IJPAR |Volume 3 | Issue 3 | July-Sep-2014 ISSN: 2320-2831

INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

Available Online at: www.ijpar.com

[Research article]

Spectral analysis of whole plant ethanol extract of Blepharisrepens (vahl) roth and its fraction

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ABSTRACT

The whole plant of Blepharisrepens (vahl) Roth, have a great medicinal value. The aim of the present study was to determine the chemical profile of ethanolic extract of Blepharisrepens (vahl) Rothand its fractions (by various spectral analysis) which will be useful for its proper identification when therapeutically used. Spectral analysis was done by different analytical methods such as UV, IR, GC-MS and HPTLC studies in ethanol extract (EE) of Blepharisrepens (vahl) Rothand its fractions. In HPTLC analysis, ethanolic extract and its fractions showed the presence of different types of compound with different RF values ranging from 0.01 to 1.05. The GC-MS analysis showed the presence of terpenoids (mono), fatty acids such as hexadecanoic acid (CAS) palmitic acid, heptadecanoic acid(CAS) palmitic acid, benzene dicarboxylic acid, cyclo heptasiloxane, Octadecene -1-ol, octadecenalin ethanol extract and its fractions ofBlepharisrepens (vahl) Roth.

Keywords: Blepharisrepens (vahl) Roth, HPTLC, GC-MS, Spectral analysis.

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INTRODUCTION

The derived material or medicinal plants, have been widely employed in all cultures, throughout history, for the prevention and treatment of diseases. The significant increase in the use of herbal medicines in recent decades may be attributed to popular wisdom, the costs of synthetic drugs and the resurgence of interest in the development of new drugs and the reestablishment of old ones from plant sources (1).medicinal plants are extensively studied for their large variety of demands for the human health.(2,3).Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra(4).Blepharisrepens (vahl) Roth is commonly known as Hadsan, In Tamil name called as elumbotti, regional name as samadana (srilanka). Some biological properties of Blepharisrepens (vahl) Roth such as aphrodisiac, diuretic, tuberculosis, diabetic, antioxidant, antimicrobial, wound healings. Nevertheless, present study was performed to characterize the antidiabetic, antioxidant, anti-microbial, wound

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healing activities of whole plant Blepharisrepens (vahl) Rothethanolic extract, together with spectral analysis is useful in differentiating the species from the adulterants and the results of this study may act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

PHARMACOGNOSTICAL STUDIES

Collection and authentication of plant materials

The plant Blepharisrepens (vahl) Roth collected from Western ghats, Erode District. Plant collections were done, during the month of January 2012. The plants were identified by Prof.P. Jayaraman, Medical plant research center, Chennai-45, voucher specimen of Herbarium sheet are kept in the plant anatomy research center west Tambram, Chennai – 45 for further reference.

PLANT PROFILE

Aerial Part of blepharisrepens (vahl.) Roth

Scientific Name Blepharisrepens
Family Name Acanthaceae
Common Name Hadsan
Local Names Samadana (Sri Lanka)

Synonyms: Acanthus repens Vahl
Regional names: Samadana (Sri Lanka)

External Features

A slender, perennial herb with prostrate, hispid-hairy, slightly branched, wiry stems;lea sessile, in a whorl, two of each whorl larger than others, oblong, 0.8-1.5cm long,0.3cm broad, obtuse or rounded at apex, glabrous or slightly scabrid, fleshy and paler beneath; flowers white , irregular, solitary and axillary, bisexual, sessile, surrounded by 4 pairs of decussiolespinous bracts supported by a pair of tiny bracteoles outside in some flowers; bracteoles ovate, 2mm long, 1.5mm broad, hairy with a single spine at the apex 3 outer pairs of bracts obovate, chartaceous, hairy, veined with long spreading spines at the margin in the upper part, the outermost pair 3mm long, 2mmbroad, the next pair inner to it 4mm long and 2.5mm broad, the 3rd pair 6mm long and 3mm broad, the innermost 4th pair boat-shaped, or oblong-ovate membranous, veined, 6mm long, 2.5mm broad, ciliated with a solitary, terminal spine; sepals 4 in two pairs, membranous, linear or linear-oblong, apiculate and hairy, the outer pair larger 9mm long, 2-2.7mm broad, one being 2-veined and the other 2-veined and bitidat apex, the inner pair linear 8.5mm long, 1.2mm broad, 1-veined; corolla 1cm long, the upper portion tubular inflated without an upper lip, lower lip obovate, as long asbroad, 3-lobed at apex and hairy; stamens 4, epipetalous in two pairs; filaments short, the lower pair borne oil stout cylindrical appendages; anther stout, 1.5mm long, base fixed with a tuft of hair at the apex; capsule small, 0.6cm long, completely enclosed in persistent sepals and bracts, ovoid, compressed, smooth, loculicidallydehiscent with 2 compressed, hairy seeds inside. Flowers during February and March (Sri Lanka).

