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IDENTIFICATION OF LIMONOID 'MELIANTRIOL' FROM AZADIRACHTA INDICA

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ABSTRACT

World population up to 80 percent, in most nations depends on traditional medicine for their primary health care. In several phytotherapy based medical practices, native medicinal plant/herbal extracts are widely used to treat various disorders and diseases. Its usage is rapidly emerging due to their beneficial effects and moreover assumed with less toxic side effects. *Azadirachta indica* (Neem) is an important medicinal plant with high medicinal values. It is a member of family *Meliaceae*, which is in cosmopolitan in distribution. In India, for centuries, its usage is common in ethno traditional medicine and also in all other medical practices. Its vegetative parts like leaves, flowers, fruits, seeds and bark are rich source for various bio-active compounds. Therefore they were termed as natural drug store or store house of

phytochemicals. Its chemical and structural composition is pretty complex. Few bioactive compounds are separated, characterized, and reported with substantial biological properties. However, in the current study, we investigated the distribution pattern of Triterpenes like Meliantriol in the vegetative parts of *Azadirachta indica* (Neem) extracts through LC–MS (Liquid Chromatography and Mass Spectroscopy) spectral analysis. The results reveal the presence of major Triterpeniods like Meliantriol ($C_{30}H_{50}O_5$) for the first time from the vegetative parts of *Azadirachta indica* collected from Tirumala Hills, Eastern Ghats, India.

KEYWORDS:- Flavonoids, Meliantriol *Azadirachta indica*, Natural products, LC –MS, ($C_{30}H_{50}O_5$).

1. INTRODUCTION

Majority of the world population in both, under developed and developing nations still depends on traditional medicine up to 80 percent for their primary health care.[1-2] Currently, application of medicinal plant/herbal medicine for treating various disorders and diseases is quickly developing, since they offer negligible toxic side effects.[1-2] The active constituents present in these Medicinal /herbal plants have been shown to efficiently hinder the disease or disorder symptoms in a synergistic manner. This active constituents 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] Moreover for the few decades, chemical entities derived from plants, termed as "phytochemicals" have gained significant attention among both the public and scientific communities for their role in regulating health and preventing disease.

Azadirachta indica is one such important medicinal plant, belongs to the family *Meliaceae,* also referred as "village pharmacy" or 'Neem tree', or nature's 'drug store' or 'store house of phytochemicals'. Hence, they are valuable targets for several phytochemical investigations.[12-13] Its distribution is cosmopolitan in nature, mostly occurs in tropics, and subtropics which covers Asia and as well as Africa.[1,11-14] Its usage is common in several health practices of rural India. It is used for centuries, in many countries, mostly in their native ethno-traditional medicinal health practices. Its vegetative such as, roots, leaves, bark, seeds and flowers have been administered to treat various acute, chronic diseases and disorders.[11-14] These vegetative parts also well known to have wide range of phytochemicals with key biological activities. Few phytochemicals are reported to have anti-cancer,

antimalarial, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory properties, while some act as insecticidal; larvicidal and even as spermicidal.[1,11-14]

Few researchers explained it possess more than 300 plus bioactive compounds, which are chemically diverse in nature and present with unique complex structural identity.[11,14-15] Majority of these molecules are categorized mainly into two types, namely isoprenoids, and non-isoprenoids. The isoprenoids type differentiated into diterpenoids, triterpenoids, vilasinins, limonoids, and C-secomeliacins whereas nonisoprenoids into proteins, polysaccharides, sulphur compounds, polyphenolics, dihydrochalcones, coumarins, tannins and aliphatic compounds.[1,11,14,16] Thus, from the above it is evident that the chemical composition of *Azadirachta indica* has been successfully studied, bio-active compounds were well characterized, and structurally elucidated in most varieties that are distributed in Asia and Africa and not in the Indian varieties.[11-14] However, the knowledge on the chemical composition on the Indian varieties is still lacking, especially on the South Indian species from the region of Eastern Ghats. Hence the current study is undertaken to assess presence of the Triterpeniods like Meliantriol ($C_{30}H_{50}O_5$) from *Azadirachta indica*.

