B. Edwin Jose et al /J. Pharm. Sci. & Res. Vol. 11(12), 2019, 3717-3720

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Identification of Phytochemical Constituents within the Leaf Extracts of *Azima tetracantha* Lam using Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

B. Edwin Jose[1], Dr. P.Panneerselvam[2]

[1] *Department of Pharmaceutical Chemistry, Sankaralingam Bhuvaneswari college of Pharmacy, Sivakasi.*

[2] *Department of Pharmaceutical Chemistry, C.L.Baid Metha college of pharmacy,Chennai. Tamilnadu Dr.M.G.R. Medical university*

Abstract

Azima tetracantha LAM. is widely used in traditional medicine for treatment of cancer and diabetic activity. Both its leaves were reported to possess antioxidant, antibacterial and anti-inflammatory activities. The aqueous extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols, and saponins in leaf extracts. In the present study Fredelin, the peak recorded in between retention time 22.2 to 22.6 min showed other significant ions at m/z 125.2, 205.3 and 273.4 were attributed to the fragmentation of A, B, C and D rings, respectively. To the molecular weight 425.5 gm/mol, the corresponding molecular formula extracted from mass data bank is found to be $C_{30}H_{50}O$. In Isorhamnetin-3-O-rutinoside the peak recorded in between retention time 14.4 to 14.5 min showed one major signal in the mass spectra. This correlates with Isorhamnetin which exhibits specific fragmentation with the loss of methyl radical, thus giving m/z 315. To the molecular weight 622.6 gm/mol, the corresponding molecular formula is found to be $C_{28}H_{32}0_{16}$ from mass data bank. In Myrectein the peak recorded in between retention time 24.5 to 24.7 min showed three major signals in the mass spectra. The peak corresponding to the electron spray ionization at m/z 151.2, m/z 179.2 and the fragment ion at m/z 317.3 and having molecular weight of 317.3 gm/mol corresponds to the molecular formula $C_{15}H_{10}O_8$.

Keywords: *Azima tetracantha* LAM, phytochemical constituents, LC-MS, compound identification.

INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs [1]. Plants produce diverse types of bioactive molecules, making them a rich source of different types of medicines. This revival of worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in healthcare[2]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha [3].

Azima tetracantha (Salvadoraceae) is a well known medicinal herb, termed '*Mulsangu*' in Tamil and '*Kundali*' in Sanskrit. Root, root bark and leaves of *Azima tetracantha* (lam) are used with food as a remedy for rheumatism, diuretic and as stimulant [4]. Traditionally Indian medical practitioners use *Azima tetracantha* (lam) in inflammatory conditions, cough, asthma, small pox and diarrhoea [5,6]. The major phyto-constituents reported in *Azima tetracantha* (lam) are azimine, azecarpin, carpine, isorhamnitine-3-O-rutinoside, friedelin, lupeol, glutinol and β-sitosterol [7,8]. *Azima tetracantha* (lam) is reported to have antifungal[9] antitumour [10], antidiabetic [11] , antidiarrhoeal [12] and hepatoprotective activities.

Azima tetracantha (lam) is a low, spinouts, highly branched bush, woody below but with pale green, herbaceous, almost quadrangular young branches. The leaves are in opposite to sub-opposite, decussate pairs. They are shortly petiolate, about 2x4cm long, entire, elliptic, acute, sharply mucronate, rigid, pale green with an acute base. Usually, there are two laterally placed spines in the axil of a leaf. The spines which morphologically represent the first pair of leaves of the auxiliary shoot are about three cm long, more or less, triangular in cross section, very sharp and with an indurate apex. The plant is dioeciously. The flowers are borne in the axils of leaves. Generally, there is cymes of three flowers in the axil of a leaf which is the upper branches, especially of the male plants become greatly reduced or even completely suppressed.

The detection and analyses of phytochemical constituents within medicinal plants extracts have relied on a number of chromatographic and spectrometric techniques such as TLC, UV, IR and NMR[13,14]. Of recent, phytochemical analysis of medicinal plant extracts involve sophisticated techniques that include Liquid chromatography instrument coupled with mass spectrometer (LC-MS).

