International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 22-10-2021, Accepted: 07-11-2021, Published: 23-11-2021
Volume 6, Issue 6, 2021, Page No. 485-491

Isolation, characterization and phytochemical evaluation of active compound lupeol from *Stobilanthes ciliatus* nees

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Abstract
Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. Plants are known to provide cures for various human illnesses and are a rich source of phytoconstituents having diversified pharmacological properties. In recent years the use of medicinal plant has increased as a low cost alternative to the expensive modern drugs. *Strobilanthes ciliatus* Nees (Bremek) Known as "Sahachara" is widely used today in indigenous Indian systems of medicine has been shown to possess a range of folk and proven biological activities such as anti-inflammatory, analgesic, anticancer, antimicrobial, antidiabetic and hepatoprotective. The roots and leaves of the plant are used as major ingredient in many of the ayurvedic preparations especially meant to relieve pain and inflammation Compounds such as lupeol, stigmasterol, betulin, stigmasterol glycosides and 4- acetyl-2, 7-hydroxy-1, 4, 8,-triphenyl-octane-3, 5-dione were reported from the acetone extract of the stem. Lupeol is the major compound reports from the various parts of the plant and is well reported for its broad pharmacological potential. The present aim of the study is to extraction and isolation of bioactive compound from *Strobilanthes ciliatus* (Bremek.). The isolation and characterization analysis included, Thin Layer Chromatography, High Performance Thin Layer Chromatography, GC-MS and Spectroscopy studies (IR, NMR, and MASS). The presences of Lupeol in the methanolic fraction revealed that the isolated constituent is most active compound. Isolation of bioactive compound Lupeol may help in identification of various pharmacological activities and carrying out further research in *Strobilanthes ciliatus* (Bremek).

Keywords: bioactive, *Strobilanthes ciliatus*, GC-MS, isolation, characterization, lupeol

Introduction
Herbal medicine is one of the most important areas of traditional Indian medicine. Many traditional medicinal plants have been used in folk remedies to treat a variety of human physical illnesses [1, 2]. *Strobilanthes* ciliatus Nees (Break.) Is a traditionally known and medicinal plant belonging to the genus "*Strobilanthes*". This plant has recently received a great deal of attention due to the presence of a wide range of secondary metabolites and various pharmacological activities. *Strobilanthes* is a genus of 350 perennial flowering herbs and shrubs commonly found in tropical Asian hills, 150 of which are available in the Indian subcontinent. Flowering plants of this genus belong to the family Acanthaceae [3]. Observed in evergreen forests of the Western Ghats up to 1200 meters, including Kerala and Karnataka [4]. Very often semi-evergreen forests are up to 1 meter high, sometimes in partial shade with round or semi-square stems, with diffuse branches and ridges on both sides when young. At nodes, hairless, lenticular, dark green or purple with white spots and often wings. The nodes are connected, prominent, and often cilia. The leaves are simple, opposite, spear-like, serrated, almost hairless, with a weak base and pointed tips [5]. Flowers are 4 rows, white or pale purple with dense spikes. Capsules are elongated and ciliates. The length of the sepal is 56.5 mm and is divided into 2/3 of the length. Non-uniform segments, linear to lanceolate, pointing to vertices, almost bald with some glandular hairs. It is an annual flowering plant and is observed from December to March [6].

Taxonomical Classification
Kingdom: Plantae
Phylum: Tracheophyta
Class: Mangoliopsida
Order: Lamiales
Family: Acanthaceae
Genus: *Strobilanthes*
Species: *Strobilanthes ciliates* [7]

S. ciliatus is a highly aromatic plant that is widely used in Ayurveda as a drug called "Sahachara" and is thought to be used in other Indian medicinal systems such as eels and Siddhas.
I am. Used in neurological disorders used. Based on this, Ayurvedic products are on the market as neuro tonic agents for neuritis and motor neuron disease [10, 11]. The roots were found to be bitter, sweet, fever-generating, emollient, diuretic, antipyretic, diuretic, cleansing, expectorant, and tonic [12].
They are also traditionally used for various diseases such as inflammation, rheumatism, low back pain, sciatica, limping, chest congestion, fever, leukoderma, skin diseases, cough, bronchitis, periodontitis, and general weakness. I have [13].