2. MATERIALS AND METHODS

Plant collection

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

Preparation of plant extracts

The pulverized powders of the germplasm, such as leaves, bark and roots were subjected to Soxhlet extraction to prepare the aqueous extracts. 15 gram of the powder is weighed from each part individually, packed in sterile cloth, further placed in soxhlet apparatus and extracted as per described protocol.[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] and the flow rate kept at 0.5 ml/min respectively. The samples comprising of aqueous extract was nebulized with nitrogen gas and the ion mass (Electro Spray Ionization) of the peaks was recorded at positive mode and negative mode as per described procedures.[1,5-7]

3. 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 has 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 has been established, through programs like in silico drug designing and synthesis of novel molecules.[7,23-30] 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 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.[27] 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 differentiated into different types based on the elimination of carbon atom from the side chain or from the ring skeletal structure of the mother compound. The triterpenoids are divided as protolimonoids, mononortriterpenoids, dinortriterpenoids, trinortriterpenoids, tetranortriterpenoids, pentanortriterpenoids, hexanortriter-penoids, octanortriterpenoids and nonanortriterpenoids. Furthermore tetranortriterpenoids were classified into two types, while the first one as ring-intact- tetranortriterpenoids and the other as ring-seco-tetranortriterpenoids. The diterpenoids also divided into 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 therapeutic potential, the chemical composition of Indian *A.indica* species, distributed in Eastern Ghats has not studied.[1] Therefore, the present study is carried in aim to report the Triterpeniods like Meliantriol ($C_{30}H_{50}O_5$) in *Azadirachta indica.*

Limonoids are a group of natural compounds primarily found in citrus fruits, especially in the seeds, peels, and leaves. They belong to the class of compounds known as triterpenoids, which are organic molecules derived from the combination of six isoprene units.[31] Limonoids are known for their diverse chemical structures and exhibit a wide range of biological activities. They have been the subject of extensive research due to their potential

health benefits and pharmaceutical applications. Some of the common types of limonoids include limonin, nomilin, obacunone, and deacetylnomilin. Research studies have suggested that limonoids possess various pharmacological properties, such as anticancer, antimicrobial, anti-inflammatory, and antioxidant activities. They have shown promising effects in inhibiting the growth of cancer cells and inducing apoptosis (cell death) in certain types of cancer, including breast, colon, and prostate cancer. Limonoids have also been investigated for their potential in preventing or treating other diseases, such as cardiovascular disorders, diabetes, and obesity.[31]

In addition to their medicinal properties, limonoids are often used as natural insecticides and pesticides due to their insect-repellent and pesticidal properties. They have been found to have toxic effects on certain insects and pests, making them a potential alternative to synthetic chemical pesticides. It's important to note that while limonoids have shown promising results in various studies, further research is needed to fully understand their mechanisms of action, potential side effects, and optimal therapeutic applications.[31] As always, it's advisable to consult with healthcare professionals or experts before using limonoid-rich products for medicinal purposes.

In the current study we examined the distribution pattern of limonoids like Meliantriol 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 attain chemical finger printing profile of the aqueous extracts of *Azadirachta indica*, an analytical protocol based on LC-MS (ESI) was deployed. The LC-MS spectral profile data reveals the presence of Meliantriol in 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 Meliantriol ($C_{30}H_{50}O_5$).

Root extract

The LC-MS spectral data of crude Aqueous root extract of *Azadirachta indica* reports the presence of a molecular ion peak of Meliantriol ($C_{30}H_{50}O_5$) at 434.43 m/z. The protonated

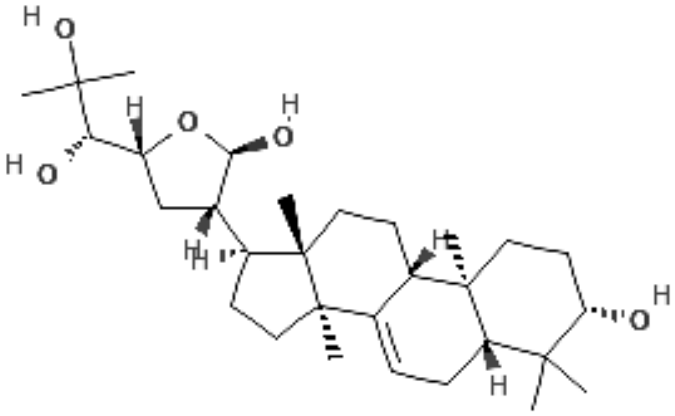
molecular ion peaks of Meliantriol was recorded in positive mode (Fig.2A) and completely unnoticed in negative mode (Fig. 2B).

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 Meliantriol ($C_{30}H_{50}O_5$) at 434.43 m/z. The protonated molecular ion peaks of Meliantriol was clearly visible in positive mode (Fig. 3A) and negative mode (Fig. 3B).

