TO ANALYZE LANTADENE FROM STEMS OF LANTANA CAMARA LINN. BY ALCOHOLIC REFLUX METHOD

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ABSTRACT : *Lantana camara L* provides a large amount of bioconstituents that are of interest to exploit for natural products in medical field. Some pentacyclic triterpenoids are known to have antibacterial, anti- inflammatory, antitumor or anti-AIDS activity. Lantadene isolated from Lantana camara L. belong to the oleanane series which have considerable application in drug discovery. Isolation of Lantadene can be done by alcoholic extraction of plant parts .However, many of these compounds are present in very small amounts and due to very little difference in their physicochemical properties, their isolation in sufficient amount is difficult .

Key words-bioconstituents, oleanane, pentacyclic triterpenoids,

INTRODUCTION:

Plants contain number of chemical constituents with varied pharmacological activities. Many potent and effective medicinal properties used for treating dreadful diseases have been isolated from various medicinal plants. Hence demand for medicinal herbal drugs is increasing day by day. So it is very clear that the study of the medicinal plants makes miracle in medicinal field. *Lantana camara* is considered as noxious weed used in folk medicine in many parts of the world The plant was reported to contain various compounds like triterpenoids, proteins, carbohydrates, lactones, furfural, flavonoids, amino acids, alkaloids, saponins, glycosides, tannins, steroids. Many of the traditional uses have been scientifically proved. [1,2,3,4,5,6]

A number of medicinal properties have also been reported of different parts of lantana, and during the past few years. Various research groups reported a number of chemical constituents from this plant, the majority of which are triterpenoids, including Lantadene (figure1&2) [11,12,13,14,16,17,18]

Recently, Lantadenes and other triterpenoids from this plant have been found to exhibit a wide array of pharmacological activities, including antitumor effects. These observations indicate that Lantadenes have the potential to be developed as an antitumor therapeutic agent [5,6] These compounds differ in the structure of the side chain attached at the C-22 position through an ester linkage, and there are indications that these structural variations may play an important role in their pharmacological activity[7,8,9,10]. However, many of these compounds are present in very small amounts and due to very little difference in their physicochemical properties; their isolation in sufficient amount is difficult. In the present paper chemical investigation of the stems from the orange and red flowering taxa was carried out.

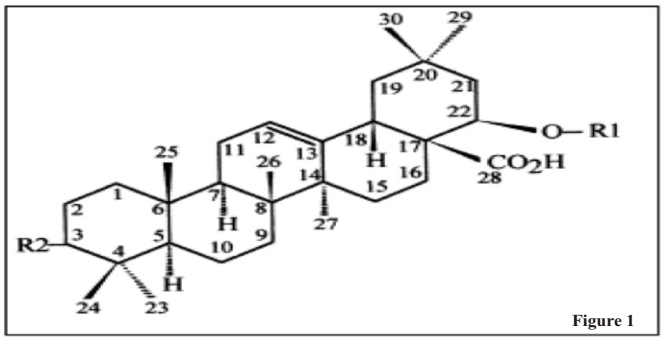


Figure 1

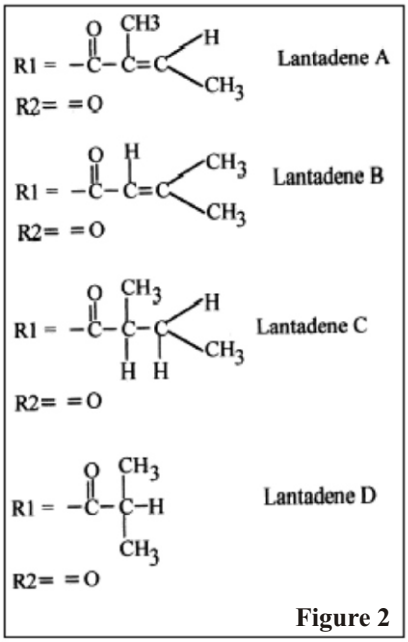


Figure 2

Collecting stems from *Lantana camara linn* :Stems from orange and red flowering taxa of *Lantana camara L*.was identified and collected from Vidya Prasark Mandal's college campus, Thane (MS) and were washed and dried under shed for 15 days. Stems are brown, hard and consisted fine hair on it. Stems are initially crushed and then powdered. Powder was packed in polythene bag and stored at room temperature. Powder was subjected to alcoholic solvent methanol[15] for the further process.

