Clinical Importance of limnoid Kulactone from *Azadirachta indica*

Pasupuleti Sreenivasa Rao[*abc], Byna Syam Sundar Rao[2], Ramalinga Viswa kumar[3]

[1a] **Senior Research Scientist, Head,** Central Research laboratory (ARC)
[1b] **Professor** Department of Biochemistry Narayana Medical College and Hospital
[1c] **Research advisory professor**,Narayana College of Pharmacy Nellore-524003,Andhra Pradesh, India
[2] **Professor** Department of Pathology Narayana Medical College and Hospital
[3] **Professor, Head** Department of Biochemistry,Narayana Medical College and Hospital

Corresponding Author: Dr. Pasupuleti Sreenivasa Rao
Professor, Department of Biochemistry,**Senior Research Scientist, Head**
Central Research laboratory (ARC),Narayana Medical College and Hospital, Nellore-524003,Andhra Pradesh, India

Abstract

Majority of global population up to 80 percent, relies on traditional medicine for their primary health care. In current phyto-therapy based medical practices, native medicinal plant/herbal extracts are employed to treat various disorders and diseases. Their application is rapidly increasing due to their positive effects and furthermore recognized with low toxic side effects. *Azadirachta indica* (Neem) is a key medicinal plant with rich medicinal values. It is in cosmopolitan in distribution and belongs to the family of *Meliaceae*. In India its usage is very common in the native ethno traditional medicinal practices along with other medical practices too. Its vegetative parts like leaves, flowers, fruits, seeds and bark are goldmine for bio-active compounds. Even sometimes fondly called as natural drug store or store house of phytochemicals. Moreover, neem chemical and structural composition is tricky and complex. A small fraction of bioactive compounds are well characterized, and demonstrated with potential biological properties. Moreover previous studies revealed the therapeutic potential of Limoniod kulactone, separated from *Melia* Species. However, in the present study, we assessed the distribution pattern of Limoniods such as kulactone in the neem extracts of various vegetative parts by LC–MS (Liquid Chromatography and Mass Spectroscopy) spectral analysis. The end results predicted the presence of Limoniod **kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z)** for the first time from the vegetative parts of *Azadirachta indica* which was collected from Tirumala Hills, Eastern Ghats, India which has clinical significance

Key words- Flavonoids, **kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z)** , *Azadirachta indica*, Natural products, LC –MS

Key words -Triterpenes, Limoniods, kulactone, LC-MS, Secondary metabolites, ***Azadirachta indica***

Introduction

Global population in developed and under developing nations rely on traditional medicine for their health care [1-2]. Now, the application of medicinal plant/herbal medicine for treating numerous disorders and diseases is quickly evolving, since they offer very low toxicity and less side effects [1-2]. The active composition in these Medicinal /herbal plants have been reported to efficiently slowdown the disease or disorder progression in a synergistic manner. This active composition from these Medicinal /herbal plants may contain either polysaccharides, pigments, steroids, terpenoids, flavonoids and or alkaloids etc. Few earlier studies too demonstrated that Medicinal/herbal plant extracts and purified molecules have considerable effects in controlling variety of diseases and disorders [1-10].

Azadirachta indica is one such key medicinal plant, placed under the family of *Meliaceae*, often refered as “village pharmacy” or ‘Neem tree’, or nature’s ‘drug store’ or ‘store house of phytochemicals’. Due to this, they are primary target for various phytochemical investigations [12-13]. Its distribution in cosmopolitan nature, largely noticed in tropics, and subtropics covering Asia to Africa [1, 11-14]. Its administration is common in various health sectors of rural India. Their application widely used for centuries, in many countries, for their ethno-traditional medicinal health practices. Its vegetative such as, roots, leaves, bark, seeds and flowers have been utilized to treat many acute, chronic diseases and disorders [11-14]. These vegetative parts contain numerous phytochemicals reported with significant biological activities. Few possess anti-cancer, antimalarial, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory properties, while some exhibited with insecticidal, larvicidal and spermicidal activities [1,11-14].

