

Chemical composition of the ethanolic extract of leaves of *Azima tetracantha* (Lam.)

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ABSTRACT

The aim of this study is to evaluate the bioactive compounds in the ethanolic leaf extract of *Azima tetracantha* by using Gas chromatography- Mass Spectrometry (GC- MS). *Azima tetracantha* leaves were collected from Ariyalur District, Tamil Nadu. The dried leaf powder was extracted with the solvent ethanol by using Soxhlet apparatus. One microlitre of the extract was subjected for analysis by GC- MS to detect the presence of bioactive compounds present in the plant. The results shown that the leaves of *Azima tetracantha* contained 39 compounds of which the major is 5-methyl-2-Furancarboxaldehyde (13.17%) followed by Maltol (8.62%).

Key words: *Azima tetracantha*, GC- MS, phytochemicals, ethanol extract, 5-methyl-2-Furancarboxaldehyde, Maltol (8.62%).

INTRODUCTION

Azima tetracantha Lam. (Family: Salvadoraceae) locally known as “*Mulsangu*”, is a rambling spinous shrub flowering throughout the year found in Peninsular India, West Bengal, Orissa, African Countries and extends through Arabia to tropical Asia. The common names of the plant are Uppimullu, Mulchangan, Needle bush, Yasanku and Kundali in Ayurvedic medicine. The leaves of the plant are elliptical in shape and are rigid, pale green colored. The flowers are small, greenish white (or) yellow colored, unisexual in axillary fascicles. The berries are white in color; usually one seeded and edible. *Azima tetracantha* root bark is used in muscular rheumatism, while the leaf juice is used for treating tooth and ear ache. In East Africa the pounded roots of *Azima tetracantha* Lam. are applied directly to snake bites and an infusion is taken orally as a treatment. In India and Sri Lanka the root, root bark and leaves are added to food as a remedy for rheumatism. It is planted as live fence in Bangalore (India). In Malaysia pickled leaves are used as an appetizer and against colds. The plant is promoted as an ornamental in the United States.

Several medicinal properties are attributed to this plant in the Indian system of medicine and included in the check list of traded medicinal plants. The ethno botanical survey reveals the usage of this plant as an unique folk medicine by the adivasis (tribal) [8, 12, 20, 9]. The root, root bark and leaves are administered with food as a remedy for rheumatism [4, 11]. It is a powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhea and as a stimulant tonic after confinement[13].The leaves are found to contain azimine, azcarpine, carpine and isorhamnitine-3-O-rutinoside etc[17, 21, 3]. Friedelin, lupeol, glutinol and β -sitosterol were isolated from the petroleum ether

extract of the leaves of *A. tetraacantha* [18]. The seeds of this plant have been found to possess novel fatty acids along with other fatty acids [6]. Antimicrobial activity was also reported for this plant [19]. *A. tetraacantha* leaf powder was assessed for its anti-inflammatory activity [10]. The benzene, chloroform and aqueous extract of leaves of *A. tetraacantha* were screened for analgesic activity in mice using hot plate method [14]. The ethanolic leaf extract of *Azima tetraacantha* Lam. was investigated for hypoglycemic and hypolipidemic activity in alloxan-induced diabetic albino rats [16].

The present study deals with the GC- MS analysis of the ethanol extract of the leaves of *Azima tetraacantha*.

MATERIALS AND METHODS

Plant material

Azima tetraacantha leaves were collected from Ariyalur District, Tamil Nadu.

Preparation of extract

The leaves were cleaned and freed from foreign materials. They were then minced, shade dried and powdered. The powdered sample was extracted with ethanol using Soxhlet apparatus, for 16 hours. The extract obtained was subsequently concentrated, under reduced pressure in a rotary vapour and maintained for further studies. One microlitre of the extract was employed in GC-MS analysis for different compounds.