Leaf extract

The leaf extract of *Azadirachta indica* was also presented with similar findings. The LC-MS spectrum of crude aqueous leaf extract of *Azadirachta indica* also reports the presence of Meliantriol ($C_{30}H_{50}O_5$) at 434.43 m/z respectively. The protonated molecular ion peaks of Meliantriol were clearly observed in positive mode (Fig. 4A) and as well as negative mode (Fig. 4B). Similar finding were reported in other studies too.[32-36] Thus, the triterpeniods like Meliantriol ($C_{30}H_{50}O_5$) identified in the current study were comparable with other studies.



Meliantriol $C_{30}H_{50}O_5$ (Mass 490.72 m/z)

Table 1: The distribution pattern of Meliantriol 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	Meliantriol	$C_{30}H_{50}O_5$	490.72	yes	yes	yes	no	yes	yes

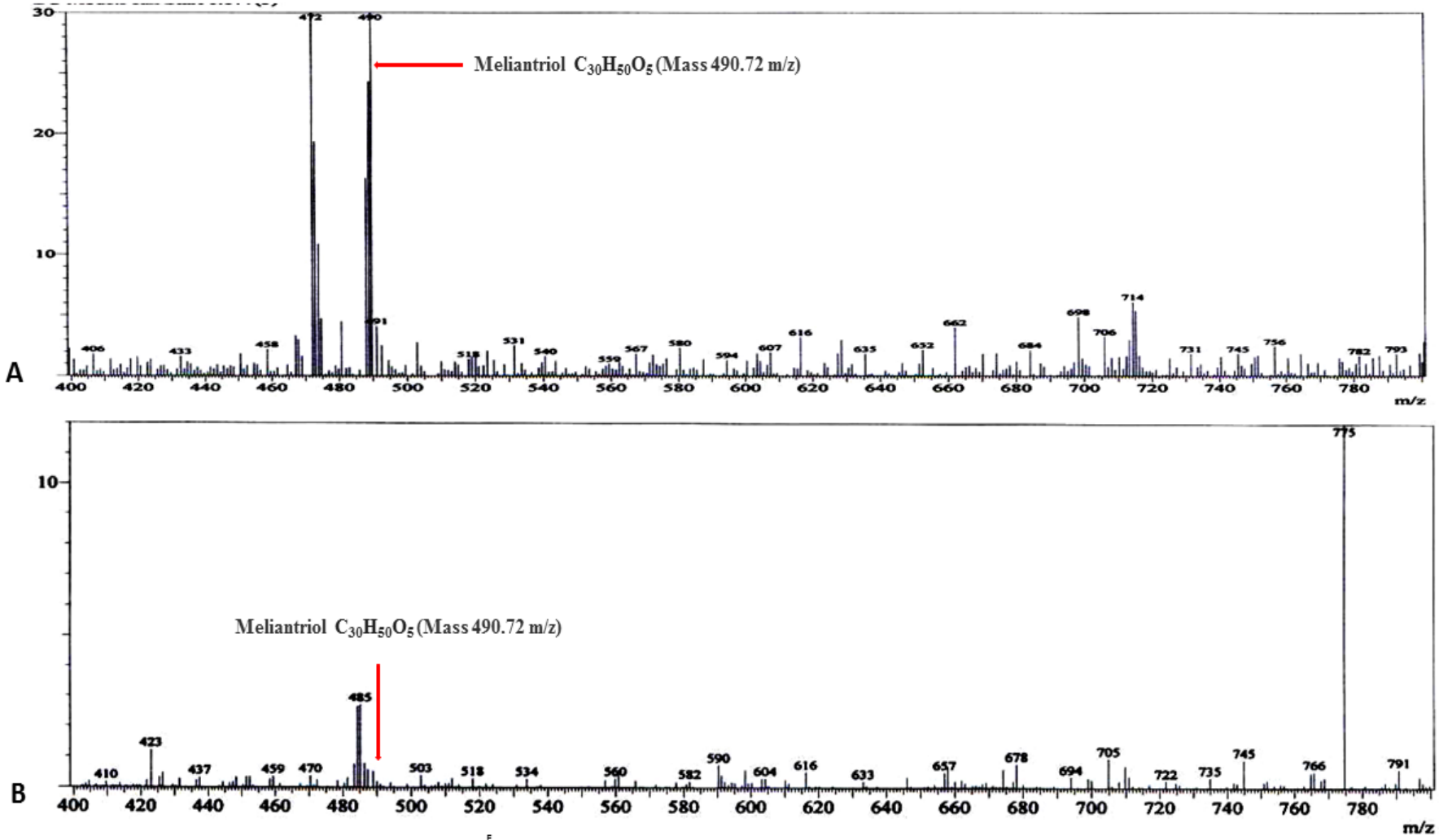


Fig 2 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) of Meliantriol from the crude aqueous root extract of *A. Indica*