For the past few decades, chemical entities reported from plants, termed as “phytochemicals” have attained global significance in public domain and also in research related scientific communities particularly to maintain good health and also preventing disease. Few studies related to *Azadirachta indica* reported that it contain more than 300 plus phytochemicals, which are diverse in nature, chemically unique and represent with complex structural identity [11,14-15]. Majority of these phytochemicals are divided mainly into two types, isoprenoids first, and non-isoprenoids. The isoprenoids are further classified as diterpenoids, triterpenoids, vilasinins, limonoids, and C-secomeliacins. The non-isoprenoids are further divided into proteins, polysaccharides, sulphur compounds, polyphenolics, dihydrochalcone, coumarin, tannins and aliphatic compounds [1,11,14,16]. Thus, from the above reports, it is crystal clear that the chemical composition of *Azadirachta indica* has been effectively studied,

phytochemicals were well characterized, and structurally elucidated in most varieties, which are distributed in Asia and Africa and exclusively completely absent in the Indian varieties [11-14]. However, the information on the phytochemical composition in the Indian native varieties is still lacking, particularly in the southern Indian species from the areas of Eastern Ghats. Moreover previous studies revealed the clinical significance of Limoniod kulactone, which was dentified from *Melia* Species. However, till date none reported the presence of Limoniod kulactone from Indian varities. Thus the present study is undertaken to assess presence of the Limoniod kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) from various vegetative partes of *Azadirachta indica* which was collected from Tirumala Hills, Eastern Ghats, India.

2. Materials and methods

Plant collection

The Fresh native germplam of *Azadirachta indica* was collected in Tirumala hills, particularly the month of March, 2017 from the region of Eastern Ghats (Andhra Pradesh) of India. Further authentication was done by the local taxonomist. The collected germplasm such as leaves, bark and roots were allowed to shade dry, followed by crushing in a grinder systematically to make a fine powder according to the described protocols [1,5-7],

Preparation of Azadirachta indica Extracts

The aqueous extracts were prepared through Soxhlet extraction with grinded powders of the freshly collected germplasm containing of leaves, bark and roots respectively. 15 gram of the powder was taken separately from each part, packed in sterile cloth, kept in soxhlet apparatus and extracted [1,5-7]. The obtained crude extract was filtered, further concentrated, final residue was dissolved in sterile water, filtered and was kept refrigerated until use. The extract concentration was acquired by calculating the dry weight per unit volume as per described procedures [1,5-7].

LC-Mass spectral analysis

The Fresh aqueous extracts of *Azadirachta indica* were chemically fingerprinted using LC-Mass spectral analysis (SHIMADZU-LC-MS-2010A) as per described procedures [1,5-7]. The LC-MS (Liquid Chromatography and Mass Spectroscopy) experiments are conducted with the combination of methanol and water as mobile phase, a gradient method was applied, using RP-C18 analytical column [240 mm× 2 cm] with a flow rate of 0.5 ml/min respectively. All the aqueous extract samples are nebulized with nitrogen gas and the ion mass (Electro Spray Ionization) of the peaks was recorded in both positive mode and negative mode as per the described protocols [1,5-7].

Results and Discussion

Several studies reveal that the chromatographic techniques are widely used in investigating and as well as characterizing the natural or synthetic molecules that fight with various diseases and disorders [1]. Recent progresses in modern molecular biological tools like DNA sequencing, genetic engineering, gene targeting and transgenic methodologies has been showcased novel approaches to better understand and estimate the infections, diseases and disorders, which could lead to deliver new options for developing modern therapeutics [17-20]. At present, to combat diseases like cancer[17-21], and disorders like diabetes [22], several efficient drug development technologies has been initiated, through programs like in silico drug designing and synthesis of novel molecules[7,23-26]. However the difficulties continue similar. Hence substitutes are essential.

Medicinal plants look as a better option. For Instance in ethno-traditional medicine, medicinal plants have been efficiently applied to treat a various ailments that includes major diseases and disorders [8-10]. Presently, usage of the medicinal plant/herbal extracts/formulations is rapidly developing, which are expected to have least side effects. The active ingredients present in this may be accountable for this outcome, which could be either polysaccharides, pigments, steroids, terpenoids, flavonoids and or alkaloids [1,7-8]. Moreover, the recent trend is exploring on secondary metabolites has become an active research area, since they are potential and rich sources for new age medications [1,4]. Several studies reveal that these secondary metabolites from plants will be separated with diverse chromatographic techniques, with appropriate methods that include extraction, separation, purification, structural elucidation and quantification [1-7]. In most cases, plant germplasm encompassing of many vegetative parts will be collected, shade dried, lyophilized, further extracted with appropriate solvents with soxhlet extractor to remove undesirable things, to obtain preferred molecules. After extraction, the preferred molecules were further separated, purified, structure elucidated and quantified with proper chromatographic techniques. Recent investigations also disclose that is an insistence to consent and introduce contemporary analytical tools for examining new age bioactive substances. Moreover implementation of novel chemical fingerprinting methods using analytical tools like LC-MS, could yield quality productivity in small period. Chromatographic fingerprinting methods must be implemented in identifying and authenticating several Phytochemicals that completely represent a particular plant or herb.

