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NIMBOCHALCIN FROM *AZADIRACHTA INDICA*

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ABSTRACT

Global population up to 80 percent, relies on traditional medicine for their primary health care. The usage of medicinal plant/herbal medicine for treating various disorders and diseases is rapidly progressing due to their less side effects. *Azadirachta indica* (Neem) belongs to the family *Meliaceae*, cosmopolitan in distribution, widely used in ethno traditional medicine. For centuries in India, it is commonly used in several native medical practices. Its vegetative parts such as leaves, flowers, fruits, seeds and bark known to possess numerous bio-active molecules, so affectionately called as store house of phytochemicals. Its chemical composure is quite complex. Few studies characterized, and separated some of the bioactive molecules and reported with substantial biological activities. However, in the present study, we identified, the presence of Dihydrochalcones like Nimbochalcin in the vegetative parts of *Azadirachta indica* (Neem) extracts using LC–MS (Liquid Chromatography and Mass Spectroscopy) spectral analysis. Moreover for the first time, we

report the presence of major Dihydrochalcones like Nimbochalcin ($C_{22}H_{26}O_9$) from the vegetative parts of *Azadirachta indica* collected from Tirumala Hills, Eastern Ghats, India.

KEYWORDS: Flavonoids, Nimbochalcin *Azadirachta indica*, Natural products, LC –MS, ($C_{22}H_{26}O_9$).

INTRODUCTION

Majority of the world population up to 80 percent relies on traditional medicine for their primary health care.[1-2] Presently, the use of medicinal plant/herbal medicine for treating various disorders and diseases is rapidly progressing, since they offer minimal side effects.[1,2] The active ingredients present in these Medicinal /herbal plants have been shown to efficiently hinder the disease or disorder symptoms in a synergistic manner. This active components from these Medicinal /herbal plants may contain of polysaccharides, pigments, steroids, terpenoids, flavonoids and alkaloids etc. Previous studies reported that Medicinal/herbal plant extracts and purified molecules have significant effects in controlling various diseases and disorders.[1-10]

Azadirachta indica is an important medicinal plant, belongs to the family *Meliaceae.* also known as "village pharmacy" or 'Neem tree', or nature's 'drug store' or 'store house of phytochemicals'. Due to this, they are prospective targets for abundant phytochemical investigations.[12-13] It is in cosmopolitan in distribution, majorly found in tropics, starting from Asia to Africa.[1,11-14] It is commonly used in various health practices of rural India. They have been used for centuries in many countries, in several of their native ethno-traditional medicinal health practices. Its vegetative parts like, roots, leaves, bark, seeds and flowers have been administered to treat various acute, chronic diseases and disorders.[11-14] They also known to possess wide range of phytochemicals with significant biological activities. Few studies revealed that these phytochemicals has anti-cancer, antimalarial, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory properties, while few act as insecticidal; larvicidal and sometimes spermicidal.[1,11-14]

Few studies state that 300 plus bioactive compounds have been identified till today, that are chemically diverse in nature with complex structures.[11,14-15] These molecules are classified into isoprenoids, and non-isoprenoids. The isoprenoids class consists of diterpenoids, triterpenoids, vilasinins, limonoids, and C-secomeliacins. The nonisoprenoids class consists of proteins, polysaccharides, sulphur compounds, polyphenolics, dihydrochalcone, coumarin,

tannins and aliphatic compounds.[1,11,14,16] Thus, from above classification is clearly visible that *Azadirachta indica* has been effectively studied, characterized, and structurally elucidated in most African and Asian species and not in Indian varieties.[11-14] Moreover, the knowledge on the chemical composure of the Indian varieties is still lacking, especially on the South Indian species from the region of Eastern Ghats. Hence the present study is undertaken to assess presence of the Dihydrochalcones like Nimbochalcin ($C_{22}H_{26}O_9$) from *Azadirachta indica.*

2. MATERIALS AND METHODS

Plant collection

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

Preparation of Azadirachta indica Extracts

The Freshly grinded powders of the germplasm comprising of leaves, bark and roots were subjected to Soxhlet extraction to prepare the aqueous extracts. From each part nearly 15 gram of the powder was taken separately and packed in sterile cloth, placed in soxhlet apparatus and extracted as per described procedure.[1,5-7] The obtained extract was filtered, further concentrated, and the residue was dissolved in sterile water and filtered and was kept refrigerated until use. The concentration of the extract was obtained by calculating the dry weight per unit volume according to the described procedures.[1,5-7]

LC-Mass spectral analysis

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

RESULTS AND DISCUSSION

Many studies reveal that the chromatographic techniques are extensively used in studying the natural or synthetic molecules that combat with various diseases and disorders.[1] Recent developments in new age molecular biological tools like DNA sequencing, genetic engineering, gene targeting and transgenic methodologies have been established a new path to better understand and evaluate the infections, diseases and disorders, which can deliver new choices for developing new age therapeutics.[17-20] Currently, to battle diseases like cancer,[17-21] and disorders like diabetes,[22] several efficient drug development technologies have been established, through programs like in silico drug designing and synthesis of novel molecules.[7,23-26] However the problems continue same. Hence alternatives are required.