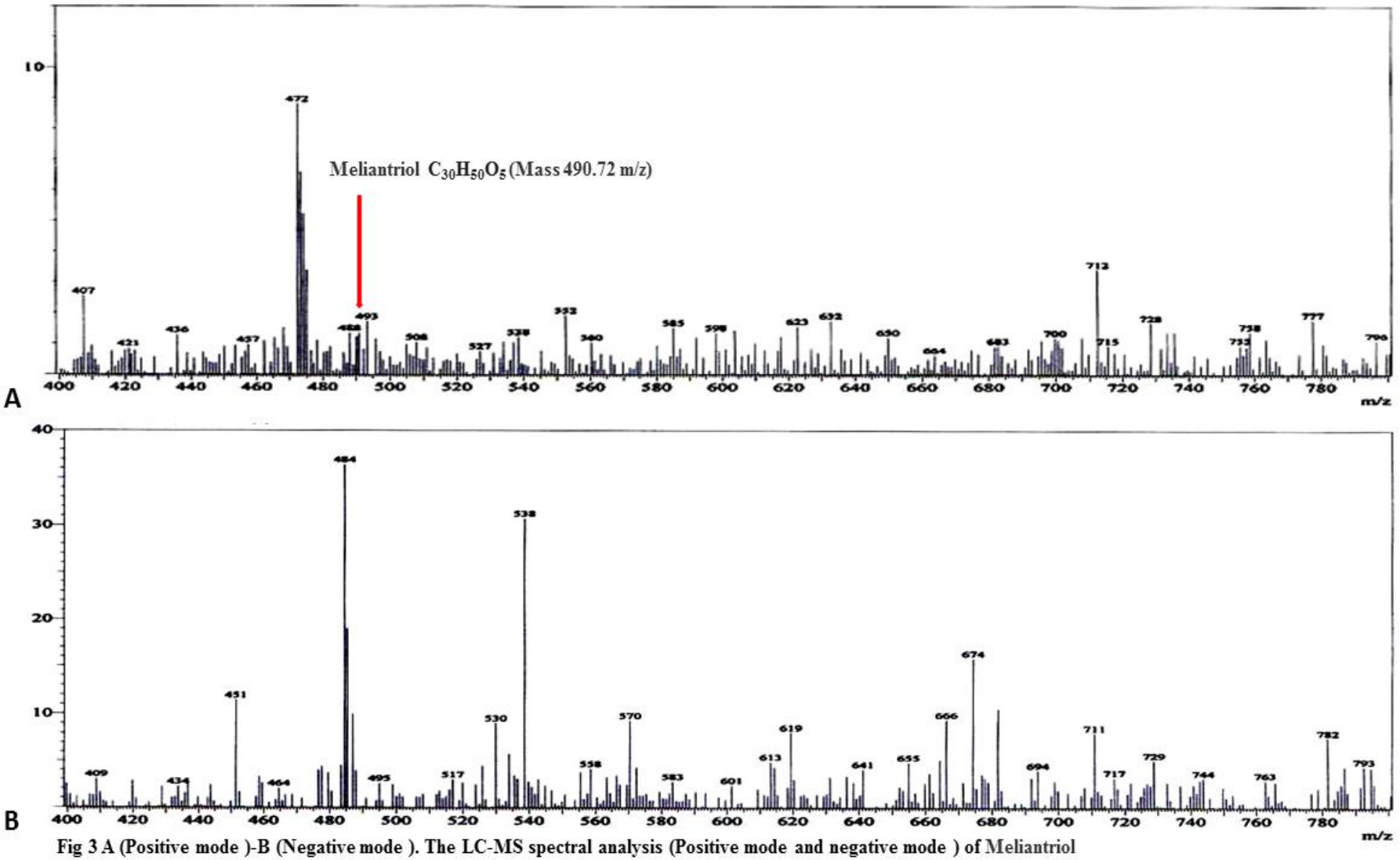


Fig 3 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) of Meliantriol from the crude aqueous bark extract of *A. Indica*