As listed above, in native ethno-traditional medicine *A. indica* is widely used in various health practices for treating many diseases and disorders [1,11-14]. Its complex chemical composition

is well studied and described [11] Patela et al 2016, classified neem active substances into two types, namely Isoprenoids and non-isoprenoids. In the isoprenoids class, the diterpenoids, triterpenoids and steroids were placed. The Falavanoids, coumarins, carbohydrates, proteins, hydrocarbons, fatty acids and esters, and other acids were kept under non-isoprenoids class. Later, the triterpenoids are further differentiated into many types based on the elimination of carbon atom neither from the side chain nor from the ring skeletal structure of the mother compound. The triterpenoids are differentiated as protolimonoids, mononortriterpenoids, dinortriterpenoids, trinortriterpenoids, tetranortriterpenoids, pentanortriterpenoids, hexanortriter-penoids, octanortriterpenoids and nonanortriterpenoids. Next the tetranortriterpenoids were again classified into two types, first one as ring-intact-tetranortriterpenoids and the later as ring-seco-tetranortriterpenoids. The diterpenoids were divided into two types, such as podacarpanoids (margolone) and abeitanoids (sugiol). However, this complex chemical composition in most plant species or varieties differs due to their geographical distribution, seasonal variations and other environmental factors [4]. In spite of its numerous therapeutic importance, the chemical composure of Indian *A.indica* species, distributed in Eastern Ghats has not been reported [1]. Therefore, the present study is carried in aim to report the Triterpeniods like kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) in *Azadirachta indica.*

Flavonoids are the largest group of plant secondary metabolites found in most plants. They have 15-carbon skeleton structure as a back bone, linked with two phenyl rings (A & B) and one heterocyclic ring (C), and abbreviated as C6-C3-C6, as per nomenclature of IUPAC [1]. In most cases they appear in conjugated forms or their hydroxyl group's connected with either one or more sugar residues. Normally they will be linked with carboxylic acids, amines, lipids and sometimes also with phenols. Many studies reveal that they exhibit wide range of biological and physiological activities [1, 27, and 28]. Few studies also reported that they possess antioxidant properties, based on their linked structure, and sometimes they also act as reducing agents, hydrogen-donating antioxidants and quenchers of singlet oxygen [28-30]. Chalcones are plant-derived polyphenolic compounds belongs to the family of flavonoids. They are aromatic ketones and enones, which forms as a central core and occur in the form of numerous biological compounds. Triterpeniods (DHCs) are typically represented as that their two C6 rings were linked by a C3 bridge, double bond is totally reduced when compared to chalcones [31]. In other words they are structurally characterization represents the benzylacetophenone skeleton, which is obtained from either phenylpropanoid and or

polyketide biosynthetic pathways. Moreover these DHCs are biosynthetically similar to other minor flavonoids like flavones and flavanones, which are open-ring derivatives [31].

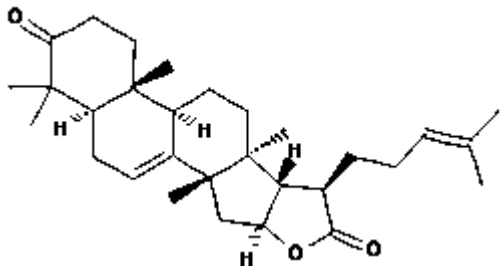

Fig-1 The Structural presentation of kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z from the crude aqueous extracts of A. Indica

S. No	Name of the Identified Molecule in the LCMS spectra	Molecular formula	Mass (*m/z*)	Presence/Absence of molecule in the LC MS spectra of Root extract		Presence/Absence of molecule in the LC MS spectra of Bark extract		Presence/Absence of molecule in the LC MS spectra of Leaf extract	
				positive mode	negative mode	positive mode	negative mode	positive mode	negative mode
1	kulactone	$C_{30}H_{44}O_3$	452.67	yes	yes	yes	yes	yes	yes

Table 1. The distribution pattern of kulactone from various vegetative parts of *A. Indica*

Moreover the phytochemicals like Limonoids have been isolated from various plants, majorly belong to families of Meliaceae and Rutaceae which has clinical significance. Hundreds of Limonoids have been identified, which are majorly they are bioflavonoids belongs to terpenoid class. Limonoids are extremely oxygenated, with prototypical structure, which possess a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton [32-33]. Limonoids In citrus fruit contain a furan ring attached to the D-ring, at C-17, as well as oxygen comprising functional groups at C-3, C-4, C-7, C-16 and C-17. Interstentingly in the present study for the first time we are reporting the Limonoid Kulactone, from the vegetative parts of of *Azadirachta indica* from Indian varieties. Earlier studies revealed that Kulactone, is an important bioflavonoid identified from Melia volkensii and also reported with anti-fungal, anti-bacterial and anti-plasmodial activities. It exhibited anti-inflammatory properties, through inhibiting the prostaglandin synthesis. It is also demonstrated cytotoxicity against human liver cancer BEL7402 cells in vitro [32-34].

