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Characteristic Change in Activity of *Lantana camara* Tripene

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The bioactive molecule tripene of *Lantana camara* exhibited therapeutic applications. It was separated by vaccum distillation followed by gel chromatography procured 16-18 h extraction time. Exhibited single of pure tripene at 4.693 and yield of tripene was 0.13, 0.27 and 0.24 mg for the year 2013, 2014 and 2015, respectively. Characteristic change of eluted fractions assessed through TLC, HPLC, UV and FTIR spectrophotometer observe tripene approach for further application.

Keywords: *Lantana camara,* Triterpene, Pharmacological activity, Behavioural activity.

INTRODUCTION

A renowned notorious weed *Lantana camara* L. (Verbanaceae) also known as wild or red sage. *It* is a perennial shrub, brought to India some 80 years ago from South America. Popular ornamental garden *Lantana camara* has become exotic and spread to different regions of the country [1]. It's constituent numerous secondary bioactive metabolites that exhibited therapeutic and traditional significance [2]. The leaf extract of *Lantana camara* is rich in different types of plant secondary metabolites such as terpenoids, steroids and polyphenols including flavonoids, tannin [2,3]. Bioactive metabolites isolated from *Lantana camara* leaves namely tripene/triterpenoids possess, anti-inflammatory [4,5], antipyretic activity [4], antifilarial activity [6], antitumor activity [7] antithrombin activity [8], antimicrobial activity [9], oxidizing activity [10] anticancer activity [11,12]. Researchers have studied the potential of bioactive triterpenes in alcoholic extract [12-15]. The main chemical constituent *i.e.*, tripene acts as promoting agent [16] and used for the treatment of chicken pox, measles, asthma, ulcers, swellings, eczema and high blood pressure [2]. Triterpene also exhibits the anticoagulant and anti-inflammatory activity and has potential to inhibit the aggregation of proteins [5,15]. Therefore, the present study focused on the extraction of tripene from *Lantana camara* leaves by conventional methods and to observe the change in behaviour of active molecule/tripene throughout the methodology for the perspectives of it in further industrial application as well chemotherapy of life. Quantification of tripene was carried out by spectrophotometric method and monitored tread by TLC, UV-visible and FTIR spectrophotometer without any further treatment. The activity of tripene was optimized with the aid of different factors such as extraction time. Optimal conditions of tripene provided good alternatives for the further pharmacological applications.

EXPERIMENTAL

Collection of plant leaves: A *Lantana camara* healthy fresh leaves was collected from the campus of Vidya Prasarak Mandal's B.N. Bandodkar College of Science, Thane (W) India in month of September 2015, September 2014 and 2013 to compare yield. Segregated leaves were washed, dried. Pulverized powder stored in self sealing bags until used.

Isolation of tripene from *Lantana camara* Linn leaves: *Lantana camara* leaves powder (100 g) was treated with increase in volume of methanol and serially refluxed for 0.5 to 18 h. Solvent was removed under vaccum (13-14 mm/Hg and distillation temperature up to 58 °C). Obtained residue was suspended in distilled water after filtration. The residue was dissolve in a methanol:water (1:7) mixture and extracted with ethyl acetate (2 × 25 cm^3) and with *n*-butanol ((2 × 25 cm^3). The ethyl acetate layer was concentrated under reduced pressure to get crude tripene. Each step was monitored timely with TLC and qualitative test. Formulated tripene was measured from 0.5 to 18 h to find out extraction time. The whole process is repeated to observe behaviour/yield active compound/tripene collected in month of September 2013, September 2014 and September 2015.

Column chromatography of *Lanatana camara* tripene: Crude tripene was loaded over silica gel (30 g) column (60-120 mesh) and 32 elutions were collected using chloroform: methanol (9:1) as eluting solvent. The active fraction 12^{th} was

rechromatogram since it showed five spot on TLC. After rechromatography active fractions (19, 22 and 23) were pulled, concentrated and rechromatography was carried out using *n*-hexane with successive increasing amount of acetone. Different fractions were monitored with TLC using ethyl acetate:methanol: water (9:3:1) throughout the process. Purity of active compound (50 µL) checked on HPLC using C-18 column having mobile phase A-0.1 % trifluoroacetic acid in water (80:20) and Mobile phase B-acetonitrile on Alliance 2695 model.

