Research Article

Isolation, Purification and Characterization of Triterpenes from *Lantana camara*

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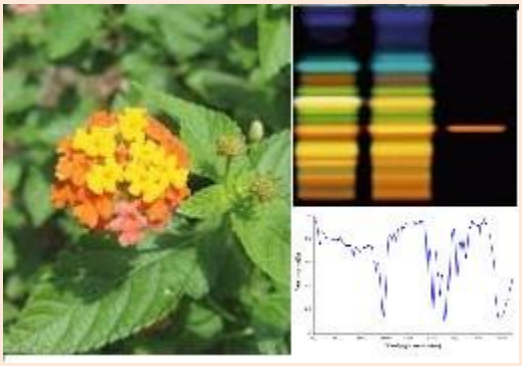
Abstract

Numerous waste lands are source of various novel bioorganic compounds. *Lantana camara* contains several bioactive molecules having antimicrobial, fungicidal and insecticidal properties and acts as alternatives in some therapeutical markets. Due to demand supply gap it is essentialto extract the bioactive substances from natural sources. Hence, present paper focuses on the separation of triterpenes from *Lantana camara* leaves by vacuum distillation followed by column chromatography and characterization by UV, IR, TLC, HPTLC, HPLC and MS.



Keywords: Phytochemistry, *Lantana camara*, Triterpenes, Spectroscopy.

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Introduction

Lantana camara is one of the natural biomass sources from nature. It belongs to verbenaceae family and found in tropical and subtropical region of world [1]. *Lantana camara* Linn contain wide array of compounds exhibiting diverse range of bioactivity [2]. Lantana's aromatic flower clusters are mix of red, orange, yellow or blue and white florets. Leaves are ovate or ovate-oblong, acute or sub-acute, crenate-serrate, rogues above, scabrid on both sides [3].

Major bioactive metabolites isolated from L. camara leaves namely triterpenoids possess antitumor activity [4], antithrombin activity [5], anti-inflammatory and antipyretic activity [6] and anti-filarial activity [7]. Lantadenes are the major triterpenoid constituent of L. camara. Mixed preparations from L. camara leaves has been found to exist in two molecular form – Lantadene A and Lantadene B, which possess a number of medicinal properties [8-14].

The main objective of the present study is to isolate these triterpenes from *Lantana camara* leaves by extraction, column chromatography followed by characterization using UV, IR, TLC, HPTLC, HPLC and MS.

Experimental

Materials and Reagents

The leaves of *Lantana camara* were collected from the campus of VidyaPrasark Mandal's B.N.Bandodkar College of Science campus, Thane (MS), India. Methanol, chloroform, hexane, ethyl acetate and formic acid were of analytical grade procured from Merck Specialities Pvt. Ltd. Mumbai. Acetonitrile HPLC grade was obtained from Hexon Laboratories, Mumbai. The spectrometer systems used were Cary 60 UV-Vis Spectrophotometer, Cary 630 FTIR spectrometer (Agilent technologies, Santa Clara, CA, USA) and API 2000 LC/MS/MS system (AB SCIEX, USA).The HPTLC system used consisted of CAMAG TLC Scanner 4 equipped with CAMAG Linomat 5 spotter and

CAMAG Reprostar system for photo-documentation. The HPLC system consisted of an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, thermostated autosampler, column compartment and photodiode-array detector.