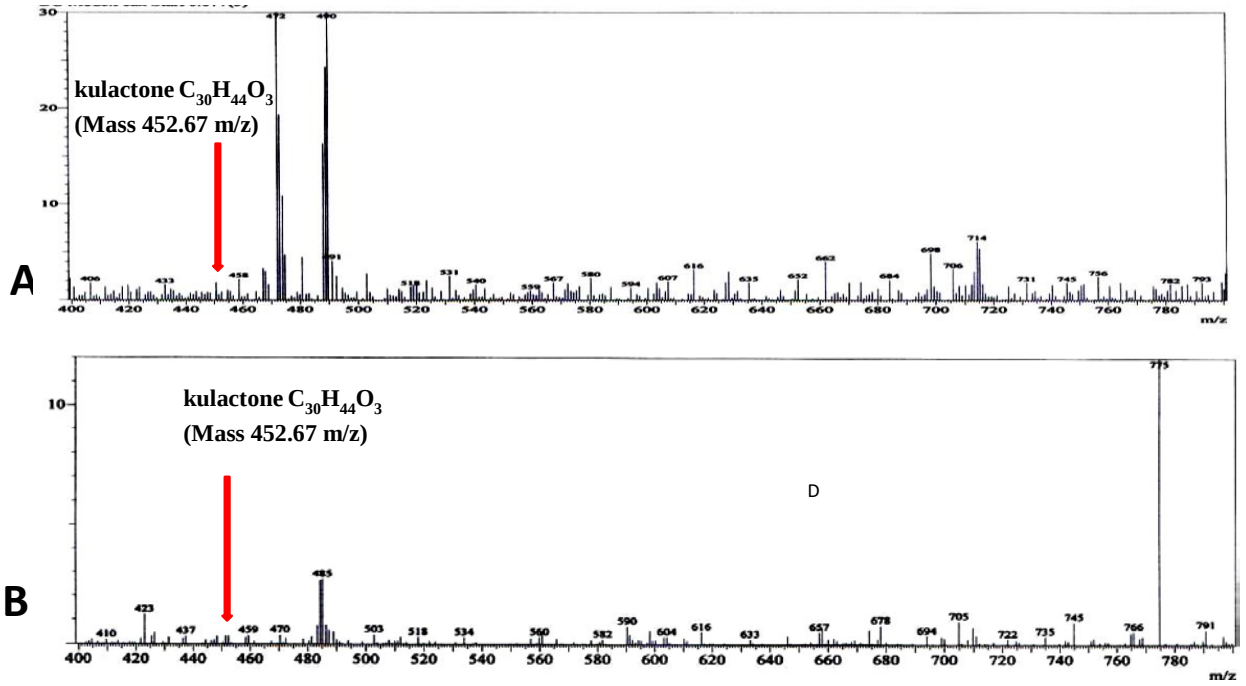

Fig 2 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) of kulactone from the crude aqueous root extract of *A. Indica*