GC-MS Analysis

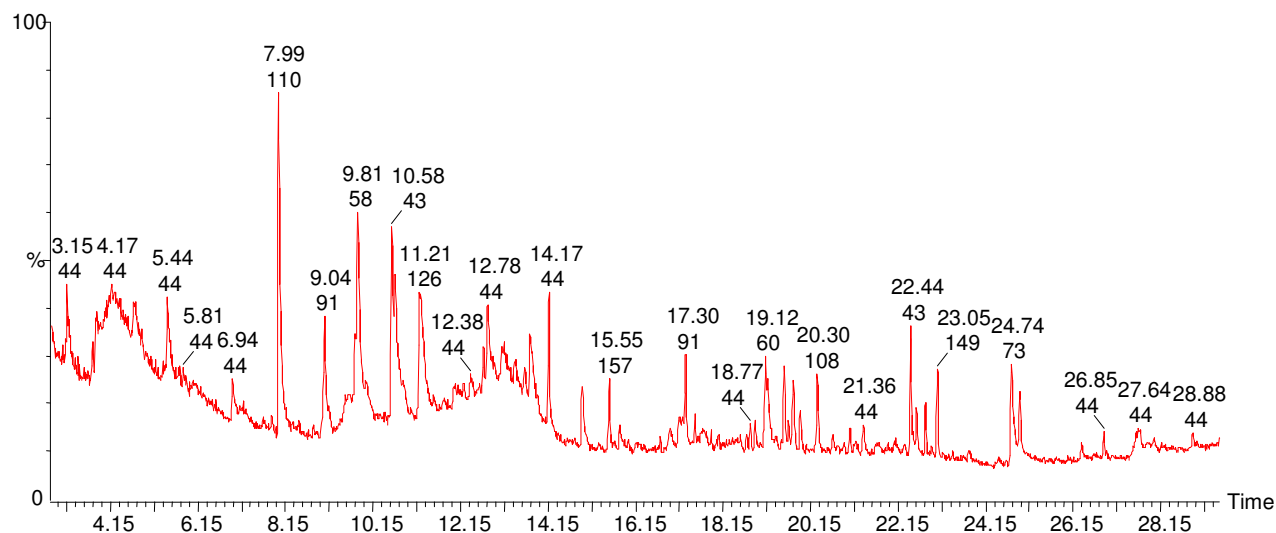
The Gas Chromatography Mass Spectrometry analysis of the extract was performed using GC-MS (Make: PerkinElmer Clarus 500) equipped with a Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane) of 30m length, 0.25mm diameter and 0.25 μ m film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. The carrier gas was Helium (99.99%), used at a constant flow rate of 1ml/min. injector and mass transfer line temperature were set at 280°C and 200°C respectively. The oven temperature was set from 50 to 220°C at 8°C/min, held isothermal for three minutes and finally raised to 290°C at 8°C/min. One microlitre of the sample was injected in a split mode with a scan range of 40 – 600 amu. The total running time of GC-MS was 35 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of components

The components in the extract were assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer and also with published literatures. NIST2005.LIB, Turbomass ver 5.2.0 library sources were used for matching the identified components in the plant material. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The GC-MS chromatogram (Figure 1) shows the peak area separation. The chromatogram reveals that the ethanol extract of *A.tetraacantha* is rich in terpenoids, especially triterpenoids. The analysis revealed the presence of 39 compounds from the ethanol leaf extract of *Azima tetraacantha* (Table 1). The major components were 5-methyl-2-Furancarboxaldehyde (13.17%), Maltol (8.62%), 1-Amino-2,6-dimethylpiperidine (6.98), Benzyl chloride (6.23), along with 35 other minor constituents. 5-methyl-2-Furancarboxaldehyde or furfural is an important renewable, non-petroleum based, chemical feestock. Hydrogenation of furfural provides furfuryl alcohol which may be further hydrogenated to tetrahydrofurfuryl alcohol. THFA is used as a nonhazardous solvent in agricultural solvent. N-Hexadeconoic acid or Palmitic acid, the major compound, is mainly used to produce soaps, cosmetics, and release agents. Recently, a long-acting antipsychotic medication, paliperidone palmitate, used in the treatment of schizophrenia, has been synthesized using the oily palmitate ester as a long-acting release carrier medium when injected intramuscularly. Retinyl palmitate is an antioxidant and a source of vitamin A added to low fat milk to replace the vitamin content lost through the removal of milk fat. Palmitate is attached to the alcohol form of vitamin A, retinol, to make vitamin A stable in milk. Oleic acid may hinder the progression of adrenoleukodystrophy (ALD), a fatal disease that affects the brain and adrenal glands. Friedelin, isolated earlier from *A.tetraacantha*, is reported to possess anti inflammatory, analgesic and antipyretic effects [1]. Nargis *et al* previously found twenty- seven compounds in ethanolic leaf extract of *Azima tetraacantha* by GC-MS analysis. The major compounds identified were tocopherol, phytol and squalene. Phytol and squalene are also terpenoids.