Preparation of sample

Dried crude powdered drug was extracted in ethanol by soxhlation method. The extract was taken and was filtered. The crude extracts obtained were concentrated by rotary evaporator at 40^{o}c. this

concentrated extracts were kept aside and used for GC-MS analysis. (Kokate (2003).

Phytochemical screening.

The phytochemical composition of the extract, fractions and isolated compound were determined using standard methods out lined by harborne (1998) and Kokate (2003).

TLC of the ethanolic extract of Blepharisrepens (vahl) roth.

The ethanolic extract of the blepharisrepens (vahl) roth plant was subjected to TLC in different solvent system. The ethanolic extract was spotted, dried and developed in the respective solvent. The solvent used are, Toluene and Ethyl acetate (93:07).

The different spots developed in various solvent system were identified by means of vanillin-sulphuric acid reagent and the solvent system Toluene and ethyl acetate (93:07) was selected to get clear separation of components.

Ultraviolet spectroscopy

UV-VISIBLE SPECTRA of EEBR and its fraction are shown in fig. 1.

U.V. analysis were been performed for the crude drugs by using the suitable solvents. The extract of the crude drugs is taken and the serial dilutions were made Blepharisrepens (Vahl.) Roth using appropriate solvents for Blepharisrepens (Vahl.) Roth, were made the serial dilution for the U.V. analysis. The U.V. region is 200 – 400 nm. The absorbance of dilution of crude drugs were as follows, 10, 20, 50, 100, 500, 1000, 2000, 3000 mcg/ml were performed by Elico SL – 164 Double Beam U.V. Spectrophotometer.

The λ_{max} for each sample are tabulated in the results.

F.T.I.R. ANALYSIS

FT - IR analysis were been performed for the crude drugs by using the suitable solvents. Instrument Shimadzo F.T – I.R. 8400S Spectrophotometer was used and Solvent Used Carbon Tetrachloride (Less Interference).The samples for the F.T – I.R. analysis were made ready with the solvent Carbon Tetrachloride, as it does not interfere in I.R. region. The I.R. instrument was activated and background scanning was done. The drug sample was loaded in the liquid cell. The vibrations of the sample was taken, from this data were interpolated the functional groups of each sample.

HPTLC finger printing

CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner 3, repro star 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software were used. All the solvents used for HPTLC analysis were obtained from MERCK. The sample(10microlitre) were spotted in the bands of width 8 mm with a Camag microliter syringe on pre-coated silica gel glass plate 60 F- 254. The sample loaded plate was kept in TLC twin trough developing mobile phase. The Toluene- Ethyl acetate (93:07) was employed as mobile phase for extract. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber saturated with the same mobile phase. The optimized chamber saturation time for mobile phase was 30 min at mobile phase for room temperature. The plate was documented at UV 366nm and white light using photo-documentation chamber and captured the images under white light, UV light at 254 and 366nm. Densitometer scanning was performed on camag TLC scanner 111 and operated by CATS software.