Materials and Methods

Collection of plant materials

Strobilanthes Fresh sample leaves of ciliates were collected from a natural habitat near Thodupuzha in the Idukki district of Kerala [14]. The sample has been certified by a pharmacologist. They were dried in the shade, packed in zippered plastic bags, and labeled.

Fig 1: *Strobilanthes ciliatus* plant extract

Extraction

The collected plant material was washed twice with tap water and dried in the shade at room temperature for 3 weeks. The leaves of the air-dried plant were crushed with an electric mixer into fine powder. A total of 3 kg of powdered *Strobilanthes* ciliatu leaves were sequentially extracted with methanol using a Soxhlet extractor until the emerging solvent became colorless. The extract of the extract was passed through Whatman filter paper (Whatman # 1) to avoid contamination and dried under vacuum at 40 ° C. The dried crude methanol extract was stored in a -4 ° C freezer for further investigation [15].

Separation of active compounds by chromatography (Thin layer chromatography)

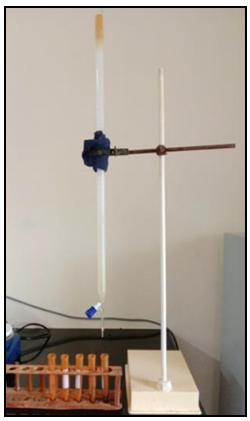

Fig 2: Column packing

The column is prepared by covering the bottom of the column with a small amount of pre-extracted quartz wool (or glass wool) and manipulating it with a non-polar solvent mixture. Following this step, activated silica gel (or alumina) is added to the column to the required amount. Finally, the organic matter that can be extracted from the sample to be fractionated should be placed at the top of the column. Cover the bottom of the column with glass wool and rinse with a mixture of polar solvents. Following this step, activated silica gel (or alumina) is added to the column to the required amount. Finally, organic matter that can be extracted from the sample to be fractionated should be added to the top of the column. The residue was chromatographed on preparative silica gel slides using a variety of solvent systems (acetone, hexane, ethyl acetate, individual or combined solvent systems). Acetone: ethyl acetate (1: 2), acetone: ethyl acetate. (2: 1), Hexane: Ethyl Acetate (1: 2) and Hexane: Ethyl Acetate (2: 1). The first raw spots were observed for migration and separation through the previously prepared mobile phase. We recorded the R-values of colored and non-colored spots obtained using visible and UV lamps. Using a silica gel plate (size 20 x 20 cm, thickness 0.50 mm, 60 GF254 fine granules), the active band was collected, dissolved in ethyl acetate and concentrated to dryness in vacuo. The dried TLC plate was observed with iodine vapor and visualized under UV light (low wavelength and high wavelength) [16].

culture (10 L) was gradually extracted with ethyl acetate (1: 1 v / v) and concentrated on a rotary evaporator at 50 ° C to give 2 g of crude brown residue. The residue was chromatographed on preparative silica gel slides using various solvent systems such as acetone, hexane, ethyl acetate, individual or combined solvent systems. Acetone: ethyl acetate (1: 2), acetone: ethyl acetate. (2: 1), Hexane: Ethyl Acetate (1: 2), and Hexane: Ethyl Acetate (2: 1). The first raw spots were observed for migration and separation through the previously prepared mobile phase. We recorded the R-values of colored and non-colored spots obtained using visible and UV lamps. Using a silica gel plate (size 20.20 cm, thickness 0.50 mm, 60GF254 fine granules), the active band was collected, dissolved in ethyl acetate, and concentrated to dryness in vacuo [17]. Methanol extracts for each fraction were purified and analyzed by GC-MS and HPLC. The structure of the isolated compounds was characterized by spectral studies, UV, FTIR, NMR, and MASS studies.

Gas chromatographic mass spectrum [18, 20]

The active fraction was analyzed using SHIMADZUGC-MSQP5050A using the CLASS5000 program at the Central Laboratory. The identification was performed using the WILEY MASS SPECTRAL DATABASE library.

Infrared [19]

The extracted mg sample of crude oil was subjected to IR spectral analysis using an infrared spectrophotometer. An average IR range of 400-4000 cm1 was used for sample analysis. The mixture of pure spectroscopic KBr had a ratio of 5:95. The pellet was fixed in the sample holder.