MATERIALS AND METHODS

Collection of plants

The aerial part (leaves) of *Azima tetracantha* (lam) was collected from the Panayur area of Madurai, Tamilnadu as raw material, during the second week of February 2015 and a voucher specimen is stored in C.L. Baid Mehta College of Pharmacy (001/ATL/CLBP) and the plant material was authenticated by a renowned botanist. About 500 g of coarse powdered leaf in 2.5 L water is boiled, cooled and filtered. The filtrate is evaporated to dryness in desiccator and stored in refrigerator (Yield- 26.5% w/w). The aqueous extract of *Azima tetracantha* (lam) (AEAT) was subjected to preliminary phytochemical analysis[15]

Various extraction methods for isolation of constituents

The whole plant will be subjected to shade drying and extraction with petroleum ether (60-80^{o}C) chloroform, Ethyl acetate and 80% ethanol in soxhlet apparatus by simultaneous extraction each for 72 hours. Concentrate the solvents in vacuum. The crude solid obtained on evaporation are to be studied for preliminary qualitative phytochemical evaluation.

Phytochemical Screening

The extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols, and saponins in leaf extracts.

Liquid Chromatography-Mass Spectroscopy (LC-MS)[16]

LC-MS analysis of the *Azima tetracantha* (lam) was carried out using Thermo/Finnigan Surveyor System consisting of a degasser, binary pump, auto sampler, and column heater. The column outlet was coupled to a Thermo fleet (LCQ-Fleet) Ion Trap mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out in a personal computer with Data Analysis software (Qual Browser; Thermo Electron, San Jose, CA). For the chromatographic separation, a phenomenex luna 5-µm C8 column (250 × 4.6 mm) was used. The column was held at 95% Solvent A (0.1% acetic acid in water) and 5% Solvent B (0.1% acetic acid in acetonitrile) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, then 4 min with 100% B. Finally, elution was achieved with a linear gradient from 100% B to 5% B for 2 min. The flow rate was 200 µl / min and injection volume was 5 µl. The following parameters were used throughout the MS experiment: for electro spray ionization with positive ion polarity the capillary voltage was set to 20 V, the capillary temperature to 300°C, the nebulizer pressure to 40 psi, and the drying gas flow rate to 15 L/ min.

RESULTS

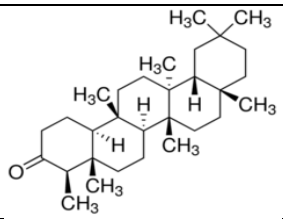
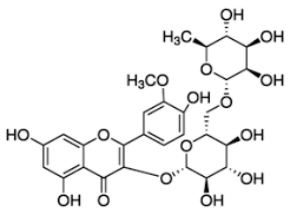
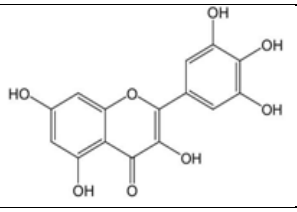
LC-MS Analysis

Preliminary phytochemical screening of AEAT showed the presence of terpenoids, alkaloids, saponins, tannins, phenolic compounds, flavonoids and steroids. The characterisations of the compounds using UHPLC ESI MS/MS analysis in negative ion mode are furnished in Table 1

The mass fragmentation pattern shows a molecular ion peak at m/z 425.5 (Figure No: 1). The loss of a methyl group was indicated by the presence of a peak at m/z 411. The peak recorded in between Retention time 22.2 to 22.6 min showed other significant ions at m/z 125.2, 205.3 and 273.4 were attributed to the fragmentation of A, B, C and D rings, respectively. To the molecular weight 425.5 gm/mol, the corresponding molecular formula extracted from mass data bank is found to be $C_{30}H_{50}O$. These results were confirmed with small molecular data base and from previous studies [17] the identified compound is Friedelin.

The peak recorded in between Retention time 14.4 to 14.5 min showed one major signal in the mass spectra (Figure No:2). This correlates with Isorhamnetin which exhibits specific fragmentation with the loss of methyl radical, thus giving m/z 315. To the molecular weight 622.6 gm/mol, the corresponding molecular formula is found to be $C_{28}H_{32}0_{16}$ from mass data bank. These results were confirmed with small molecular data base and from previous studies [18] and the identified compound is Isorhamnetin-3-O-rutinoside.