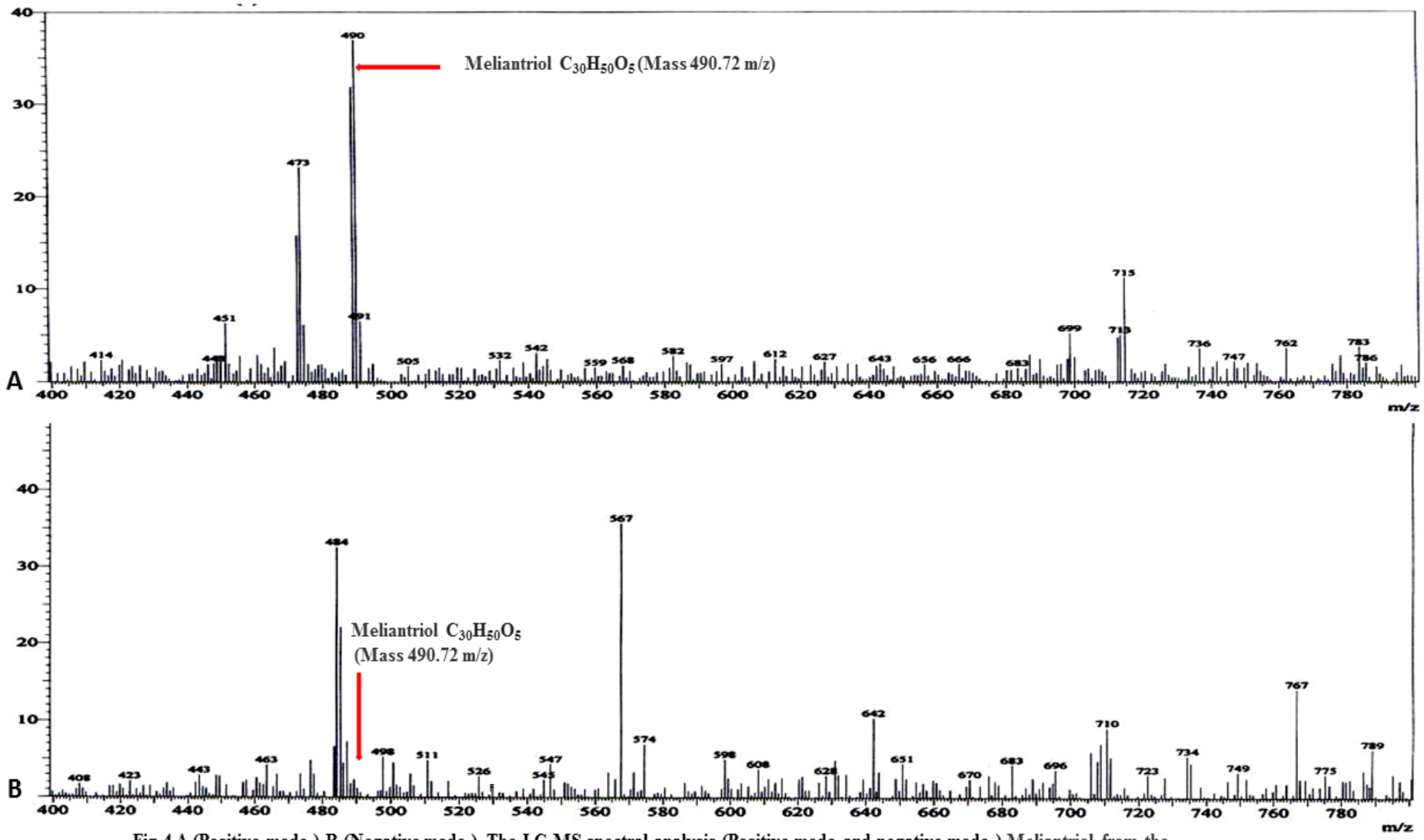


Fig 4 A (Positive mode)-B (Negative mode). The LC-MS spectral analysis (Positive mode and negative mode) Meliantriol from the of crude aqueous leaf extract of *A. Indica*

CONCLUSIONS

Thus from the above study for the first time, we conclude and confirm the presence of Triterpeniods like Meliantriol ($C_{30}H_{50}O_5$) from various vegetative parts of *Azadirachta indica* collected from Tirumala hills, Eastren Ghats, India.

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