Methods

Leaves were dried, powdered, sieved through mesh and preserved in airtight containers at room temperature for further analysis.100 g of leaf powder was subjected to reflux condensation using 500 ml methanol for 3 hours. The solvent was further distilled under vacuum (13-14 mm/Hg and distillation temperature up to 60oC) to get concentrated residue. Dark green residue was further suspended in 10 ml acetonitrile. The extract was filtered through Whatmann filter paper No.1 in dry stoppered test tubes and the filtrate was concentrated under reduced pressure to get crude lantadene which was further purified by column chromatography. Crude triterpene was loaded over silica gel column (60–120 mesh, 30g) and eluted using chloroform and chloroform–methanol (9:1) as eluting solvent. The fractions collected were analysed using UV/VIS spectrometer and TLC followed by IR, HPLC and MS analysis. HPTLC analysis was performed by spotting TLC – sample volume - 10 µL sample volume on TLC plate with band and space width of 8 mm and 7 mm and mobile phase saturation time of 30 minutes. The mobile phase used was Hexane- methanol- ethyl acetate (85:10:5). Liebermann Burchard reagent was used for derivitisation and scanning was performed using mercury lamp at 366 nm. For HPLC analysis, Phenomenex Luna C18 column (4.6 × 250 mm) (Phenomenex, USA) packed with 5 µm diameter particles was used. The solvent system: 0.25% formic acid (%, v/v) (A) and Acetonitrile (%, v/v) (B) with an isocratic elution ratio of 20:80 for 10 minutes, flow rate: 1.5 ml/min; injection volume: 10 µL, column temperature: 35oC and detection: 192 nm was used for analysis.

Results and Discussion

The selected source for the present study is shown in **Figure 1**. Number of researches gave the direction for the modification to the conventional study for the isolation of triterpene (**Figure 2**). The subjected dried leaves powder for isolation gave variations in the final product when it was repeated thrice. This may be due to seasonal change or laboratory conditions. At optimum conditions results obtained from the air dried *Lantana camara* leaves are represented. All bioactive compounds presented are compared with standard.

The UV analysis of standard Lantadene and crude triterpene extract showed absorbance maxima (λmax) at 192 nm (**Figure 3A**). HPTLC analysis of shows triterpene spot at Rf value – 0.82 in standard Lantadene and crude triterpene extract (**Figure 3 B**)

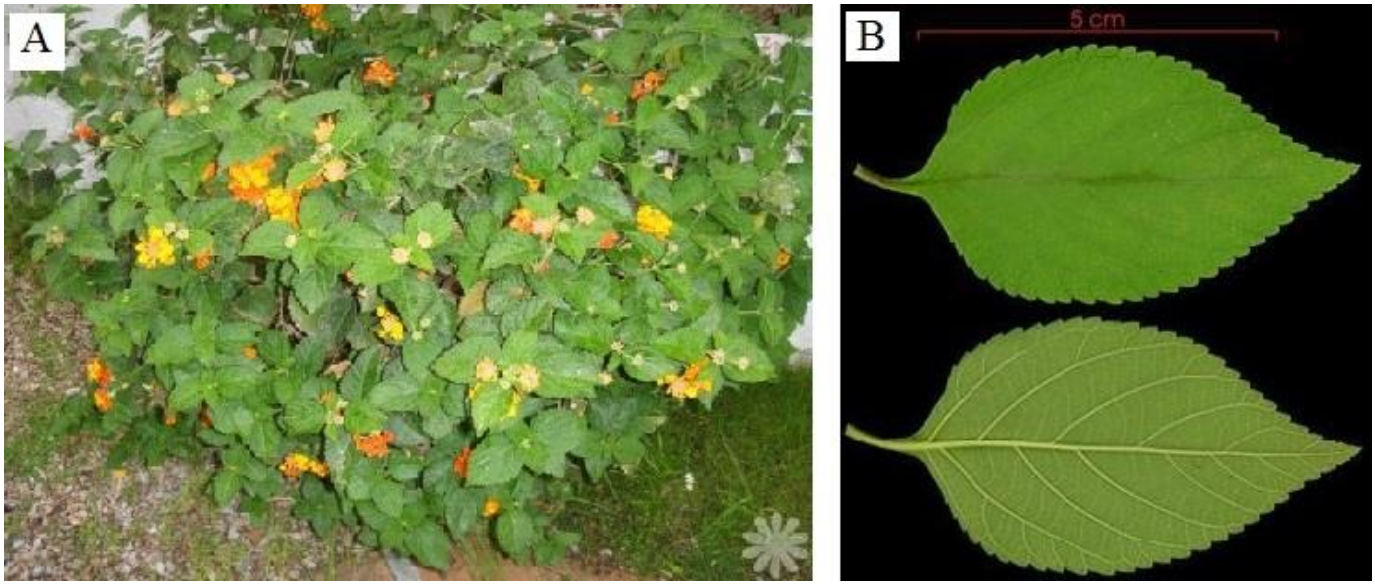

Figure 1 *Lantana camara* (A) whole plant (B) leaves.