GC-MS and NMR

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO 6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite – 5 capillary column measuring 30 x 0.25mm with a film thickness of 0.25mm composed of 95% dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml /min. 1ul (microliter) sample injection volume was utilized. The inlet temperature was maintained as 250^{o}c. the oven temperature was programmed initially at 110^{o}c for 4 min , then an increase to 240^{o}c .And then programmed to increase to 280^{o}c at a rate of 20^{o}c ending with a 5 min . Total run time was 90 min. The MS transfer line was maintained at a temperature of 200^{o}c the source temperature was Maintained at 180^{o}c. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantitation. The spectrums of the components stored in the GC-MS library. Measurement of peak area and processing were carried out by Turbo- Mass – OCPTVS-Demo SPL software.

RESULTS AND DISCUSSION

UV – visible absorption

UV – visible spectra of EE and its fraction are shown in fig .1.The UV spectrum of EE of blepharisrepens (vale) Roth showed absorption maxima at 413.2, 413.6, 413.4, 413.0, 413.0 and 413.5 nm. The λ_{max} for each sample were **413.2nm, absorbance = 0.154 (u.v)**

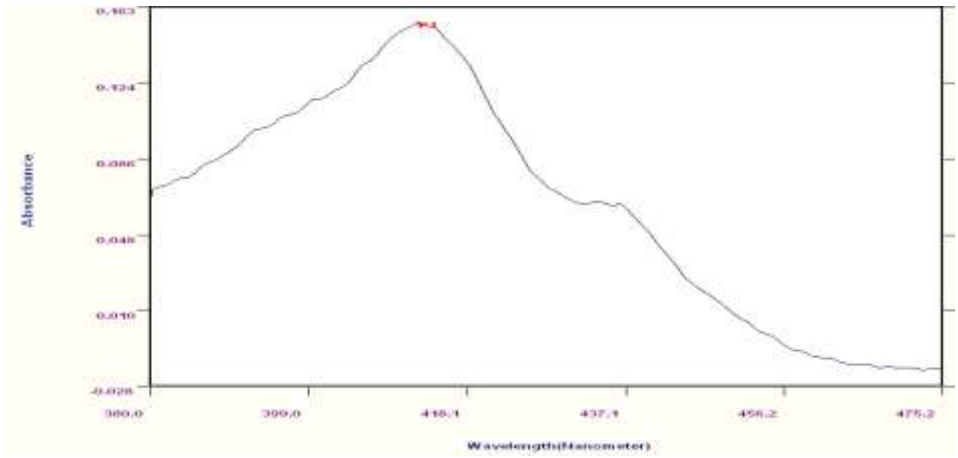
FTIR Spectroscopy

IR spectra Of EE and its fractions are shown in fig .2. The mid-infrared, approximately 3028 $cm^{-1}$was used to study the fundamental vibrations and its fraction were given in Table -2.

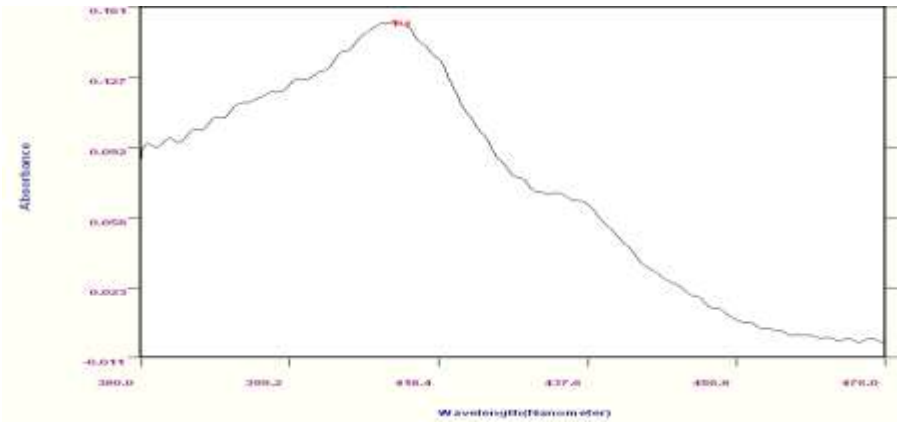
HPTLC fingerprinting

High performance Thin Layer Chromatography (HPTLC) technique is most simple and fastest separation technique available today which gives better precision and accuracy with extreme flexibility for various steps. The results showing number of peaks, maximum RF value and total % area are given in Table 3. The EE showed 0.5 peaks in 200-800 nm spectral range (R_F value 0.31, 0.34, 0.78, 0.79, 0.95 respectively) (fig. 4).This HPTLC technique may be useful for both the identification and the quality evaluation of preparation containing blepharisrepens (vahl) Roth.