Figure 1: GC- MS chromatogram of ethanol extract of *Azima tetraacantha* Lam.Table 1: Chemical composition of ethanol extract of *Azima tetraacantha* Lam

S. No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: 1-Propen-2-ol, acetate Formula: C ₅ H ₈ O ₂ MW: 100	3.15	1784263	2.0668
2.	Name: Furfural Formula: C ₅ H ₄ O ₂ MW: 96	5.44	4008796	4.6436
3.	Name: Ethanone, 1-(2-furanyl)- Formula: C ₆ H ₆ O ₂ MW: 110	6.94	1367985	1.5846
4.	Name: 2-Furancarboxaldehyde, 5-methyl- Formula: C ₆ H ₆ O ₂ MW: 110	7.99	11368380	13.1686
5.	Name: 2(3H)-Furanone, 3-acetyldihydro- Formula: C ₆ H ₈ O ₃ MW: 128	8.78	328885	0.3810
6.	Name: Benzyl chloride Formula: C ₇ H ₇ Cl MW: 126	9.04	5400048	6.2551
7.	Name: 1-Amino-2,6-dimethylpiperidine Formula: C ₇ H ₁₆ N ₂ MW: 128	9.81	6022708	6.9764
8.	Name: 2,5-Dimethyl-4-hydroxy-3(2H)-furanone Formula: C ₆ H ₈ O ₃ MW: 128	10.58	1471322	1.7043
9.	Name: Maltol Formula: C ₆ H ₆ O ₃ MW: 126	11.21	7439552	8.6176
10.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: C ₆ H ₈ O ₄ MW: 144	12.02	805518	0.9331
11.	Name: Benzoic acid, 2-hydroxy-, methyl ester Formula: C ₈ H ₈ O ₃ MW: 152	12.67	183958	0.2131
12.	Name: 2-Pentanol, 5-(2-propynyloxy)- Formula: C ₈ H ₁₄ O ₂ MW: 142	12.78	1602958	1.8568