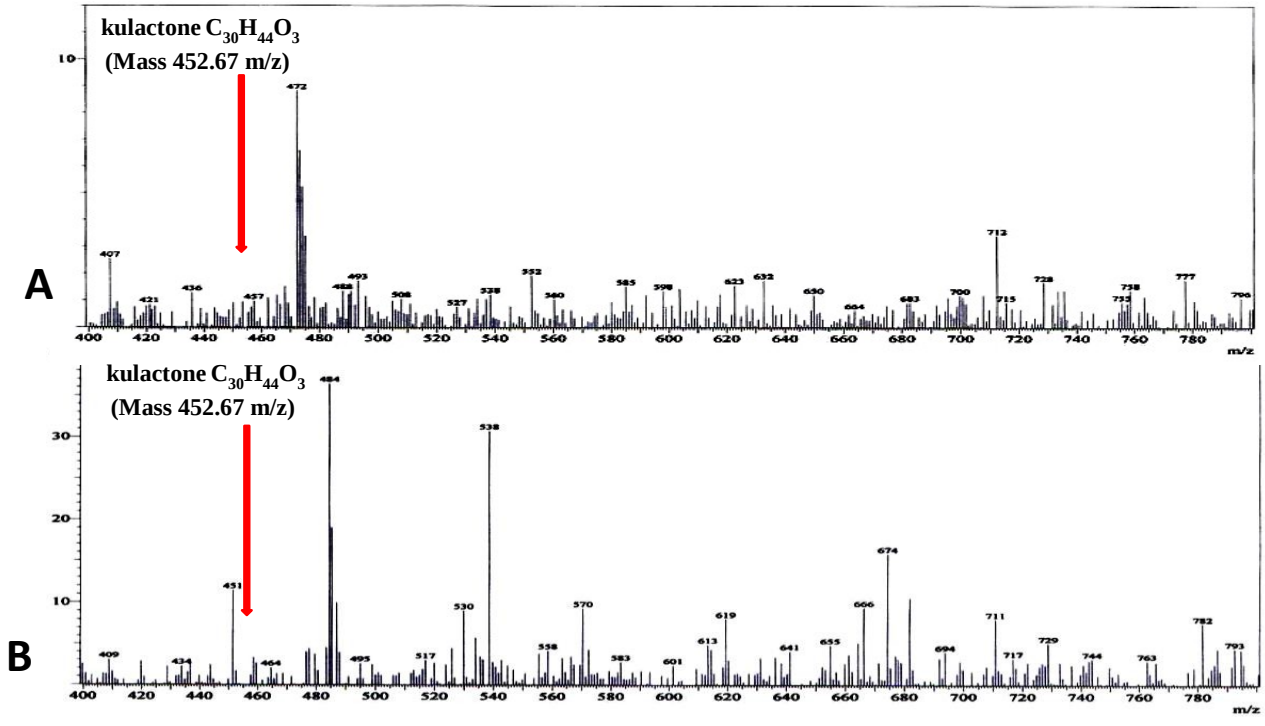

Fig 3 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) of kulactone from thecrude aqueous bark extract of *A. Indica*

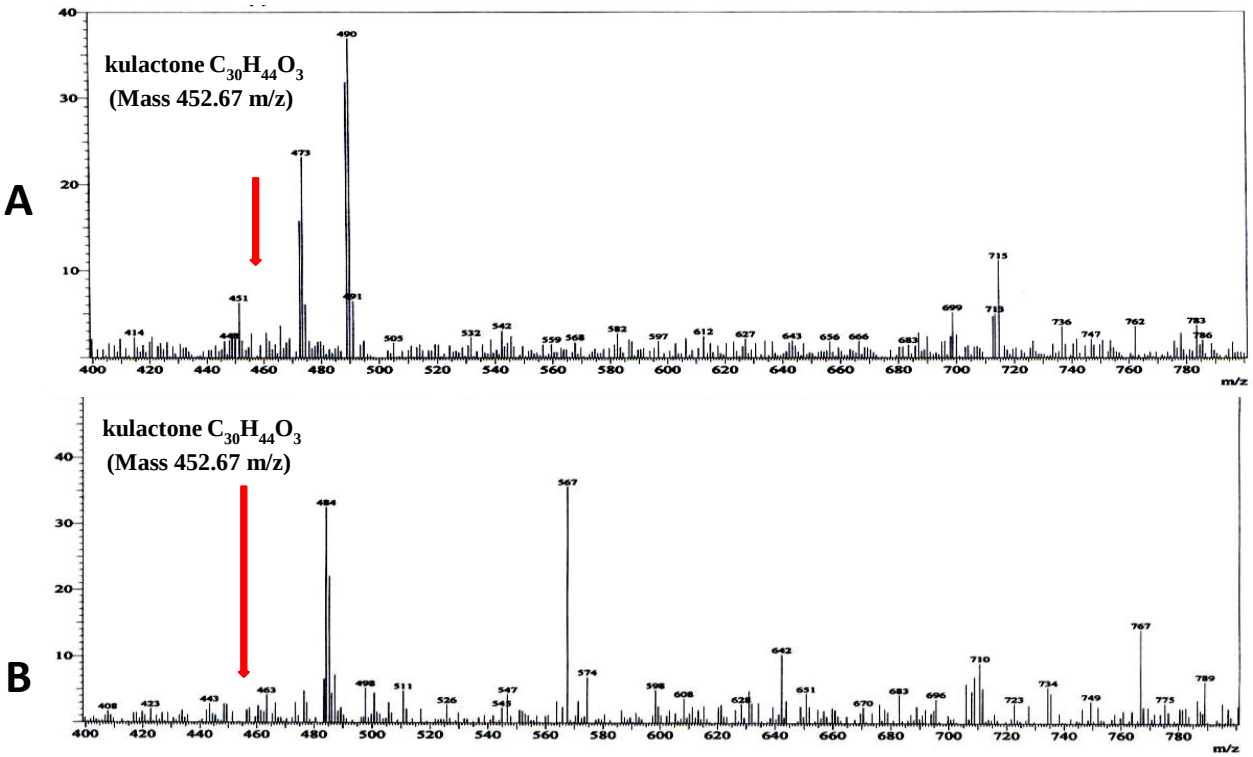

Fig 4 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) kulactone from the of crude aqueous leaf extract of *A. Indica*