Characterization of *Lantana camara* tripene

Qualitative test for triterpene: Active fraction from silica gel column (50 µL) was treated with 0.5 cm^3 chloroform and warmed for about 0.5 h and 2-3 drops of concentrated H_2SO_4 was added and mixed well. The presence of triterpenoid was observed by change in colour [16].

Spectrophotometric study: The active fractions from silica gel column chromatography were explored to UV-1800 Shimadzu spectrophotometer to observed exact λ_{max}. The observe spot on TLC were dissolved in diethyl ether. UV and FTIR (Thermo scientific Nicolet iS5) were carried out for each fractions monitored from 190 to 1100 nm wavelength.

RESULTS AND DISCUSSION

Lantana camara Linn. is renowned weed for therapeutic activity and economical wellness. Hence, role of its secondary metabolites need to facilitate in drug delivery system. Attention of present research especially on the change in behaviour of active molecule throughout the processing. Behavioural activity depends on seasonal changes and attribution of time, volume of solvents and effective concentration of reactant used to achieved the tripene yield (Tables 1 and 2). Organic solvents contributed exceptionally low yield showing very little difference in physicochemical properties of pentacyclic tripene [9,10]. Problems with the separation of the complex triterpene mixtures result in a very small quantities, usually a few milligrams of individual compounds being isolated. Thus, for the structure elucidation non-degradative techniques are preferred. The compound is being a new structure or to confirm its similarity to the known standards. TLC analysis may be of a great help along with UV, IR.

Ethyl acetate does not able to extract tripene completely consequently to achieve tripene the methanol-water mixture was used. Extracted yield (10.34 g) showing its λ_{max} 228 nm (Fig. 1A) that was treated with butanol and ethyl acetate procured producible results (0.95 mg) of tripene. The qualitative test was conducted for crude compound showing red colour after adding of H_2SO_4.

The obtained crude tripene is 0.95 mg from 100 g of dried leaves powder in 18 h refluxed time. Serially refluxed and process 100 g of leaves dried powder treated with as per process shown increase in yield of product till 16 and 18 h (Table-1).

TABLE-1
EXTRACTION TIME OF *Lantana camara* Linn.

Time (h)	Colour	Weight (mg)
0.5	Greenish colour liquid	–
1	Green brown colour	–
2	Green brown colour	0.12
4	Faint brown colour	0.16
8	Brown colour	0.23
10	Brown colour	0.23
12	Brown colour	0.24
14	Dark brown colour	0.48
16	Dark brown colour	0.53
18	Dark brown colour	0.95
36	Black colour	0.54
40	Black colour	0.54
48	Mask clear solution	0.51

There is no change in product and colour of the compound up to 40 h after the product is drops its quantity and characteristics including colour. At 48 h the obtained product is very dark and sticky mass can be removed out from the dish. The maximum extraction time is 16 to 18 h was preferred for characterization.

The process of separation of tripene was carried out for the collected in the year serially September 2013, 2014 and 2015 to ensure yield that is shown in Table-2. Ratio of crude to pure compound isolated consistently shown negligible difference, which may be due to geographical activity and human analytical error. HPLC of fraction 19 showed single peaks at 4.693' and 4.980' min (Fig. 2).

After crude extract (0.95 mg) was loaded on column chromatography and rechromatogram, the active fraction 12^{th} was monitored by TLC. Five yellowish coloured spot observed for fraction 12^{th} having R_f values are 0.35, 0.54, 0.76, 0.85, 0.93 and respective UV spectra was 294, 416, 236, 235 and 211 nm. After rechromatography of fraction 12^{th}; procured three active fractions (19, 22 and 23). Fraction 19 and 23 procured the single spot (R_f 0.97 and 0.45) and fraction 22 showed three spot (R_f 0.36, 0.50, 0.84) on TLC. The fraction 12^{th} was segregated in three active fractions on rechromatography may be due to the matrix may functionalized affinity support works on spacer and support matrix which eliminates toxic reagents which used during isolation and reachers occurs if any.