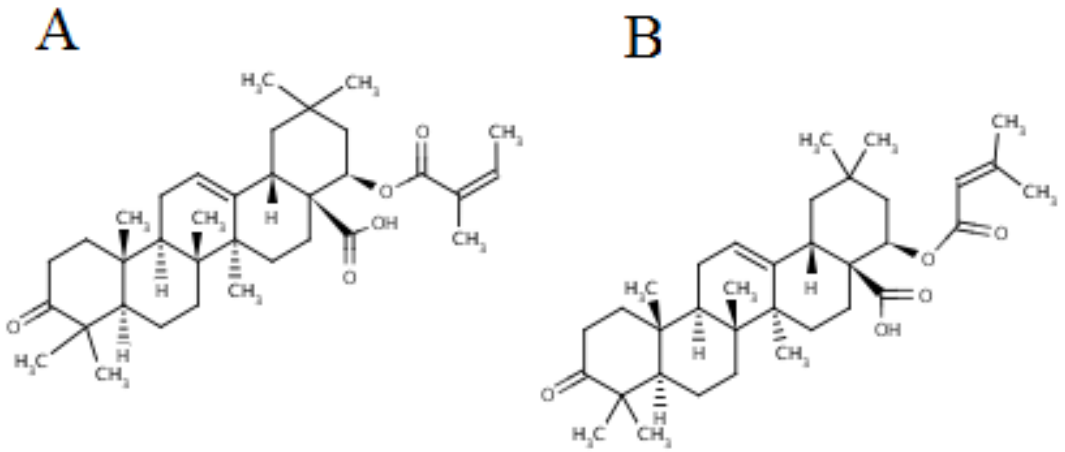

Figure 2 (A) Lantadene A; (B) Lantadene B.

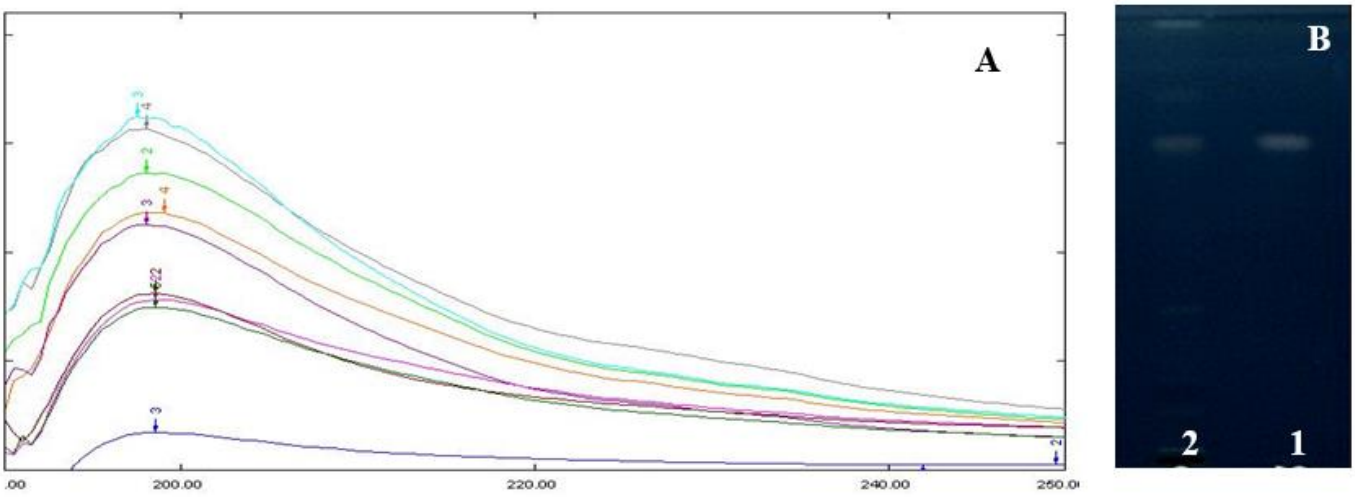

Figure 3 (A) UV spectra of standard Lantadene and crude triterpene sample; (B) HPTLC of Standard Lantadene (Lane 1) and crude triterpene sample (Lane 2).