In the current study we observed the distribution pattern of Limoniods like kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) in the vegetative parts of *Azadirachta indica*. The fresh Plant germplasm of *Azadirachta indica* were collected from Eastern Ghats (Andhra Pradesh, India), shade dried, subjected to crushing and made into fine powder. Later powdered material from various vegetative parts were extracted with water in soxhlet apparatus and aqueous water residues were obtained. Next, these aqueous water residues were filter sterilized individually and subjected to LC-MS spectral analysis. In order to obtain chemical finger print profile of the aqueous extracts of *Azadirachta indica*, an analytical method based on LC-MS (ESI) was employed. The LC-MS spectral profile data reveals the presence of kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) the extracts, exhibiting with the protonated molecular ions, respective *m/z* observed in both positive mode (Fig.2A-4A) and as well as in the negative mode (Fig. 2B-4B, Table-1). The figure (Fig. 1A) demonstrates the structural representation of kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z).

Root extract

The LC-MS spectral data of crude Aqueous root extract of *Azadirachta indica* reports the presence of a molecular ion peak of kulactone $C_{30}H_{44}O_3$ at 452.67 m/z. The protonated molecular ion peaks of kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) was recorded in positive mode (Fig.2A) and as well as in negative mode (Fig. 2B).

Bark extract

The bark extracts revealed too followed the same path but the bioactive compound found in both modes. The LC-MS data of crude Aqueous bark extract of *Azadirachta indica* clearly depicts the presence of a molecular ion peak of kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) at 434.43 m/z. The protonated molecular ion peaks of kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) was clearly visible in positive mode (Fig. 3A) and negative mode (Fig. 3B).

Leaf extract

The leaf extract of *Azadirachta indica* was too repeated with similar findings. The LC-MS spectral profile of crude aqueous leaf extract of *Azadirachta indica* identifies the presence of a molecular ion peak of kulactone $C_{30}H_{44}O_3$ at 452.67 m/z. The protonated molecular ion peaks of kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) were clearly observed in positive mode (Fig. 4A) and as well as negative mode (Fig. 4B).

Thus, the Limoniods like kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) identified in the current study were correlated with other studies that demonstrated the presence of these molecule [32-36]. Moreover, the present study supports the current investigations that are majorly focused on developing novel drugs from medicinal plants to treat various diseases like cancer, and disorders like diabetes [37-39] and also supports that *Azadirachta indica* collected from Tirumala hills is rich source for secondary metabolites like kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) along with other secondary metabolites [40-42].

Conclusions.

Thus from the above study for the first time, we conclude and confirm the presence of Limoniods like kulactone $C_{30}H_{44}O_3$ (Mass 452.67 m/z) from various vegetative parts of *Azadirachta indica* collected from Tirumala hills, Eastren Ghats, India.

References

1. Rao PS, Subrahmanyam G, Ramu Sridhar P (2018) Flavonol glycosides from Azardicta Indica. Drug intervention Today 10: 8, 1421-1426

2. Cowan, M.M (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12,

564–582.

3. Rao PS, Subrahmanyam G (2017) Induction of diabetes by alloxan in male wistar albino rats –a simplified methodology. Euro J Biomed Pharmace sci 6: 13, 68-75

4. Rao PS, Subrahmanyam G, Bhaskar M (2017). U-HPLC (Ultra-High-pressure Liquid Chromatography) Separation of Indole Alkaloid Strychnine. World J of Pharmace Res 4: 11,1022-35.

5. Rao PS, Prasad MNV (2008) Extraction, Purification and Characterization of Indole Alkaloids from Strychnos wallichiana L. – an Endangered Medicinal Plant from India. Medici Aroma Plant Sci Biotech 2: 63-67

6. Rao PS, Ramanadham M, Prasad MNV (2009). Anti-proliferative and cytotoxic effects of Strychnos nux-vomica root extract on human multiple myeloma cell line - RPMI 8226. Food Chem Toxic 47: 283–288.