Table No: 1 UHPLC-ESI MS/MS Analysis of AEAT

[M-H] m/z (g/mol-^{1})	Retention Time (min)	Fragmentation in MS HPLC-ESI-MS^{n}	Identified Compound	Molecular Formula	Structural Formula
425.5	22.2-22.6	125.2, 205.3, 273.4	Friedelin	$C_{30}H5_{0}O$	
622.6	14.4-14.5	315	Isorhamnetin-3-O-rutinoside	$C_{28}H_{32}O_{16}$	
317.3	24.5-24.7	151.2, 179.2, 317.3	Myricetin	$C_{15}H_{10}O_{8}$	

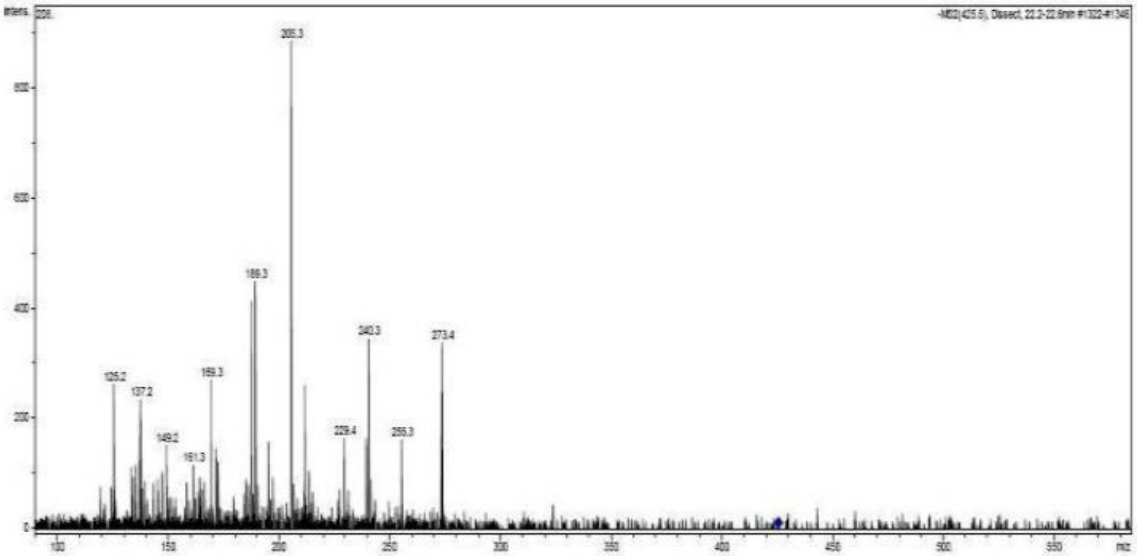


Figure No: 1 LCMS spectra of Friedelin from AEAT

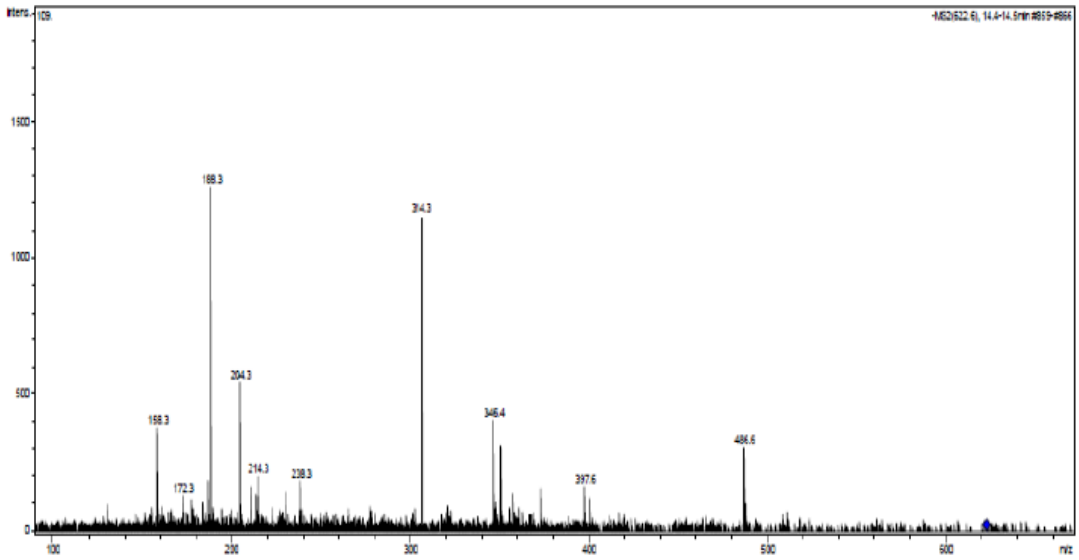


Figure No: 2 LCMS spectra of Isorhamnetin-3-O-rutinoside from AEAT

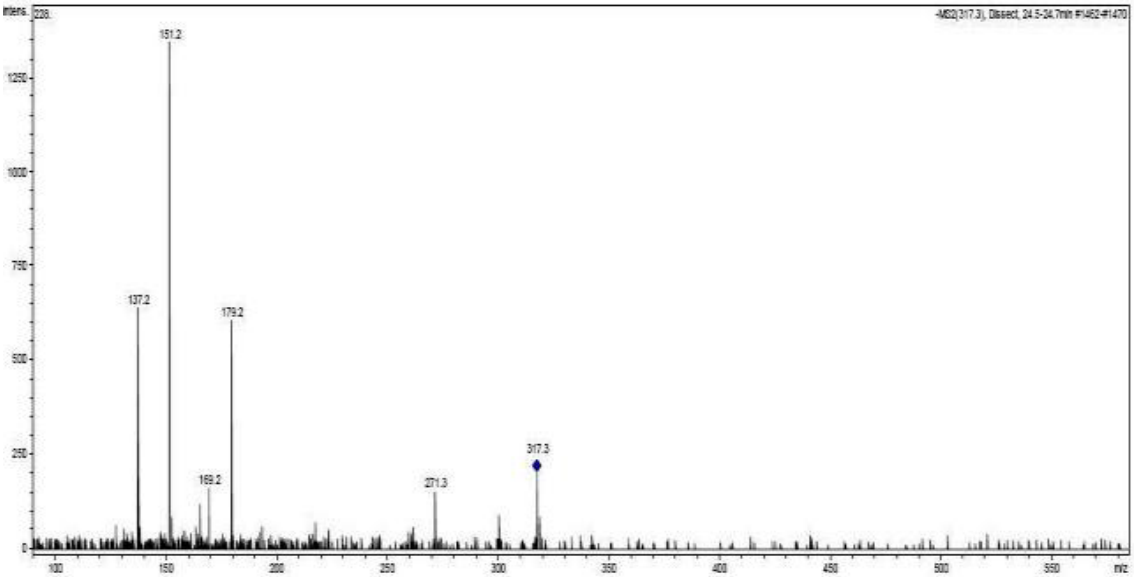


Figure No: 3 LCMS spectra of Myricetin from AEAT

The peak recorded in between Retention time 24.5 to 24.7 min showed three major signals in the mass spectra (Figure No: 3). The peak corresponding to the electron spray ionization at m/z 151.2, m/z 179.2 and the fragment ion at m/z 317.3 and having molecular weight of 317.3 gm/mol corresponds to the molecular formula $C_{15}H_{10}O_8$. These results were confirmed with small molecular data base and from previous studies [19] and the identified compound is Myricetin.

DISCUSSION

Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health [20]. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug [21]. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay[22]. Many reports are available on the antiviral, antibacterial, antifungal and anti-inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. According to authors[23] LCMS is the best way for chemical characterization. HPLC profile differentiation is one such important and powerful procedure which has often employed for this purpose. Each and every metabolite has a specific role and functions in harmony with other metabolites within the organizational framework of cells in the defence mechanism of the plants [24].