Medicinal plants appear as a better choice. For Instance in ethno-traditional medicine, medicinal plants have been effectively used to treat a various ailments that includes diseases and disorders.[8-10] Presently, administration of the medicinal plant/herbal extracts/formulations is speedily progressing, which are supposed to have minimal side effects. The active substances present in this may be accountable for this outcome. The active elements may be polysaccharides, pigments, steroids, terpenoids, flavonoids and alkaloids.[1,7,8] Moreover, now a days studying secondary metabolites has become an active field, since they are prospective sources for novel drugs.[1,4] In most cases, these plant secondary metabolites will be separated with different chromatographic techniques, by suitable methods that include extraction, separation, purification, structural elucidation and quantification.[1,7] At first, different vegetative parts of the plant germplasm will be collected, shade dried, lyophilized, further extracted with proper solvents in soxhlet extractor to eliminate unwanted substances in order to obtain desired bioactive compounds. After extraction, needed bioactive molecules were separated, purified, structure elucidated and quantified with appropriate chromatographic techniques. Recent studies states that is an urgency to accept and introduce contemporary analytical tools for investigating novel bioactive substances. Moreover implementation of novel chemical fingerprinting methods with analytical tools like LC-MS, could yield quality output in short period. Chromatographic fingerprinting methods could be applied in identifying and validating various bioactive molecules that completely represent a particular plant or herb.

As stated above, in native ethno-traditional medicine *A. indica* is extensively used in various health practices for treating various diseases and disorders.[1,11-14] Its chemical composure is well studied and characterized.[11] Patela et al 2016, classified neem active substances into

two types, namely Isoprenoids and non-isoprenoids. In the isoprenoids type, the diterpenoids, triterpenoids and steroids were positioned. The Falavanoids, coumarins, carbohydrates, proteins, hydrocarbons, fatty acids and esters, and other acids were positioned under the type of non-isoprenoids. Later, the triterpenoids are further classified into various types based on the removal of carbon atom either from the side chain or from the ring skeletal structure of the parent compound. The triterpenoids are further divided as protolimonoids, mononortriterpenoids, dinortriterpenoids, trinortriterpenoids, tetranortriterpenoids, pentanortriterpenoids, hexanortriter-penoids, octanortriterpenoids and nonanortriterpenoids. Furthermore tetranortriterpenoids were categorized into two types, namely ring-intact-tetranortriterpenoids and ring-seco-tetranortriterpenoids. The diterpenoids were further categorized into two types, such as podacarpanoids (margolone) and abeitanoids (sugiol). However, this chemical composure 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 Dihydrochalcones like Nimbochalcin ($C_{22}H_{26}O_9$) 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.[1,27,28] 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. Dihydrochalcones (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]

In the current study we examined the distribution pattern of DHCs like Nimbochalcin 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 Nimbochalcinin 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 Nimbochalcin ($C_{22}H_{26}O_9$).

Root extract

The LC-MS spectral data of crude Aqueous root extract of *Azadirachta indica* reports the presence of a molecular ion peak of Nimbochalcin ($C_{22}H_{26}O_9$) at 434.43 m/z. The protonated molecular ion peaks of Nimbochalcin was recorded in positive mode (Fig.2A) and completely unnoticed in negative mode (Fig. 2B).

Bark extract

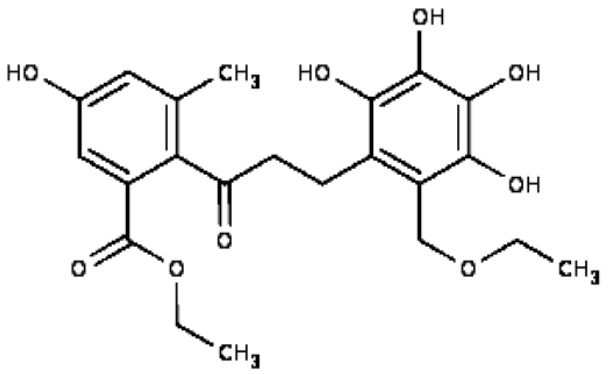
The bark extracts revealed too followed the same path but the bioactive compound found in both modes. The LC-MS data of crude Aqueous bark extract of *Azadirachta indica* clearly depicts the presence of a molecular ion peak of Nimbochalcin ($C_{22}H_{26}O_9$) at 434.43 m/z. The protonated molecular ion peaks of Nimbochalcin 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 Nimbochalcin ($C_{22}H_{26}O_9$) at 434.43 m/z. The protonated molecular ion peaks of Nimbochalcin were clearly observed in positive mode (Fig. 4A) and as well as negative mode (Fig. 4B). Thus, the Dihydrochalcones like Nimbochalcin ($C_{22}H_{26}O_9$) identified in the current study are well correlated with other studies that also carried in similar approach in identifying other molecules using LC-MS[32-36].