Result and Discussion

Thin layer chromatography separation

A compound standard separation by TLC using a methanol extract of S. ciliatus was dissolved in 50 ml of hot water and extracted 3 times with 50 ml of ethyl ether and ethyl acetate, respectively. Solvents from all fractions were removed on a rotary evaporator to give ethyl ether and ethyl acetate extracts. The ethyl ether extract was separated by TLC (normal phase plate, ethyl ether-hexane, 1: 5, v / v) and 9 zones of the TLC plate could be visualized under 254 nm UV light. Each zone was scraped off the plate and extracted with methanol. The methanol extracts of each fraction were analyzed by GC-MS and HPLC.

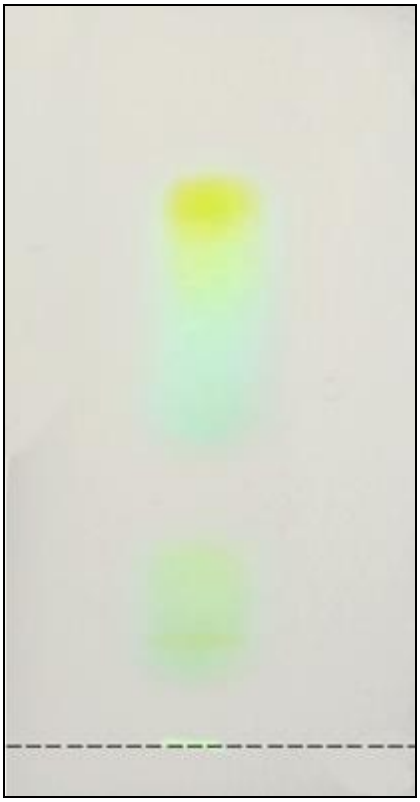

Fig3: TLC of lupeol

Based on the Rf value of the band, the active ingredient was identified as standard. Under UV light (in the case of a chromonic system, Lupeol's TLC visualized by UV light) shows spots, and based on relative Rf values, the purity of each fraction after elution is tested by analytical TLC and the fractions are clear. Showed separation. This fraction was scraped off and collected for further analysis (Figure 4).

GCMS analysis

The GC-MS analysis identifies the major bioactive compounds and is called Lupeol. Here, the sample passed through a unipolar GC analyzer and the separated, purified process, and structure with the extracted bioactive compound were passed through the separated compound, and lupeol was identified by spectral studies [20].

