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Short Communication

# CHEMICAL COMPOSITION OF THE HEXANE EXTRACT OF LEAVES OF *AZIMA TETRACANTHA* (LAM)

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**ABSTRACT**

**Objective:** In the present study, the phytochemical constituents of hexane extract from *Azima tetracantha* (AT) leaves were done by using Gas Chromatography Mass Spectrometry analysis technique (GC-MS). *Azima tetracantha* has been an important medicinal herb being used in tribal medicines since long, but chemical constituents of its bark responsible for the activities are still not studied in depth.

**Methods**: *Azima tetracantha* leaves were collected from Ariyalur District, Tamil Nadu. The dried leaf was powdered and was extracted with the solvent hexane by using a Soxhlet apparatus. One microlitre of the extract was subjected to analysis by GC-MS to detect the presence of bioactive compounds present in the leaves of the plant.

**Results:** The results showed that the leaves *of Azima tetracantha* contained 47 compounds, of which the major is n-hexadecanoic acid (39.10%) followed by oleic acid (11.54%). Analysis and identification of the presence of the compound in the extract were done by using the database of the National Institute of Standards and Technology (NIST) library.

**Conclusion:** In the present study, 47 chemical constituents have been identified from the hexane leaf extract of *Azima tetracantha* by GC-MS analysis. The hexane extract is mainly composed of terpenoids and sterols. Thus, *Azima tetracantha* is found to possess significant phytonutrients, which attribute to its medicinal worth.

**Keywords:** *Azima tetracantha*, GC-MS, Phytochemicals, Hexane extract, Hexadecanoic acid, Oleic acid.

*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 as well as 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. *A. 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 *A. 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 fences 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) [1-4]. The root, root bark and leaves are administered with food as a remedy for rheumatism [5-7]. It is a powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhea and as a stimulant tonic after confinement [8]. The leaves are found to contain azimine, azcarpine, carpine and isorhamnitine-3-O-rutinoside [9-11]. Friedelin, lupeol, glutinol and β-sitosterol were isolated from the petroleum ether extract of the leaves of *A. tetracantha* [12]. The seeds of this plant have been found to possess novel fatty acids along with other fatty acids [13]. Antimicrobial activity was also reported in this plant [14, 15]. *A. tetracantha* leaf powder was assessed for its anti-inflammatory activity [16]. The benzene, chloroform and aqueous extract of leaves of *A. tetracantha* were screened for analgesic activity in mice using a hot plate method [17]. The ethanolic leaf extracts of *A. tetracantha* Lam. was investigated for hypoglycemic and hypolipidemic activity in alloxan-induced diabetic albino rats [18]. In the present study, investigations were carried out to determine the possible phytochemical components in the hexane extract of AT leaves by GC-MS analysis. The results show that the hexane extract of the plant *A. tetracantha* contains significant quantities phytoconstituents compared to the other solvent extracts, which can be used for multiple medicinal therapy.

*A. tetracantha* leaves were collected from by the first author Abirami Hariharanfrom Ariyalur District, Tamil Nadu. The leaves were cleaned and freed from foreign materials. They were then minced, shade dried and powdered. The powdered sample was extracted with hexane using a Soxhlet apparatus, for 16 h. 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 of different compounds.

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, 5% dimethylpolysiloxane) of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The carrier gas was helium (99.99%), used at a constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 280 °C and 200 °C respectively. The oven temperature was set from 50 °C to 220 °C at the 8 °C/min, held isothermal for 3 min 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 a percentage with peak area normalization.

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.

The GC-MS chromatogram (fig. 1) showed the peak area separation. The chromatogram revealed that the hexane extract of *A. tetracantha* is rich in terpenoids, especially triterpenoids. The analysis revealed the presence of 47 compounds from the hexane leaf extract of *A. tetracantha* (table 1). The major components are n-hexadecanoic acid (39.10%), oleic acid (11.54%), hexadecane (7.47%), tetracosane (6.76%), along with 43 other minor constituents.