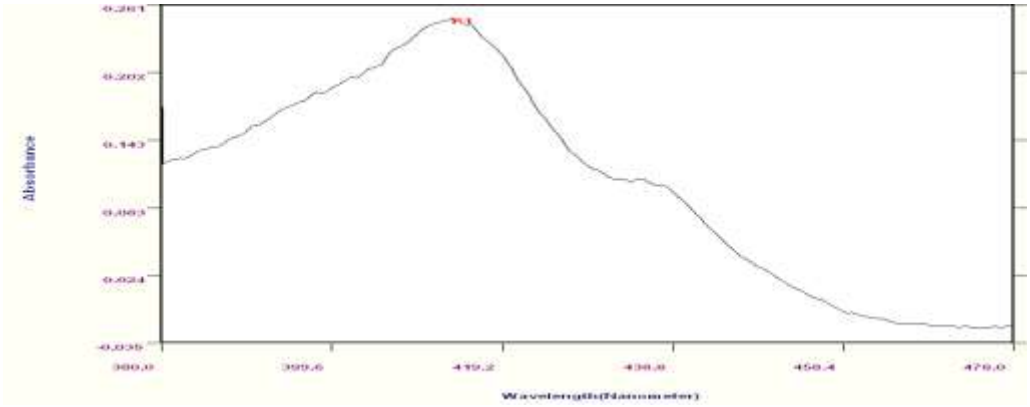
Gas Chromatography mass spectroscopy analysis was carried out in ethanolic extract of blepharisrepens (vahl) Roth .The total ion chromatography (TIC) of ethanol extract, showing the GC-MS profile of the compounds. In NMR spectrum, gives Yield: 2.25g; MP 128-130^oC; H^1 NMR ($CDCl_3$): δ 0.96-0.97 (s, 6H, $2CH_3$), 1.37-1.38 (m, 32H, $16CH_2$), 5.48 (s, 2H, 2CH). MS (m/z) 280 (M^+).