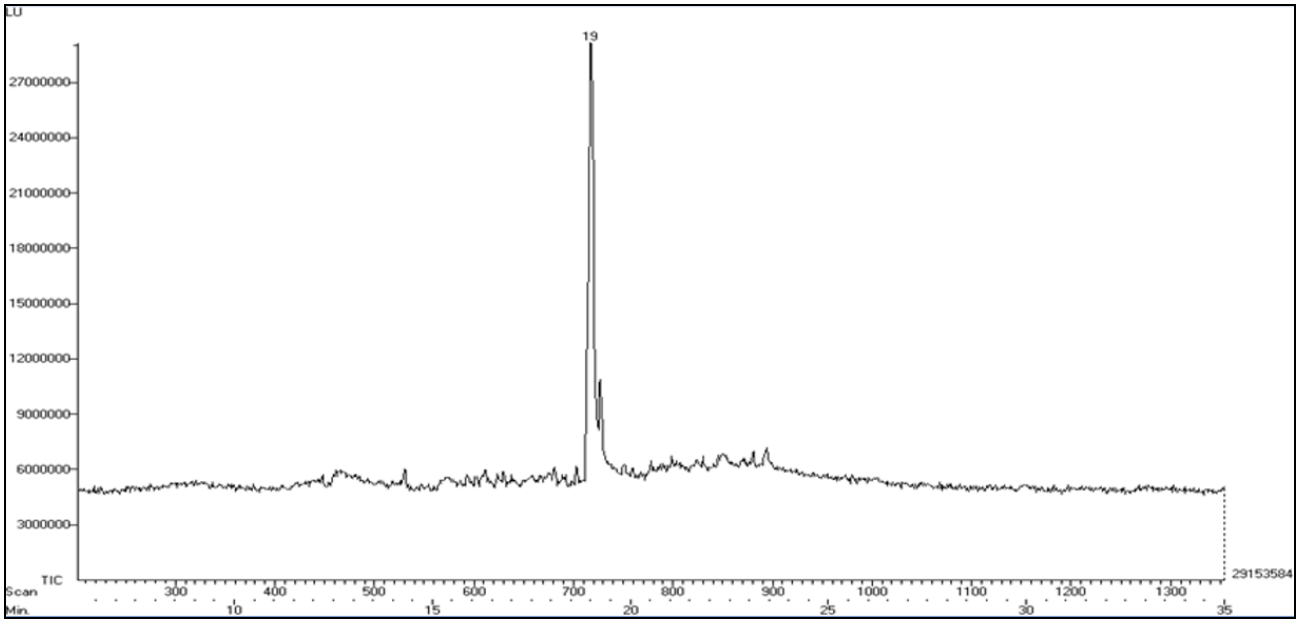

Fig 4: GC-MS- analysis of *Strobilanthes ciliatus*

GC-MS analysis identifies the major bioactive compounds and designates them as (726-Lupeol). Here, the sample passes through the Unipolar GC analyzer, which is further focused on the Lupeol and the isolation procedure is performed.

HPLC analysis

The purity of the separated compounds was checked by HPLC analysis and the spectra were recorded and shown in Figure 6. In both cases it is standard. The purity of the

active ingredient (coumarin) was shown as a single sharp peak.

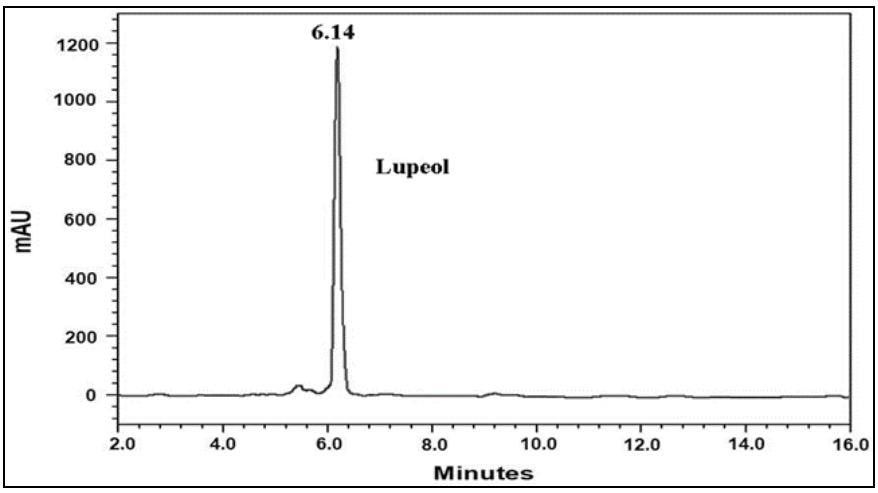

Fig 5: HPLC (Lupeol separation)

UV- spectrum analysis of isolated compound

As per the reference the UV-VISspectrum (Fig.7) analysis the isolated fraction of Lupeol shows 335nm shallow peak, confirmed as Lupeol with the reference [21].

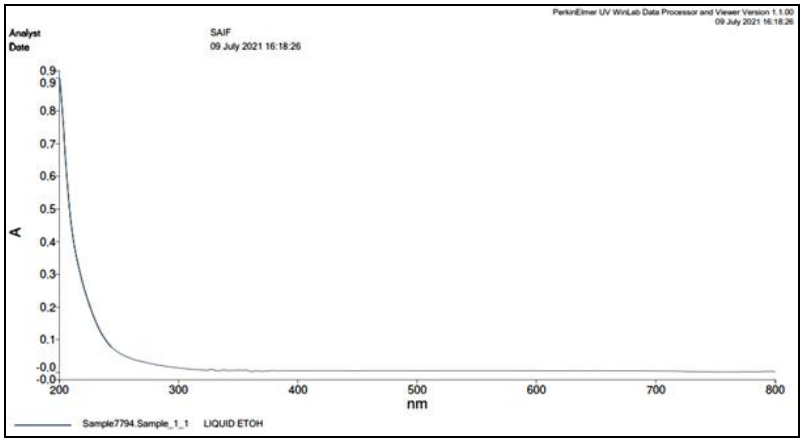

Fig 6: UV-Vis spectrum of Lupeol

FTIR spec of Lupeol

FTIR spectrum of purified Lupeol shows many peaks corresponding to functional groups many peaks corresponding to functional groups which are present in structure of isolated compound there were a broad peak at 3319.55 cm−1, which corresponds to the Hydroxyl group (O-H). The aromatic C–H stretching peak was observed at 2973.32 cm−1 while aliphatic C-H stretching was observed in 2881.70 cm−1, C=C stretching frequency was found at 1420.92 cm−1. The absorption band at 1274.97 and 1087.87 cm−1 corresponds to the C=O stretch of Benzene ring and C-C bend, respectively [21].