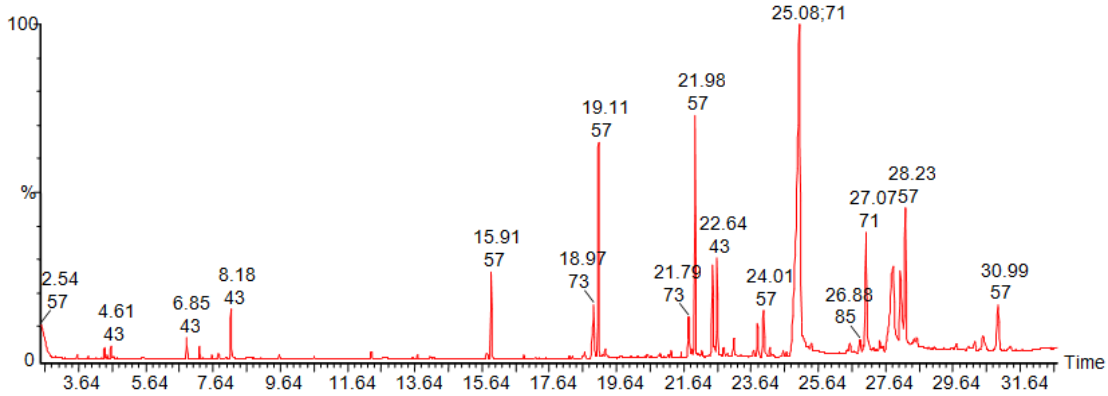


**Fig. 1: GC-MS chromatogram of hexane extract from the leaves of *Azima Tetracantha***

**Table 1: Chemical composition of hexane extract of *AzimaTetrecantha***

| S. No. | Peak name | Retention time | Peak area | %Peak area |
|---|---|---|---|---|
| 1. | Name: 3-Hexanol, 4-methyl-<br>Formula: C7H16O<br>MW: 116 | 3.60 | 2897512 | 0.1643 |
| 2. | Name: Acetamide, N-2-propenyl-<br>Formula: C5H9NO<br>MW: 99 | 4.08 | 335469 | 0.0190 |
| 3. | Name: 2H-Pyran, 3, 4-dihydro-6-methyl-<br>Formula: C6H10O<br>MW: 98 | 4.16 | 1146636 | 0.0650 |
| 4. | Name: 2-Hexanone<br>Formula: C6H12O<br>MW: 100 | 4.41 | 4993533 | 0.2831 |
| 5. | Name: 2-Hexanol<br>Formula: C6H14O<br>MW: 102 | 4.68 | 8646148 | 0.4902 |
| 6. | Name: Acetic acid, butyl ester<br>Formula: C6H12O2<br>MW: 116 | 4.80 | 737689 | 0.0418 |
| 7. | Name: 1-Heptene<br>Formula: C7H14<br>MW: 98 | 6.55 | 355983 | 0.0202 |
| 8. | Name: Pentan-2-ol, 4-allyloxy-2-methyl<br>Formula: C9H18O2<br>MW: 158 | 6.85 | 15316752 | 0.8684 |
| 9. | Name: Hexylene Glycol<br>Formula: C6H14O2<br>MW: 118 | 7.34 | 412153 | 0.0234 |
| 10. | Name: Benzaldehyde<br>Formula: C7H6O<br>MW: 106 | 8.03 | 2111855 | 0.1197 |
| 11. | Name: 1-Butanone, 1-cyclohexyl-<br>Formula: C10H18O<br>MW: 154 | 8.18 | 37284364 | 2.1138 |
| 12. | Name: 2-Hexene, 1-(pentyloxy)-, (E)-<br>Formula: C11H22O<br>MW: 170 | 8.75 | 238643 | 0.0135 |
| 13. | Name: Octanoic acid, 7-oxo-<br>Formula: C8H14O3<br>MW: 158 | 9.61 | 5029741 | 0.2852 |
| 14. | Name: Nonanal<br>Formula: C9H18O<br>MW: 142 | 10.65 | 2072578 | 0.1175 |