Crude tripene showed λ_{max} 228 nm while after purification it showed fraction 19 (λ_{max} 409 nm), fraction 22 (λ_{max} 235 nm) and fraction 23 (λ_{max} 232 nm), respectively. The fraction 22 and 23 may be the bifurcation of elution. Hence, the observed λ_{max} 235 nm and λ_{max} 232 nm. This may be due to ($\pi \rightarrow \pi^*$) transition which is stabilized by H-bonding in diethyl ether. It lowers the energy and shift wavelength from 232 nm to 235nm (Fig. 1B). Crude tripene showed after purification it showed fraction 12^{th} (λ max 416 nm) and rechromatography fraction 19 (λ_{max} 409 nm) showed the divergence of elutions. The observed λ_{max} 416 nm and λ_{max} 409 nm it showed the π^* level will be higher and $n \rightarrow \pi^*$ transition will be higher energy.

TABLE-2
SEASON WISE EXTRACTION YIELD OF CRUDE AND PURE ACTIVE COMPOUND FROM 100 g OF *Lantana camara* Linn.

Year	Crude (mg)	Pure (mg)	Ratio	R_f value	UV (nm)
September 2013	0.83	0.24	0.29	0.72, 0.73, 083	435
September 2014	0.95	0.27	0.28	0.76 0.85	409
September 2015	0.53	0.13	0.25	0.36, 0.45, 0.50, 0.84, 0.97	409, 235, 232