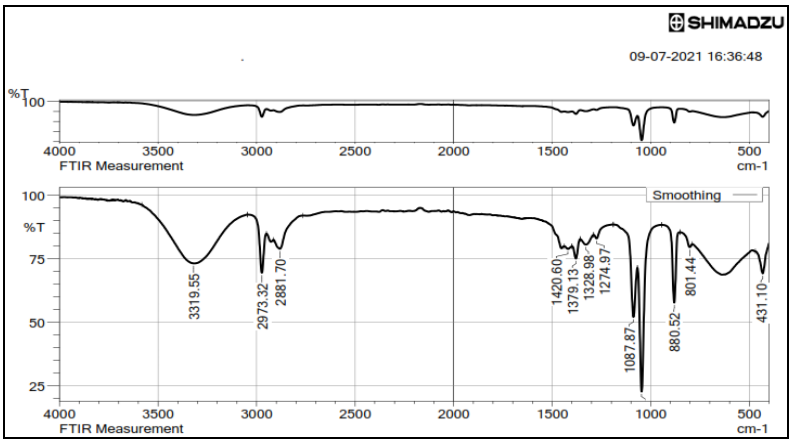

Fig 7: FTIR spec of lupeol

NMR- Spectrum Analysis of isolated compound

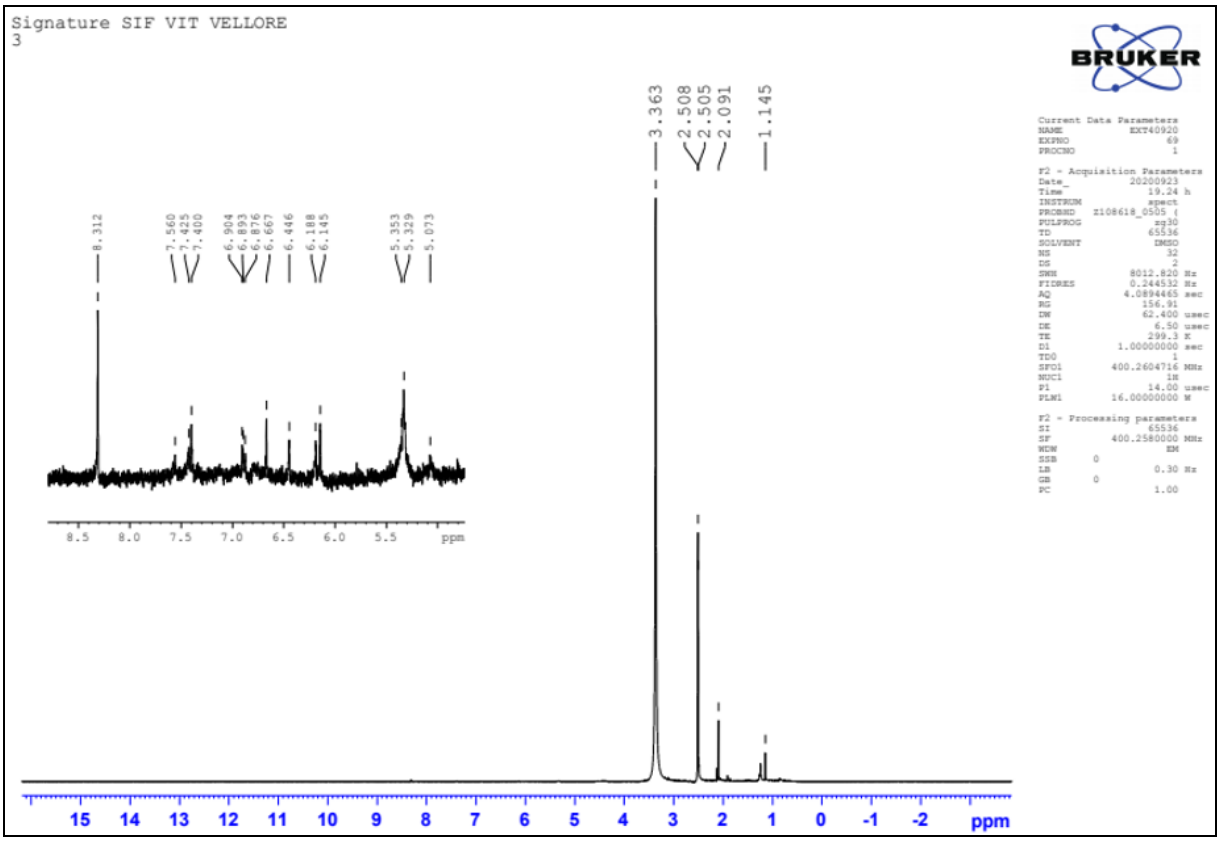

Fig 8: H^1 NMR spectrum of isolated compound

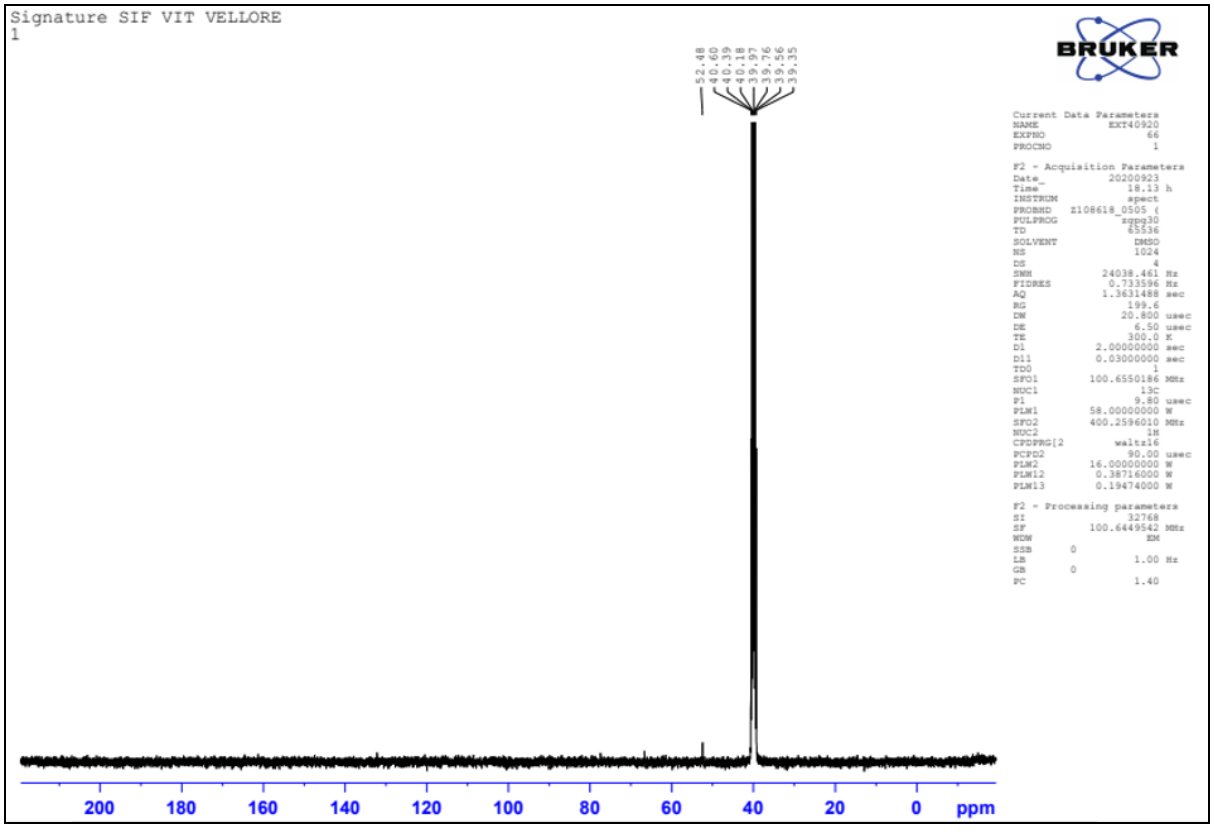

Fig 9: C^{13} NMR spectrum of isolated compound

Mass of lupeol

Through the Mass spectrometric analysis the isolated compound identified as Lupeol with the molecular weight of 426.12. Mass spectral analysis suggested MS at m = z 134 of compound. It was represented in this data that this compound corresponds to a molecular formula of $C_{30}H_{50}O$