REFERENCES

1. N. Savithramma, M. Ling Rao and S. Ankanna: Preliminary Phytochemical Screening of Some Important Medicinal Plants. Middle-East Journal of Scientific Research 2012; 2-1:139-145.
2. N. Md. Azmathullah, M. Asrar Sheriff and A.K. Sultan Maiden: Phytochemical Screening of *Calotropis procera* Flower Extracts and their Bio-Control Potential on *Culex sp.* Mosquito Larvae and Pupae. International Journal of Pharmaceutical & Biological Archives 2011; 26:1718-1721.
3. N. Savithramma, M. Linga Rao and D. Suhrulatha: Screening of Medicinal Plants for Secondary Metabolites. Middle-East Journal of Scientific Research 2011; 8(3):579-584.
4. Abirami H, Muhammad Ilyas MH, Syed Jahangir H, Prem Kumar K, Nargis Begum T. *Azima tetracantha*: A treasure to be hunted. International Journal of Recent Scientific Research.2013;4(5):597-602.
5. Venugopala Rao Konda, Ruckmani Arunachalam, Madhavi Eerike, Ramesh Rao K, Arun Kumar Radhakrishnan. Nephroprotective effect of ethanolic extract of ***Azima*** *tetracantha* root in glycerol induced acute renal failure in Wistar albino rats. J Tradit Complement Med. 2016; 6(4): 347–354.
6. Nargis Begum T, Muhammad Ilyas MH, Vijaya Anand A. Hepatoprotective activity of *Azima tetracantha* Lam. in experimental animals. Journal of Pharmacy Research.2011;4(7):2359-2360.
7. Nargis Begum T, Muhammad ILyas MH, Kalavathy S, Vijaya Anand A, Senthil R.Effect of ethanolic leaf extracts of *Azima tetracantha* (lam) on EAC tumor bearing mice.Research Journal of Medicine and Medical sciences.2009;4(2):351-354.
8. Ramesh CK. Hepatoprotective and antioxidant effect of *Azima tetracantha* (lam) leaves extracts against $CC1_4$ induced liver injury in rats. Indian Journal of Products and Resources.2010;1(4):493-499.
9. Hazeena Begum V, Dhanalakshmi M, Muthukumaran P . *In-vivo* evaluation of antidiarrhoeal activity of the leaves of *Azima tetracantha* (lam). International Journal of Nutrition and Metabolism.2013;5(8):140-144.
10. Nargis Begum T, Muhammad ILyas MH, Kalavathy S, Vijaya Anand A, Senthil R. Hypoglycemic and antihyperlipidimic activity of ethanolic leaf extract of *Azima tetracantha* (lam) on alloxan induced diabetic rats. Journal of Cell and Tissue Research.2009; 9(1):1681-1685.
11. Gowthami M, Tamil Selvi S, Senthil Kumar G , Panneerselvam A. Phytochemical analysis and anti bacterial properties of leaf extract of *Azima tetracantha* (lam). Asian Journal of Plant Science and Research, 2012, 2 (2):110-114.
12. Hema TA, Shiny M ,Parvathy J. Antimicrobial activity of leaves of *Azima tetracantha* against clinical pathogens. International Journal of Pharmacy and Pharmaceutical Sciences.2012;4:317-319.
13. Shunmuga jothi, R., Uthayakumari, F., Bharathy, V. *Biosci. Discovery* 2015, 6, 106-111
14. Oyugi, D.A., Ayorinde, F.O., Gugssa, A., Allen, A., Izevbigie, E.B., Eribo, B., Anderson, W.A. *J. Biosci. Tech.* 2011, 2, 287-304
15. Harborne, Phytochemical Methods, Edition –III, Pub: Springer (India) Private Limited, 2005, p. 1-32.
16. Zabin K. Bagewadi, Siddanagouda R.S and Praveen G. Baligar Phytoconstituents investigation by LCMS and evaluation of anti-microbial and anti-pyretic properties of cynodon dactylon. International Journal of Pharmaceutical Sciences and Research. 2014, 5(7): 2874-2889.
17. McRae J, Yang Q, Crawford R, Palombo W. Review of the methods used for isolating pharmaceutical lead compounds from traditional medicinal plants. Environmental list. 2007;27:165–174.
18. Fellows L, Scofield A. Intellectual Property Rights and Biodiversity Conservation—An Interdisciplinary Analysis of the Values of Medicinal Plants. University Press; Cambridge, UK: 1995. Chemical diversity in plants.
19. Farnsworth NR, Akerele RO, Bingel AS, Soejarto DD, Guo Z. Medicinal Plants in Therapy. Bull. WHO. 1985;63:965–981.
20. Suresh K, Deepa P, Harisaranraj R and Vaira Achudhan V: Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. Ethnobotanical Leaflets 2008; 12:1184-1191.
21. B. Mahesh and S. Satish: Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. World Journal of Agricultural Sciences 2008; 4 (S): 839-843.
22. Sofowara A: Medicinal plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria, 289.
23. Patil AG, Koli SP, Patil DA and Chnadra N. Phamacognostical standardization and HPTLC finger print of *Crataeva tapia* Linn. SSP. Odora (Jacob.) Almeida leaves. International Journal of Pharma and Bio Sciences 2010; 1(2):1-14.
24. Saraf A: Phytochemical and antimicrobial studies of medicinal plant Costus speciosus (Koen.). Journal of Chemistry 2010; 7(1):405-413.