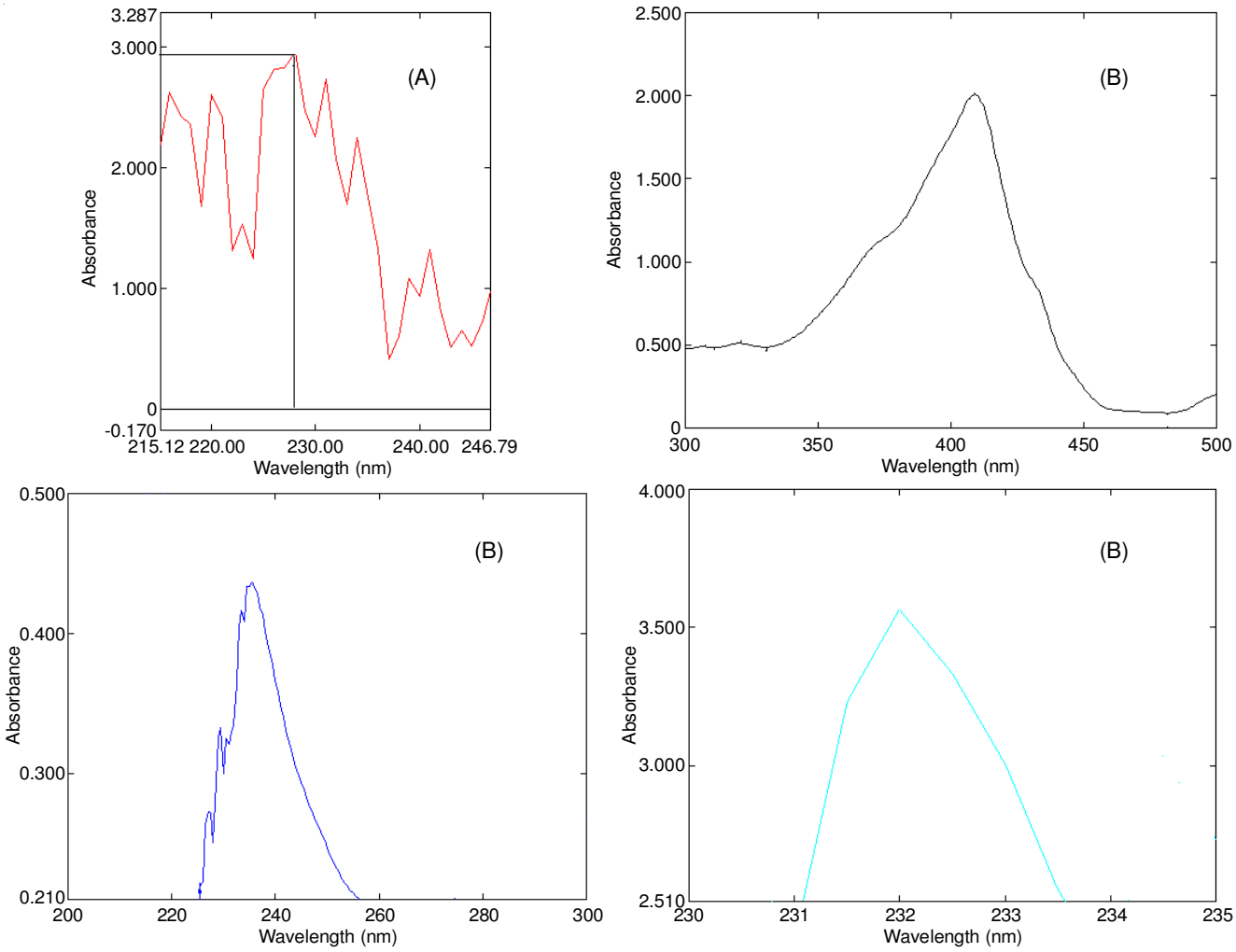

Fig. 1. UV maxima of *Lantana camara* leaves (A) crude active fraction/tripene before column chromatography showed λ_{max} 228 nm (B) after rechromatography showed fraction 19 (λ_{max} 409 nm, fraction 22 (λ_{max} 235 nm) and fraction 23 (λ_{max} 232 nm)

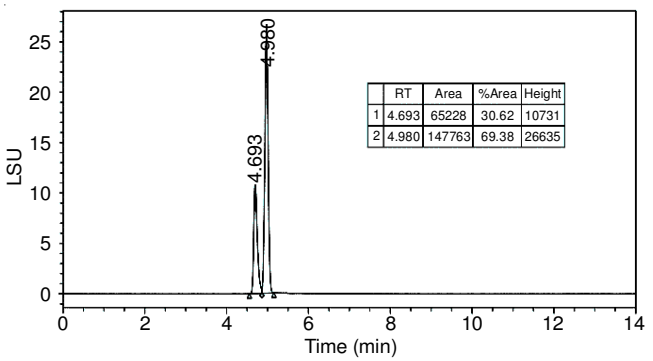

Fig. 2. Pure tripene compound (fraction19 50 µL) provoked single peak on HPLC

Hence, shifting of lower energy (red shift) to higher energy (blue shift). It is higher energy and shift wavelength from 409 nm to 416 nm. When neutral solvent was used as solvent system, tripene has not exhibited any identifiable spot in iodine and also under long wavelength and short wavelength in UV chamber while ethyl acetate:methanol:water (9:3:1) was procured yellowish colour spot. The colour observed in visible light and the ultraviolet fluorescence found more distinguishing. After vaccum distillation, the fraction 22 observed brown greenish in colour having R_f value 0.85.

FTIR after and before column chromatography: The intermolecular H bond dose not takes place as the molecules are widely separated. Increasing concentration of solvent for extraction was produce sharp band at frequency 2927 cm^{-1}; due to O-H stretching in vaccum distillation fraction. The bending vibration of methyl group occur at 1462 cm^{-1} and 1467 cm^{-1} (fraction 22). The experiment reveals that the band 1462 cm^{-1} and 1467 cm^{-1} are due to α-methylene group while 1456 cm^{-1} represent β-methylene group (Fraction 19). Confirming the compound is aromatic in nature substitution pattern of the ring C-H bond in the region 668 cm^{-1}. Strong absorption band at the region around 1700 cm^{-1}. Consistently, all fractions due to C=O stretching indicates that the compound may be ketone, aldehyde, ester *etc*. At frequency 1716 cm^{-1} pentanone shown C=O stretching vibration in conjugated ketone. The C-O stretching in vaccum distilled fraction at 1232 cm^{-1} increases the resonance character of double bond in the ring, which is disappearance the frequency in fraction spectra since tripene/triterpenoids have diverse chemical structures and bioactivities [10].

FTIR spectrum before column chromatography: 3695 cm^{-1} (O-H stretching (acid free), 3683 cm^{-1} (O-H str), 3654 cm^{-1} (O-H str), 2927 cm^{-1} (CH_2, CH_3 str, =CH stretching), 1710 cm^{-1} (C=O stretching), 1606 cm^{-1} (C=C str, NH_2 bending), 1513

cm^{-1} (C=C aromatic, NO_2 gr), 1462 cm^{-1} (CH_2, CH_3 bend), 1378 cm^{-1} (CH_2,CH_3 bend, C-O-C stretching ether), 1232 cm^{-1} (C-OH stretching acid).