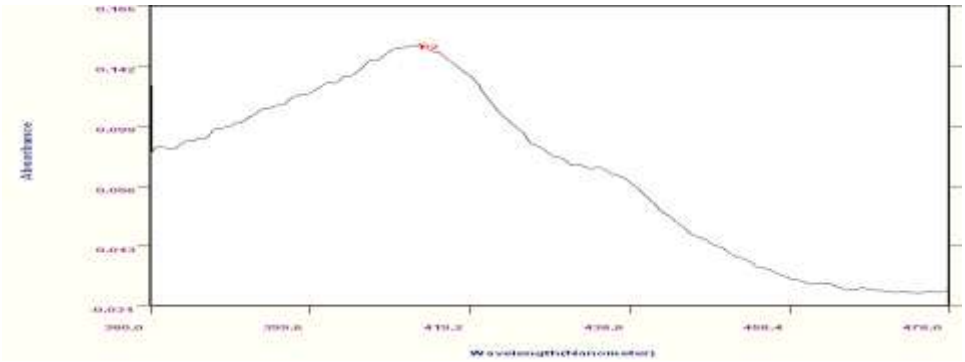
413.6nm, absorbance = 0.153



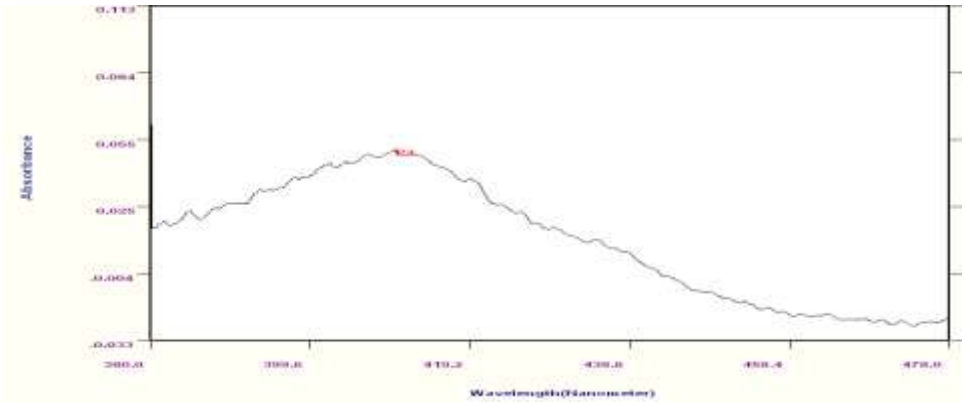
413.4nm, absorbance = 0.248



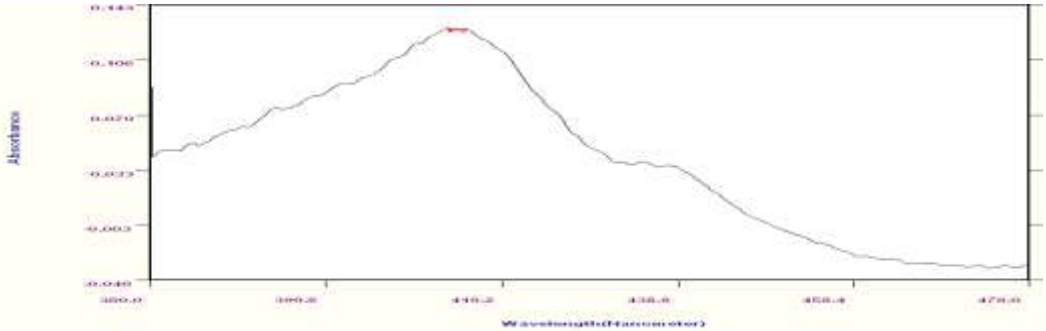
413.0nm, absorbance = 0.157



413.0nm, absorbance = 0.046

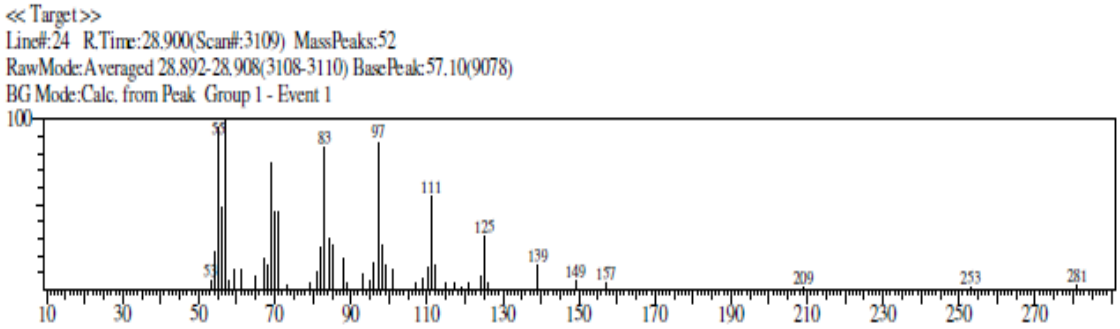


413.2nm, absorbance = 0.127



STRUCTURAL ELUCIDATION OF ISOLATED COMPOUND.

GC-MS



Formula: C20 H40 Mol. Weight: 280

Compound Name: 9-Eicosene

CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH=CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3