Table 1: The distribution pattern of Nimbochalcin from various vegetative parts 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	Nimbochalcin	$C_{22}H_{26}O_9$	434.43	yes	no	yes	yes	yes	yes



Nimbochalcin $C_{22}H_{26}O_9$ (Mass 434.43 m/z)

Fig. 1: The structural presentation of identified Dihydrochalcones like Nimbochalcin from *A. Indica.*

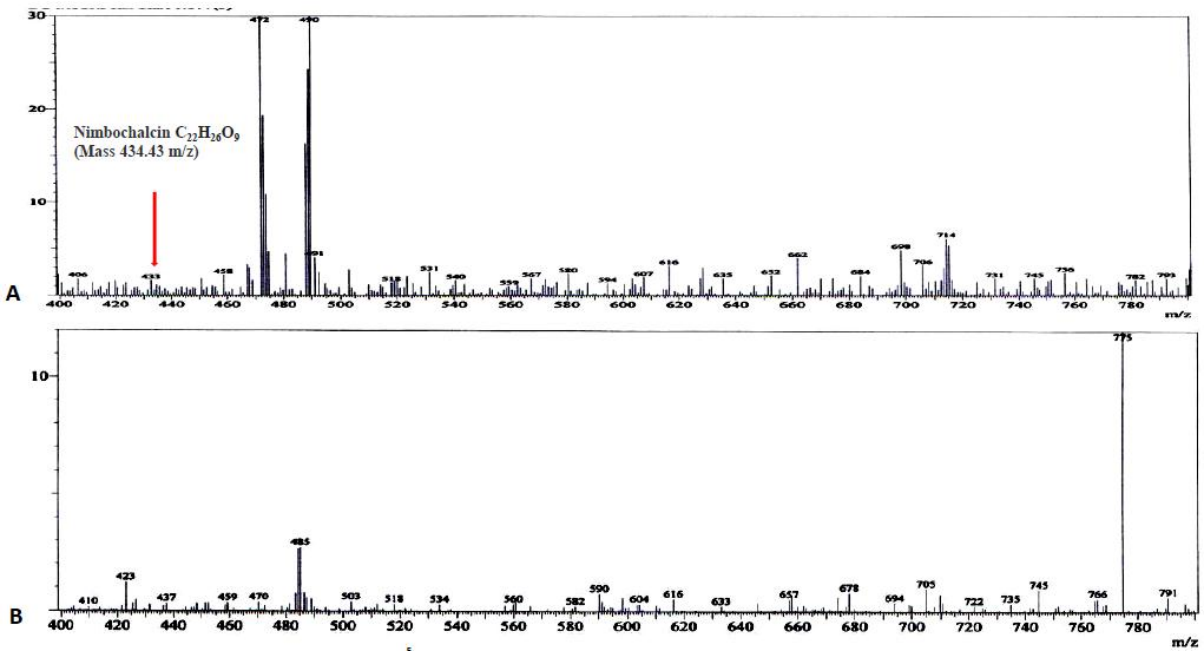


Fig 2 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) of Nimbochalcin from the crude aqueous root extract of *A. Indica.*