The FTIR spectra were carried out identify the possible biomolecules in selected plant *Lantana camara* Linn. The IR spectrum of the lantana tripene peak was observed as 1750 cm^{-1} frequency of a C=O observed. Alkane group observed in 2900 cm^{-1} frequency and ether group 1378 cm^{-1} O-H stretching acid free 3695 cm^{-1}, =CH str 2927 cm^{-1}, C=C 1600 cm^{-1} and OH, -C-O stretching acid is a1232 cm^{-1} frequency observed.

Disappearance of strong intensity absorption 1378 cm^{-1} band in the rechromatogram. However, these bands are rarely useful they overlap other stronger absorption.

FTIR of after column chromatography

Fraction 19: 3504 cm^{-1} (aromatic O-H), 2925 cm^{-1} (alkanes strong intensity aldehyde weak intensity), 2360 cm^{-1} (alkanes m-w intensity, nitrile), 2341 cm^{-1} (C=C), 1733 cm^{-1} (conjugated aldehyde, C=O), 1699 cm^{-1} (aldehyde, amide), 1652 cm^{-1} (C=O ester, alkenes), 1635 cm^{-1} (alkenes C=C, amide), 1558 cm^{-1} (C=C, N=O), 1540 cm^{-1} (R-NO_2 group strong intensity), 1507 cm^{-1} (C-Cl, C-N, C-C, C-O R-NO_2 group strong intensity), 1457 cm^{-1} (CH_3-CH_2 bend), 668 cm^{-1} (C-Cl, C-O, C-N, C-C).

Fraction 22: 3773 cm^{-1} (O-H), 3457 cm^{-1} (C-H, N-H, O-H bonding), 2924 cm^{-1} (C-H str), 2359 cm^{-1} (alkanes m intensity, nitrile), 1738 cm^{-1} (aldehyde, ketone, C=O g), 1659 cm^{-1} (C=O amide, C=C alkenes isolated), 1640 cm^{-1} (C=O, alkanes isolated), 1534 cm^{-1} (C-C, C-N, C-O, N-H str vib), 1502 cm^{-1} (C-C, C-N, C-O, amine), 1467 cm^{-1} (CH_2 bend), 1414 cm^{-1} (CH_3 bend), 1351 cm^{-1} (C-C, C-N, C-O, C-Cl), 1022 cm^{-1} (C-O alcoholic, ester, ether, C=O anhydride, C-N amine), 669 cm^{-1} (C-C-C medium).

Fraction 23: 3441 cm^{-1} (C=O band, overtone C=O str), 2952 cm^{-1} (C-H str), 1716 cm^{-1} (ketone), 1699 cm^{-1} (ketone), 1653 cm^{-1} (C=C str), 1558 cm^{-1} (N=N, C=O), 1540 cm^{-1} (N=N, aliphatic N-O stronger), 1507 cm^{-1} (C-Cl, C-N, C-C, C-O R-NO_2 group strong intensity), 1489 cm^{-1} (C=C aromatic), 1457 cm^{-1} (carbonate ion), 1378 cm^{-1} (CH_2, CH_3 bend sp^3), 1233 cm^{-1} (C-O-C strong, C-O-H), 1180 cm^{-1} (C-N str, O-H bending), 1038 cm^{-1} (C-O ester, ether, alcohol), 679 cm^{-1} (=C-H bending alkenes strong), 662 cm^{-1} (CO_2 medium, NH_2), 473 cm^{-1} (CHO, C-N).

Infrared region 4000-650 cm^{-1} have large number of bands. The possibility that two different compound shows same spectrum. The pure sample of TLC gives different infrared spectra must be different. It they passes supreme spectra there they represent same spectra. The region to the right of 1500-600 cm^{-1} is usually complex showing stretching and bending band. Hence, correlation of an individual bend with specific functional group of offends difficult. (*Lantana camara* active fraction after vaccum distillation followed by column chromatography shows bands 1539, 1463, 1378, 1075, 797, 721 and 465 cm^{-1}.

Conclusion

Yearly assessed bioactive molecule/tripene from *Lantana camara* leaves produced similar yield with negligible difference. Change in extraction time in relation to colour and R_f values, UV spectra, arrival and departure of FTIR exhibits richness of tripene that assists the diversified pharmacological application of active fraction/tripenes in processing.

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