97%), (+-)-Lavandulol acetate (0.97%), 6-Octen-1-ol, 3,7 Dimethyl-, Propanoate (3.01%) and 1-Butanol, 3-Methyl- (1.74%), these are very minor compounds mainly used in the industries as flavouring agent. They are generally considerd to smell faint [15]. It is beneficial to further separate the compounds and determine their specif activity.

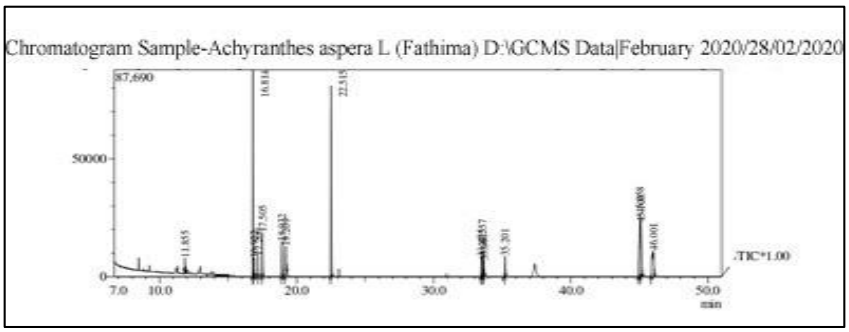

Fig 2: GC-MS Chromatogram of ethanolic leaf extract of *Achyranthea aspera* L.

Table 5: GC-MS analysis of ethanolic leaf extract of *Achyranthes aspera* Linn

Peak#	R.Time	Area	Area %	Height	Height %	Name	Base m/z
1	11.855	11136	0.97	4788	1.43	Butanoic acid, 2-oxo-	57
2	16.814	196597	17.1	87690	26.26	Phytol, acetate	68.1
3	16.922	20046	1.74	7947	2.38	1-BUTANOL, 3-METHYL-	55
4	17.2	11136	0.97	8879	2.66	(+-)-LAVANDULOL ACETATE	82.1
5	17.505	34737	3.01	18786	5.63	6-OCTEN-1-OL, 3,7-DIMETHYL-, PROPANOATE	82.1
6	18.932	39081	3.39	15080	4.52	DECANOIC ACID	73.05
7	19.209	36747	3.19	12111	3.63	6-METHYLFURO[2,3-C]PYRID-5-ONE	149
8	22.515	257282	22.3	81016	24.26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	57.05
9	33.475	46824	4.06	8494	2.54	2-DECEN-1-OL	57.05
10	33.557	46636	4.04	12728	3.81	3-BROMO-2-METHOXYCYCLOHEXANONE	67.05
11	33.667	29983	2.6	6743	2.02	2-NONEN-1-OL, (E)-	55.1
12	35.201	19374	1.68	8783	2.63	CYCLOHEXANE, 1-METHYL-2-METHYLENE-, 5-	69.1
13	45.058	166332	14.4	26690	7.99	1-HEXADECYNE	81.05
14	45.1	123464	10.7	23036	6.9	6-OCTEN-1-OL, 3,7-DIMETHYL-, PROPANOATE	79.1
15	46.001	113627	9.85	11190	3.35	6-OCTEN-1-OL, 3,7-DIMETHYL-, PROPANOATE	57.1
		1153002	100	333961	100		

Conclusion

In the present study, twenty two chemical constituents have been identified from the ethanolic leaf extract of *Achyranthes aspera* L. The obtained phytochemical compounds were identified as pharmacologically active and can be used for the treatment of various diseases. So that isolation of individual phytochemical constituents and subjecting it to the biological activity will be definitely giving prolific results and will open a new area of researche of individual components and their pharmacological potency.

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