13.	<u>Name:</u> 1,4:3,6-Dianhydro- α -D-glucopyranose <u>Formula:</u> C ₆ H ₈ O ₄ <u>MW:</u> 144	13.40	849166	0.9836
14.	<u>Name:</u> 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- <u>Formula:</u> C ₇ H ₉ NO ₂ <u>MW:</u> 139	13.62	550579	0.6378
15.	<u>Name:</u> Benzaldehyde, 3-methyl- <u>Formula:</u> C ₈ H ₈ O <u>MW:</u> 120	13.74	3036599	3.5174
16.	<u>Name:</u> 2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl- <u>Formula:</u> C ₁₃ H ₂₀ O <u>MW:</u> 192	14.17	3322997	3.8492
17.	<u>Name:</u> 2-Methoxy-4-vinylphenol <u>Formula:</u> C ₉ H ₁₀ O ₂ <u>MW:</u> 150	14.92	2599309	3.0109
18.	<u>Name:</u> Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl- <u>Formula:</u> C ₁₃ H ₁₆ <u>MW:</u> 172	15.55	1679511	1.9455
19.	<u>Name:</u> 3-(4-Isopropylphenyl)-2-methylpropionaldehyde <u>Formula:</u> C ₁₃ H ₁₈ O <u>MW:</u> 190	15.98	61187	0.0709
20.	<u>Name:</u> 5-Amino-2-methoxy-4-picoline <u>Formula:</u> C ₇ H ₁₀ N ₂ O <u>MW:</u> 138	16.93	935337	1.0834
21.	<u>Name:</u> 5-Methylene-4,5,6,6a-tetrahydro-3ah-pentalen-1-one <u>Formula:</u> C ₉ H ₁₀ O <u>MW:</u> 134	17.30	1805354	2.0912
22.	<u>Name:</u> 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one <u>Formula:</u> C ₁₃ H ₁₈ O <u>MW:</u> 190	17.51	463609	0.5370
23.	<u>Name:</u> Naphthalene, 1,2,3,4-tetrahydro-5-methyl-1-(1-methylethyl)- <u>Formula:</u> C ₁₄ H ₂₀ <u>MW:</u> 188	18.77	212564	0.2462
24.	<u>Name:</u> 2-Butanone, 1-(2,3,6-trimethylphenyl)- <u>Formula:</u> C ₁₃ H ₁₈ O <u>MW:</u> 190	19.12	4089788	4.7374
25.	<u>Name:</u> Benzenebutanoic acid, 2,5-dimethyl- <u>Formula:</u> C ₁₂ H ₁₆ O ₂ <u>MW:</u> 192	19.55	2682996	3.1078
26.	<u>Name:</u> 2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)- <u>Formula:</u> C ₁₀ H ₁₄ O <u>MW:</u> 150	19.75	2440675	2.8272
27.	<u>Name:</u> 3-(2-Isopropyl-5-methylphenyl)-2-methylpropionic acid <u>Formula:</u> C ₁₄ H ₂₀ O ₂ <u>MW:</u> 220	19.91	937600	1.0861
28.	<u>Name:</u> 2-Cyclohexen-1-one, 3,5,5-trimethyl-4-(3-oxobutyl)- <u>Formula:</u> C ₁₃ H ₂₀ O ₂ <u>MW:</u> 208	20.05	42214	0.0489
29.	<u>Name:</u> Acetic acid, m-tolyl ester <u>Formula:</u> C ₉ H ₁₀ O ₂ <u>MW:</u> 150	20.30	2236716	2.5909
30.	<u>Name:</u> 2-Cyclohexen-1-one, 3,5,5-trimethyl-4-(3-oxobutyl)- <u>Formula:</u> C ₁₃ H ₂₀ O ₂ <u>MW:</u> 208	20.65	344720	0.3993
31.	<u>Name:</u> Bicyclo[6.3.0]undeca-1,7-dien-3-one, 5,5-dimethyl- <u>Formula:</u> C ₁₃ H ₁₈ O <u>MW:</u> 190	21.05	603654	0.6992
32.	<u>Name:</u> 2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl- <u>Formula:</u> C ₁₃ H ₂₂ O ₂ <u>MW:</u> 210	21.36	918754	1.0642
33.	<u>Name:</u> 3,7,11,15-Tetramethyl-2-hexadecen-1-ol <u>Formula:</u> C ₂₀ H ₄₀ O <u>MW:</u> 296	22.44	2995860	3.4703
34.	<u>Name:</u> 2-Pentadecanone, 6,10,14-trimethyl-	22.57	1274429	1.4762

	Formula: C ₁₈ H ₃₆ O MW: 268			
35.	Name: Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester Formula: C ₁₅ H ₂₈ O ₂ MW: 240	23.05	2223478	2.5756
36.	Name: n-Hexadecanoic acid Formula: C ₁₆ H ₃₂ O ₂ MW: 256	24.74	4562341	5.2848
37.	Name: Hexadecanoic acid, ethyl ester Formula: C ₁₈ H ₃₆ O ₂ MW: 284	24.92	2164052	2.5067
38.	Name: 2,4-Pentadienoic acid, 5-(1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-, methyl ester, (R)-(Z,E)- Formula: C ₁₆ H ₂₂ O ₄ MW: 278	26.33	712417	0.8252
39.	Name: Palmitoyl chloride Formula: C ₁₆ H ₃₁ ClO MW: 274	28.88	799449	0.9260

CONCLUSION

In the present study, 39 chemical constituents have been identified from the ethanol leaf extract of *Azima tetraantha* by GC-MS analysis. The ethanol extract is mainly composed of terpenoids and sterols. Thus, *Azima tetraantha* is found to possess significant phytonutrients, which attribute to its medicinal worth.

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