7. Rao PS, Prasad MNV (2013) The Strychnos nux-vomica root extract induces apoptosis in the human multiple myeloma cell line-U266B1. Cell Biochem Biophy 66:443–450

8. Satyanand V, Reddy CB, RamaMohan P, Kumar MR, Narayanaswamy DL, Seelam A, Ramalingam K, Rao PS (2013a). Effects of Garlic extract (Allium sativum) in combination with Amlodipine in mild to moderate essential hypertensive patients: An Open randomized parallel group study. J Pharmace Res Dev 2(4):181-188.

9. Satyanand V, Venkata Krishnan, Ramalingam K, Rao PS , Priyadarshini S (2013b) Blockade of voltage dependent calcium channels lowers the high blood pressure through ginger Int J Analy, Pharmace Biomed Sci, 2(1): 64-66.

10. Satyanand V, Venkat Krishnan, Madhavi D, Revathi, Indira S, Shaik AB, Rao PS (2013c) The effect of peppermint juice for indigestion among old age people- A preliminary study. J Pharmace Res Dev 2(7): 238-243

11. Akhila, A., Rani, K., (1999) Chemistry of the neem tree (Azadirachta indica A. Juss.).Fortschritte der Chemie organischer Naturstoffe=Progress in the chemistry of organic natural products. Progres dans la chimie des substances organiques naturelles 78: 47–149.

12. Patela SM., Venkata KCN, Bhattacharyya P, Sethi G, Bishayee A (2016). Potential of neem (Azadirachta indica L) for prevention and treatment of oncologic diseases Seminars in Cancer Biology 40–41: 100–115

13. Gupta SC, Prasad S, Tyagi AK, Ajaikumar B,. Kunnumakkara, Aggarwal BB (2017). Neem (Azadirachta indica): An indian traditional panacea with modern molecular basis. Phytomedicine 34: 14-20

14. Biswas, K., Chattopadhyay, I., Banerjee, R.K., Bandyopadhyay, U (2002) Biological activities and medicinal properties of neem (Azadirachta indica). Curr. Sci. 82, 1336–1345.
15. Subapriya, R., Nagini, S (2005). Medicinal properties of neem leaves: a review. Curr. Med. Chem. Anti-Canc. Agents 5: 149–146
16. Brahmachari, G (2004) Neem–an omnipotent plant: a retrospection. Chembiochem: 5, 408–421.
17. Suresh G, Gopi Krishna S, Nayudu N, Sravanthi M, Rao PS, Kumar VKM, Sowdamani G, Rudramadevi K, Nagesh N, Manjula B, Kondaiah K, Varadacharyulu N (2014) Contribution of cyclin D1 (CCND1) and E-cadherin (CDH1) alterations to colorectal cancer susceptibility: a case–control study. Tumor Bio doi 10.1007/s13277-014-2505-9.
18. Suresh G, Sravanthi M, Bulle S, Dasi D, Prathap BN, Bramhachari PV, Nagesh N, Shivaji S, Bhanoori M, Rao RT, Rao PS, Varadacharyulu N (2016a) Manganese-superoxide dismutase (Mn-SOD) overexpression is a common event in colorectal cancers with mitochondrial microsatellite instability. Tumor Biol 2-4: doi 10.1007/s13277-016-4918-0.
19. Suresh G, Bulle S, Sravanthi M, Krishna MT, Nagesh N, Manjula B,Varadacharyulu N, Rao PS (2016) Association of Mitochondrial Displacement Loop Polymorphisms with Risk of Colorectal Cancer in South Indian Population. Mitochondrial DNA .doi.org/10.3109 /24701394.2016b.1160076.
20. Singh S, Kotakonda A, Kapardar R, Kankipati H, Rao PS, Mambatta PS, Vetaikorumagan SR, Gundlapally S, Nagappa R, Shivaji S (2015). Response of bacterioplankton to iron fertilization of the Southern Ocean, Antarctica. Front. Microbiol 6: 863. doi:10.3389/ fmicb.2015.00863
21. Chetan R, Veeresalingam B, Kumar KM, V, Teja PD, Rao PS (2013) A study on the clinical manifestations and the incidence of benign and malignant tumors in a solitary thyroid nodule. Int J Res Medi Sci Nov;1(4):429-434
22. Reddy S A, Dasu K, Venkat Krishnan, Reddy MR, Jithendra K, Rao PS (2013). Prevalence of asymptomatic bacteriuria and its antibiotic sensitivity in type-2 diabetic women along the sea coast. Int J Res Medi Sci 1(4):487-495
23. Rao PS, Muvva C, Geethanjali K, Babu BS, Kalashikam R (2012) Molecular docking and virtual screening for novel protein tyrosine phosphatase 1B (PTP1B) inhibitors. Bioinformation 8(17): 834-837.
24. Bola BR, Rao PS, Satish S (2017) Ligand Docking Based Identification of Novel Drug Analog for an Effective Treatment against Filaria , Haya: Saudi J. Life Sci. 2, -9: 335-348.