1 2 3 4 5 6 7 8 **9** 10 11 12 13 14 15 16 17 18 19 20

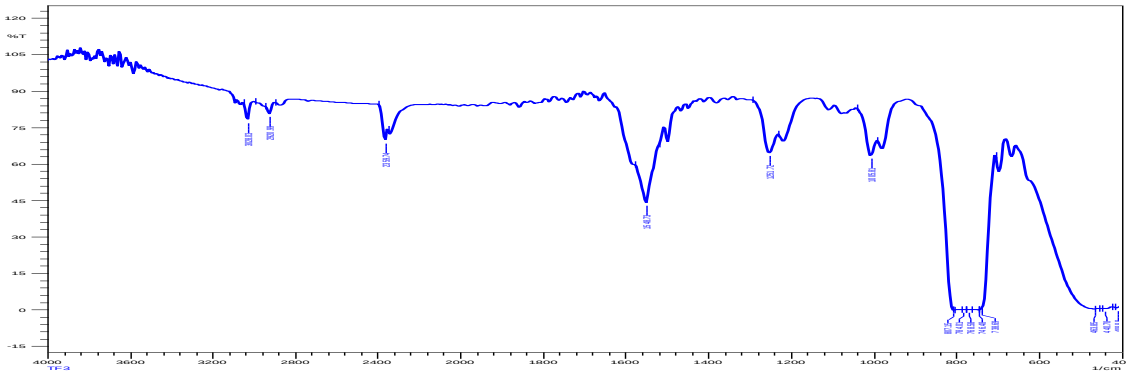
Fragment Peak	Fragment	Pattern
M-27	CH=CH_2	280-27 = 253
M-14	CH_2	139-14 = 125
		125-14 = 111
		111-14 = 97
		97-14 = 83
M-42	-CH_2-CH_2-CH_2-	97-55 = 42
M-71	-CH_2-CH_2-CH_2-CH_2-CH_3	280-209 = 71
M-140	CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH=CH_2	281-140 = 139

Elucidation

- M-27 fragment indicate CH=CH_2 which is unique for terpenes
- Numerous M-14 peaks indicates –CH_2- methylene groups
- M-42 fragment indicates large fragment of -CH_2-CH_2-CH_2- which shows the presence of larger fragment.
- M-71 fragment indicates the presence of terminal methyl and methylene group
- M-140 fragment indicates the presence of = bond at 9th position CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH=CH_2

Hence the isolated compound for the given spectrum of GC-MS indicates the presence of **9-EICOSENE**

FT IR SPECTRUM ANALYSIS



BAND	FREQUENCY	COMPOUND
1	3028 CM^{-1}	Alkyne C=H Stretching
2	2920 CM^{-1}	Aliphatic C-H Stretching
3	1548 CM^{-1}	Aliphatic C=C stretching

Important IR frequencies indicates presence of CH=CH, C-H of CH_2, AND C=C of monoterpenes.

CONCLUSION

Structural elucidation of the compound using GCMS and IR Spectrum indicates that the compound may be **9-eicosene**.

DISCUSSION

The ultraviolet spectroscopy method was used very much useful for identification of unsaturated bonds present in a plant component, which can be used to distinguish between conjugated and non-conjugated system. Using the principle of absorption maxima, the structure of compounds can be deduced. Gas chromatography, mass spectrometry (GC-MS) method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Application of GC-MS includes drug detection, fire investigation, environment analysis, explosive investigation and identification of unknown samples. The presence of terpenoids and flavonoids, fatty acid in EEBR and its fractions shows the pharmacological properties of the plant. In the presence study, GC-MS data showed saturated and un saturated fatty like hexadecanoic acid (Anti-inflammatory) linoleic acid, mono terpenoid like Eicosene (antidiabetic, anticancer) benzene dicarboxylic acid, cyclooctasiloxane, Octadecene in EEBRs and its fractions of blepharisrepens(vahl) Roth. In all, 30 compounds were present in EEBR and its fractions. In HPTLC technique gives a valuable tool for reliable identification .This method was emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations. In the present study, EE and its fraction showed peaks respectively in chromatogram which was produced by HPTLC. Therefore, HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and The spectral analysis will help the manufacturer for quality control and standardization of herbal formulation. Such type of analysis were useful in differentiating the species from adulterants and other sub species and the result of this study will become the fingerprint of this plant and it may act as a biochemical marker which will be useful for pharmaindustry.

CONCLUSIONS

Spectral analysis is useful in differentiating the species from the adulterants and the results of this study may act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

ACKNOWLEDGMENT

The authors are thankful to Dr.Munirajah, M.tech Bolton), Chairman, JKKMMRF college of Pharmacy, B.Komarapalayam. and Dr.N.Senthil kumar, M.pharm, Ph.D, Dean &Principal, JKKMMRF college of Pharmacy, B.Komarapalayam, Nammakal district, TN, India. for providing facilities to carry out this work.

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