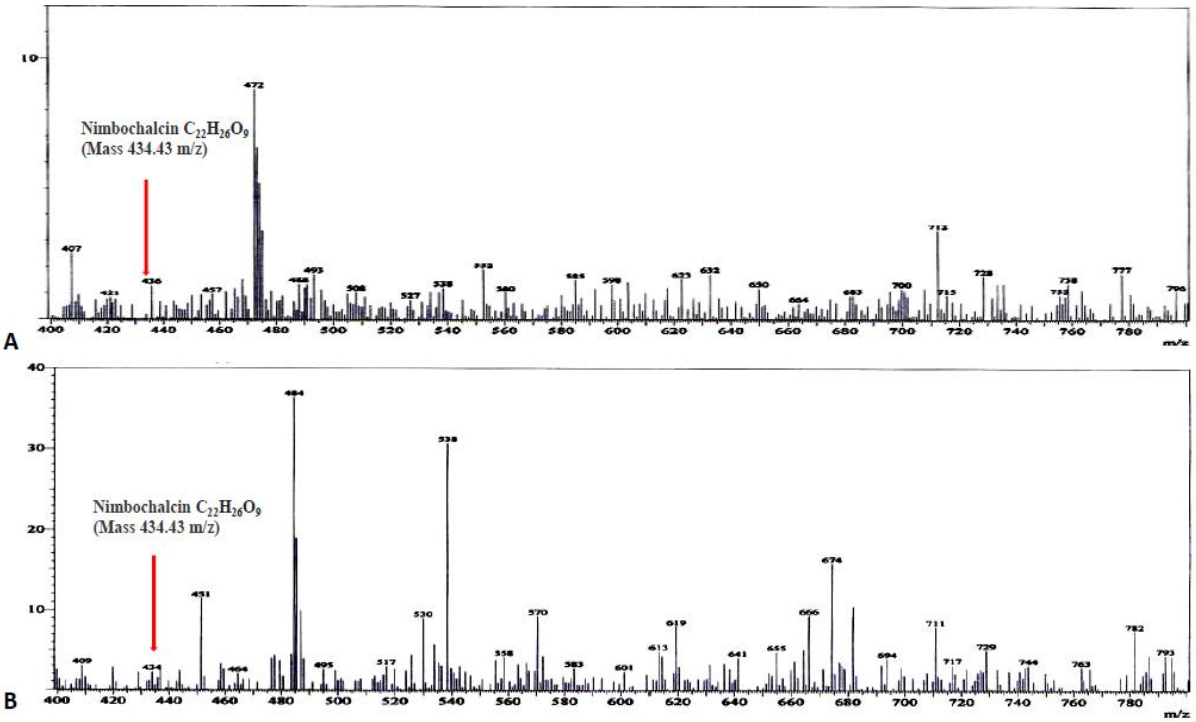


Fig 3 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) of Nimbochalcin from the crude aqueous bark extract of *A. Indica.*

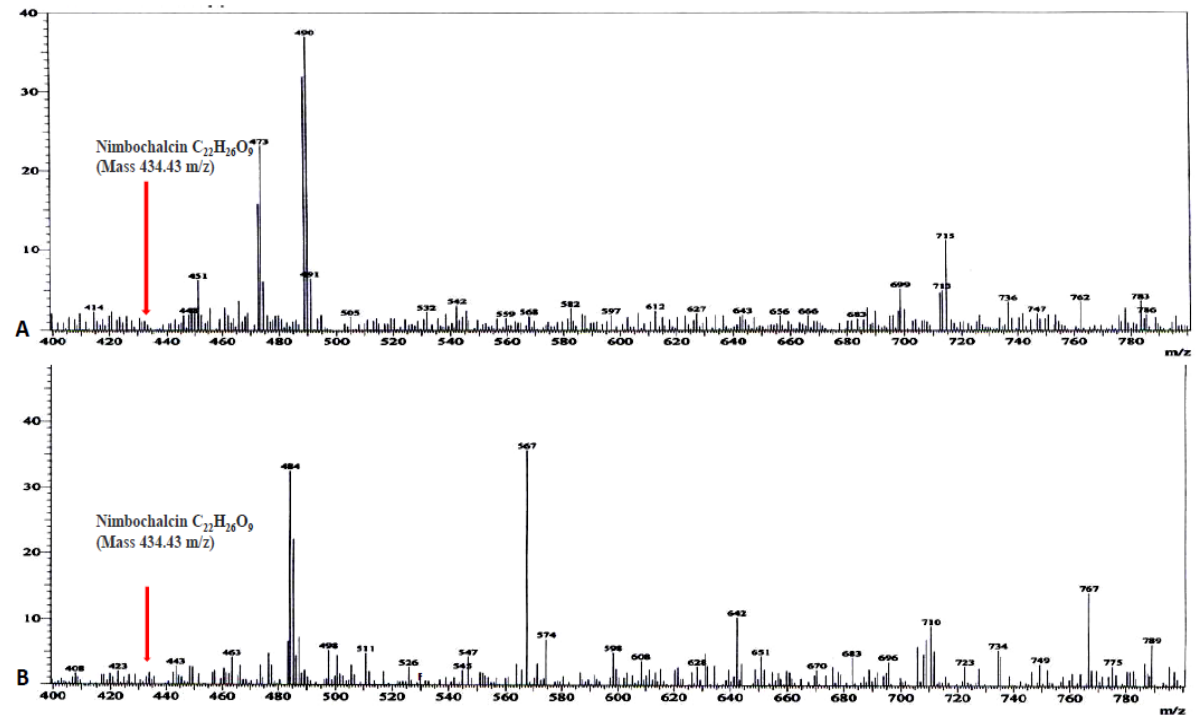


Fig 4 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) Nimbochalcin from the of crude aqueous leaf extract of *A. Indica*

CONCLUSIONS

Thus from the above study for the first time, we conclude and confirm the presence of Dihydrochalcones like Nimbochalcin ($C_{22}H_{26}O_9$) from various vegetative parts of *Azadirachta indica* collected from Tirumala hills, Eastren Ghats, India.

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