| | | | | |
|---|---|---|---|---|
| 15. | Name: Decanal<br>Formula: C10H20O<br>MW: 156 | 12.61 | 153472 | 0.0087 |
| 16. | Name: Benzenecarboxylic acid<br>Formula: C7H6O2<br>MW: 122 | 12.72 | 3352666 | 0.1901 |
| 17. | Name: 1H-Pyrrole-2, 5-dione, 3-ethyl-4-methyl-<br>Formula: C7H9NO2<br>MW: 139 | 13.59 | 1463913 | 0.0830 |
| 18. | Name: 2-Tridecenal, (E)-<br>Formula: C13H24O<br>MW: 196 | 13.72 | 5171545 | 0.2932 |
| 19. | Name: Nonanoic acid<br>Formula: C9H18O2<br>MW: 158 | 14.11 | 3503981 | 0.1987 |
| 20. | Name: 2, 4-Nonadienal, (E, E)-<br>Formula: C9H14O<br>MW: 138 | 14.82 | 433213 | 0.0246 |
| 21. | Name: n-Decanoic acid<br>Formula: C10H20O2<br>MW: 172 | 15.78 | 6429240 | 0.3645 |
| 22. | Name: Tetradecane<br>Formula: C14H30<br>MW: 198 | 15.91 | 50713572 | 2.8752 |
| 23. | Name: Hexadecanal<br>Formula: C16H32O<br>MW: 240 | 16.22 | 515982 | 0.0293 |
| 24. | Name: Propenylguaethol<br>Formula: C8H8O3<br>MW: 152 | 16.64 | 645591 | 0.0366 |
| 25. | Name: 1-Hepten-4-ol, 4-propyl-<br>Formula: C10H20O<br>MW: 156 | 16.98 | 2369550 | 0.1343 |
| 26. | Name: 2, 5-Cyclohexadiene-1, 4-dione, 2, 6-bis(1, 1-dimethylethyl)-<br>Formula: C14H20O2<br>MW: 220 | 17.24 | 1016214 | 0.0576 |
| 27. | Name: 3-Buten-2-one, 4-(2, 2, 6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-<br>Formula: C13H20O2<br>MW: 208 | 17.60 | 1500287 | 0.0851 |
| 28. | Name: 2-Butenedioic acid (Z)-, dibutyl ester<br>Formula: C12H20O4<br>MW: 228 | 18.24 | 2503177 | 0.1419 |
| 29. | Name: 2(4H)-Benzofuranone, 5, 6, 7, 7a-tetrahydro-4, 4, 7a-trimethyl-<br>Formula: C11H16O2<br>MW: 180 | 18.68 | 4776733 | 0.2708 |
| 30. | Name: Dodecanoic acid<br>Formula: C12H24O2<br>MW: 200 | 18.97 | 43867364 | 2.4870 |
| 31. | Name: Hexadecane<br>Formula: C16H34<br>MW: 226 | 19.11 | 131775488 | 7.4710 |
| 32. | Name: 3-Buten-2-ol, 3-methyl-4-(2, 6, 6-trimethyl-2-cyclohexen-1-yl)-<br>Formula: C14H24O<br>MW: 208 | 20.08 | 2700914 | 0.1531 |
| 33. | Name: Heptadecane<br>Formula: C17H36<br>MW: 240 | 20.56 | 1474714 | 0.0836 |
| 34. | Name: Tetradecanoic acid<br>Formula: C14H28O2<br>MW: 228 | 21.79 | 32902962 | 1.8654 |
| 35. | Name: 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol<br>Formula: C20H40O<br>MW: 296 | 22.50 | 59233884 | 3.3582 |
| 36. | Name: 2-Pentadecanone, 6, 10, 14-trimethyl-<br>Formula: C18H36O<br>MW: 268 | 22.64 | 52833764 | 2.9954 |
| 37. | Name: 3-Eicosene, (E)-<br>Formula: C20H40<br>MW: 280 | 23.84 | 25274728 | 1.4329 |
| 38. | Name: 7, 9-Di-tert-butyl-1-oxaspiro(4, 5)deca-6, 9-diene-2, 8-dione<br>Formula: C17H24O3<br>MW: 276 | 24.01 | 42144528 | 2.3894 |
| 39. | Name: Nonadecane, 3-methyl-<br>Formula: C20H42 | 24.60 | 4989560 | 0.2829 |

| | | | | |
|---|---|---|---|---|
| | MW: 282 | | | |
| 40. | Name: n-Hexadecanoic acid<br>Formula: C16H32O2<br>MW: 256 | 25.08 | 689725760 | 39.1037 |
| 41. | Name: Sulfurous acid, hexyl nonyl ester<br>Formula: C15H32O3S<br>MW: 292 | 26.88 | 7288409 | 0.4132 |
| 42. | Name: Oleic Acid<br>Formula: C18H34O2<br>MW: 282 | 27.87 | 203550432 | 11.5402 |
| 43. | Name: Hexadecanoic acid, butyl ester<br>Formula: C20H40O2<br>MW: 312 | 28.09 | 81494200 | 4.6203 |
| 44. | Name: Tetracosane<br>Formula: C24H50<br>MW: 338 | 28.23 | 119286104 | 6.7629 |
| 45. | Name: 4, 8, 12, 16-Tetramethylheptadecan-4-olide<br>Formula: C21H40O2<br>MW: 324 | 30.55 | 27030822 | 1.5325 |
| 46. | Name: Tetracosane<br>Formula: C24H50<br>MW: 338 | 30.99 | 66182508 | 3.7522 |
| 47. | Name: 1-Tetracosanol<br>Formula: C24H50O<br>MW: 354 | 31.35 | 5954085 | 0.3376 |

N-Hexadecanoic 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. tetracantha,* is reported to possess anti inflammatory, analgesic and antipyretic effects [19].

Nargis *et al.* previously found twenty-seven compounds in ethanolic leaf extract of *Azima tetracantha* by GC-MS analysis [20]. The major compounds identified were tocopherol, phytol and squalene. Phytol and squalene are also terpenoids.

In the present study, 47 chemical constituents have been identified from the hexane leaf extract of *A. tetracantha* by GC-MS analysis. The hexane extract is mainly composed of terpenoids and sterols. Thus, *A. tetracantha* is found to possess significant phytoconstituents, which attribute to its medicinal worth.

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**CONFLICT OF INTERESTS**

All authors disclose no conflict of interest

**ABBREVIATION**

AT-*Azima tetracantha*; NIST-National Institute of Standards and Technology; RT-Retention Time; MF-Molecular Formula; MW-molecular Weight; Amu-Atomic mass unit; MS-Mass spectroscopy/mass spectrum.

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