The bands located in the high frequency region of the IR spectra of crude triterpene extract sample belong to O-H and C–H stretching modes. The characteristic- OH stretching vibration isexpected to appear at 3465 cm-1 (PM3), which is in good agreement with the standard data.The characteristic CH stretching vibrations of heteroaromatic structure are expected to appear in 2700-3100 cm-1 frequency ranges. These vibrations were observed at 2925 cm-1 in the standard and the corresponding bands were seen are given at 2928 cm-1 in the sample. In middle region, the H-C–H scissor predicted at 1457 cm−1 in standard is in reasonable agreement with the 1455 cm−1 observed in sample. (C–C–H) angle-bending mode observed at 1302 and 1396 cm−1 in standard and corresponding band at 1302 and 1394 cm-1 in sample FTIR spectrum (**Figure 4**).

The HPLC analysis shows peak at 1.9 minutes in standard Lantadene and crude triterpene extract sample (**Figure 5**). The molecular characterization performed by mass spectrometric analysis by direct probe method in positive polarity showed major peak (M+H)+ at 552.9 Da in the standard Lantadene and crude triterpene extract sample confirming the presence of Lantadene triterpenes in the sample (**Figure 6**).

Figure 4 IR spectra of (A) – Standard Lantadene; (B) Crude Triterpene extract sample.

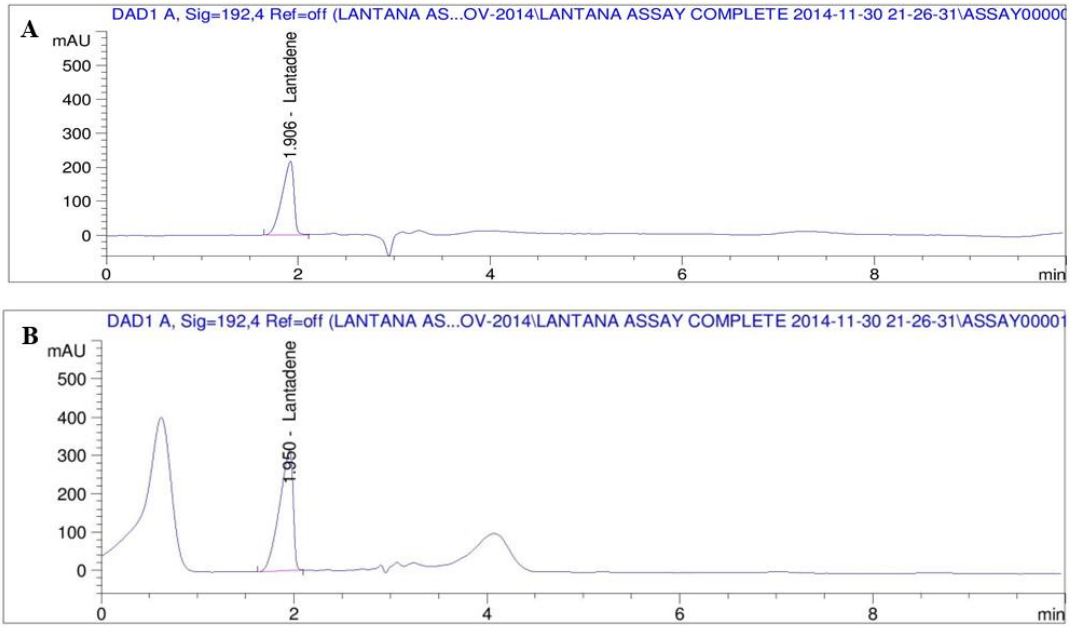

Figure 5 HPLC chromatogram of (A) – Standard Lantadene; (B) Crude Triterpene extract sample.