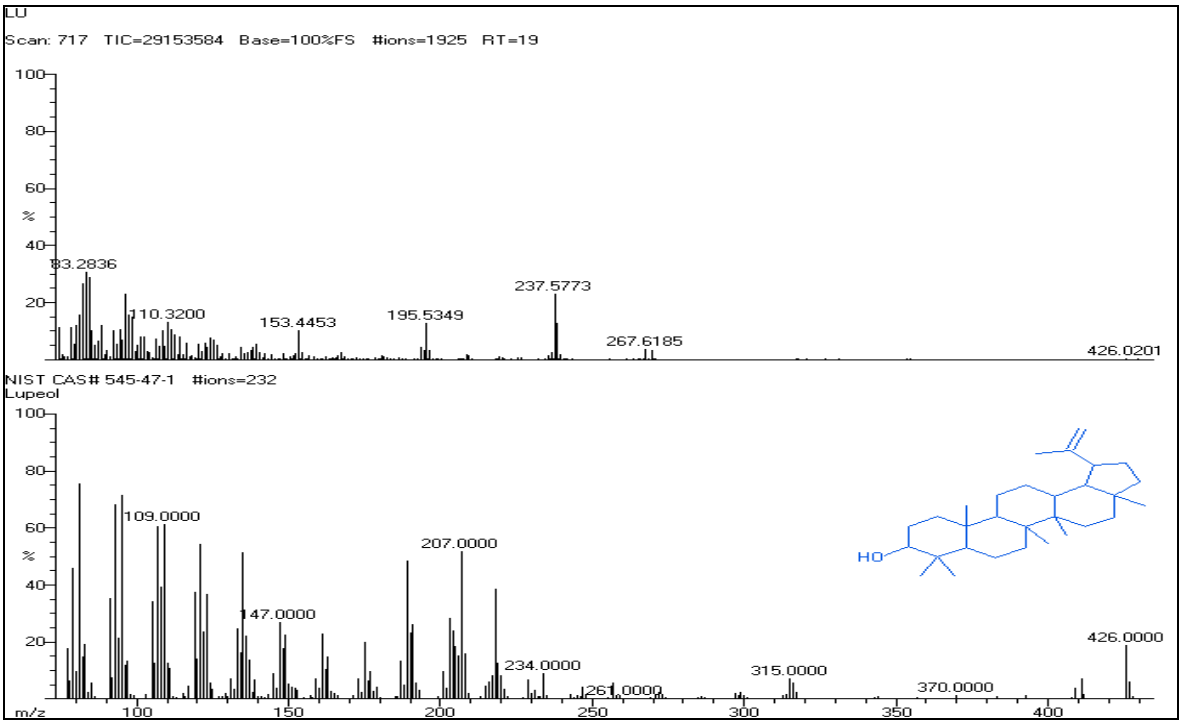

Fig 10: Mass spectrum of lupeol

Conclusion

There has been a worldwide interest in scientific validation of the old traditional medicines for their therapeutic efficacy. However research up to now has shown that that the plants are valuable sources for novel compounds. Emergence of combinatorial chemistry opens a wide platform to create natural product libraries from the base molecules isolated. The main objective of the presentwas isolation of bioactive compound by chromatographic techniques (TLC), Purified using HPLC and GC-MS analysis.

Finally the structure of the compound was appraised by FTIR, NMR and MASS spectroscopy studies which revealed that the isolated compound is Lupeol compare with the standard. From the above result conclude that the methanolic extract of plant *Strobilanthes ciliatus*is good source of Lupeol. Hence, this study recommends that the isolated active compound Lupeol can be used as a prototype molecule for medicinal drug. The plant needs to be further evaluated in combination with other plants of same family to establish this common weed as a pharmacologically potential herb.

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