25. Avinash A, Swarupa SS, Siva K, Sirisha D, Riyaz S, kumar ND, Sreenivasulu M, Rao PS (2015). Design and Evaluation of Famotidine Floating Tablets. Int J Innov Pharmace Res 6 (1):440-445

26. Hymavathi, R, Suresh, G, Sumanth, K.M, Swapna, V.K, Sravanthi, M, Rao, P.S, Manjula, B, Varadacharyulu, N (2017). Therapeutic effect of green tea extract on alcohol induced hepatic mitochondrial DNA damage in albino wistar rats. J Adv Res 8(3): 289-295.

27. Cuyckens F, Claeys M. (2004). Mass spectrometry in the structural analysis of flavonoids. J Mass Spectrom. 39:1–15.

28. Cook NC, Samman S (1996). Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 7:66–76.

29. Moure A, Cruz JM, Franco D, Domınguez JM, Sineiro J, Domınguez H, Nu~nez MJ, Parajo JC (2001). Natural antioxidants from residual sources. Food Chem. 72:145–171.

30. Bravo L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56:317–333.

31. Ce´ line Rivie` re. (2016) ,Triterpeniods: Occurrence in the Plant Kingdom, Chemistry and Biological Activities. Studies in Natural Products Chemistry, Vol. 51. Studies in Natural Products Chemistry,51: 253-381

32. DENG, Z.-P., LIU, S.-C., CUI, S.-X., ZHOU, L., & YAO, Q.-Q. (2012). Cytotoxic activity of five limonoids from Meliae cortex and their structure-activity relationship. Chinese Journal of Natural Medicines, 10(3), 238–240. doi:10.3724/sp.j.1009.2012.00238

33. Rahab W Kamau, et al. Antimicrobial compounds from root, stem bark and seeds of Melia volkensii. Nat Prod Res. 2016 Sep;30(17):1984-7.

34. https://www.biosynth.com/p/FK74058/22611-36-5-kulactone

35. Keskes H, Belhadj S, Jlail L, Feki A E, Damak M, Sayadi S, Allouche N (2017). LC-MS–MS and GC-MS analyses of biologically active extracts and fractions from Tunisian Juniperus phoenice leaves, Pharmaceutical Biology, 55:1, 88-95.

36. Francescato LN, Debenedetti SL.,.Schwanz TG, Bassani VL., Henriques AT(2013). Identification of phenolic compounds in Equisetum giganteum by LC–ESI-MS/MS and a new approach tototal flavonoid quantification Talanta 105:192–203

37. Shaik SB, Meesala GS, Shaik SF, Shaik MI, Sarbon NMhd, Mohammed A, Sudheer Aluru S, Rao PS, Rahman SA, Nannepaga JS, Mohiddin SS, Ramesh B, and Bhaskar M. Combinational antidiabetic effects of Curcuma longa L. and Trigonella foenum-graecum L. extracts in alloxan induced type-1 diabetic rats RJPT, 2023 16(11). 5432—5438

38. Sravanthi M, Neelam A, Devalaraju RS, Trivedi KVSR, Srinivasulu C , Tamanam RR Suresh G, Rao PS, (2024). Role of GSTP1 functional polymorphisms in molecular pathogenesis of colorectal cancer. Human Gene. 42. 201335. 10.1016/j.humgen.2024.201335

39. Divyamaanasa D, Nallabelli N, Devalaraju RS, Sushma KN, Ghosh S, Karnati R, Rao PS Curcumin attenuates replicative senescence in human dental follicle cells and restores their osteogenic differentiation." Journal of Oral Biosciences (2023).

40. Rao PS , Kumar SV, Arumugam I, Aruna G, Kumar RV, Basha SS, Shaik MI. Nimbochalcin from Azadirachta indica. WJPR, 2021 Volume 10, Issue 14, 1887-1898

41. Rao PS, Harshita SS , Kumar SV, Aruna G , KumarRV , Basha SS, Shaik MI. Identification of limonoid 'meliantriol' from Azadirachta Indica. WJPR, 2022, Volume 11, Issue 16, 2296-2309.

42. Rao PS. limonoid 'Azadirachtin' from Azadirachta Indica. WJPR, 2023, Volume 12, Issue 15, 943-954.