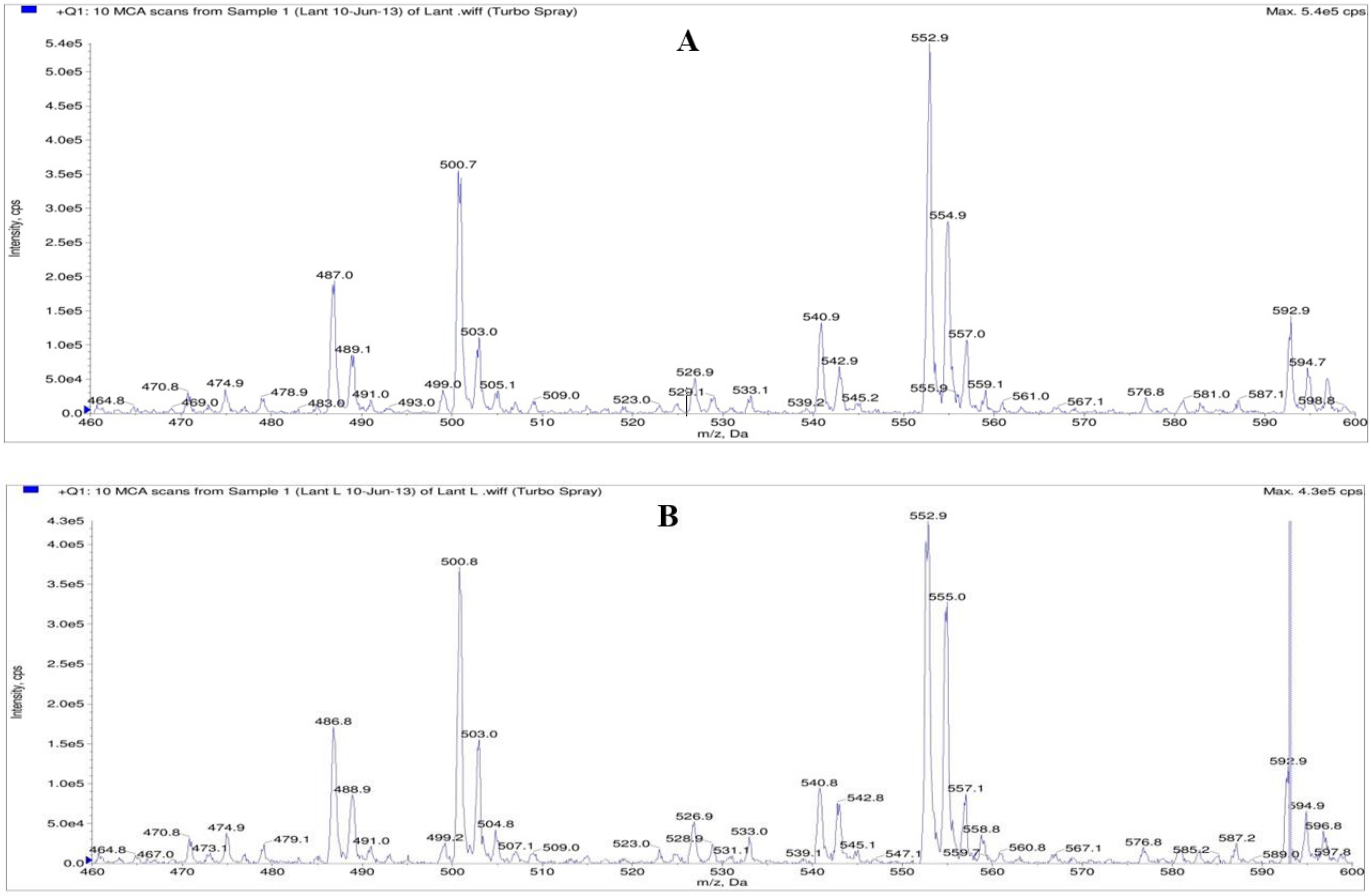

Figure 6 MS spectra of (A) Standard; (B) Crude triterpene extract sample

Conclusion

The developed method is useful for isolation, purification and characterization of triterpenes found in *Lantana camara*. The method does not require any elaborate treatment and tedious extraction procedure for isolation and purification. It is simple, precise and reproducible approach for further characterisation of extracted triterpenes.

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