Extraction and isolation of Lantadene :

From stem powder : To 100g of *lantana* stem powder, 500 ml methanol was added and the mixture was extracted with boiling methanol for 3 hours. Colour of the mixture was light green color. Mixture was allowed to cool at R.T and then subjected to vacuum distillation to remove methanol from the mixture. Distillation was carried out under 13-14 mm/Hg and distillation vapor temperature was upto 58°c.The residue was suspended in 500 ml distilled water. The extract was separated by filtration through Whatman paper 1.Clear liquid was extracted with ethylacetate (2 × 25 ml) and with n-butanol((2 × 25 ml) The light green ethylacetate layer was concentrated to get crude Lantadene 0.052 gms. Ethyl acetate, n-butanol and aqueous fractions were analyzed by HPLC.

For isolation of Lantadene, partially purified Lantadene fraction was subjected to column chromatography. Chloroform methanol (9:1) was used as eluting solvent and was chromatographed over silica gel column (30 g, 60 120 mesh).

Second fraction was loaded on a silica gel column with n-

Hexane by increasing amount of acetone. Different fractions were collected. Among these greyish eluent was checked with TLC. TLC system $CHCl_3$ CH_3OH; 9.8:0.2
Rf 0.72
Solvent was removed and Lantadene (0.013 g) as whitish solid was obtained. Melting point was checked (It doesn't gives sharp melting point.)

Methods for the identification of Lantadene (pentacyclic triterpenoids)

1. Ethyl acetate extract (300mg) was mixed with 5 ml chloroform and warmed for 30 minutes . Few drops of concentrated sulphuric acid was added and mixed well. The appearance of red colour indicates the presence of triterpenoids
2.I.R.spectra shows peaks,3465 cm^{-1}(-OH),,3076.5cm^{-1} (cyclic),2925cm^{-1}(aliphatic C-H),2536 cm^{-1}(_COOH),1925cm^{-1}, 1834 cm^{-1}(carbonyl group,3 Keto), 1455 cm^{-1}(aliphatic double bond),1303cm^{-1}(O-C=O) linkage, 997.3 (Trans olefin).

3. HPLC method-Standard compound was purchased from chemical agent.

The specifications of HPLC ELSD:

Mobile phase A - 0.1 % Trifluoroacetic acid in water
Mobile phase B - ACN
Elution mode (Isocratic/Gradient) Gradient
Mobile phase composition
Column- Sunfire C-18
Dimensions -250 X 4.6 mm, 5 µm
Column Temp. - 35 °C
ELSD Conditions
Sample preparation procedure
GAIN: 10, Drift tube temp: 50°C, Gas pressure: 50 psi
2000 ppm concentration of sample solution in ACN: water (80:20)
Injection volume- 5 µl.
Calculation for area normalization
System Make Waters
Model Alliance 2695

4. Retention time of Lantadene (pentacyclic triterpenoids) is 4.98 minutes and HPLC of Standard, ethylacetate extract, aqueous extract and n-butanol extract are listed below-

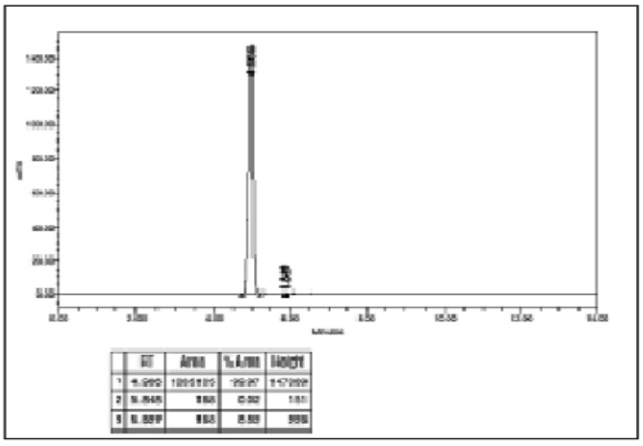


Figure 3 Standard compound shows single peak at 4.98 minutes

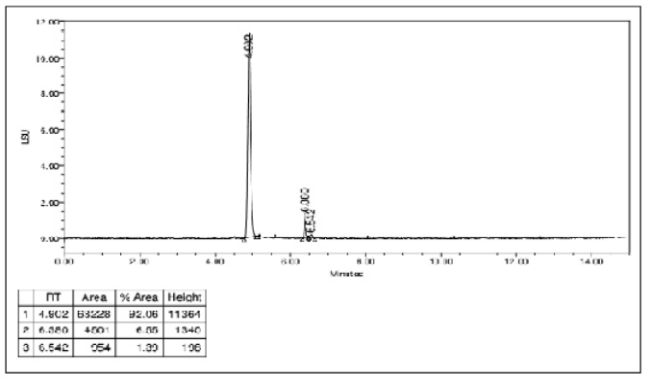


Figure 4 Aqueous extract shows peak at 4.98 minutes

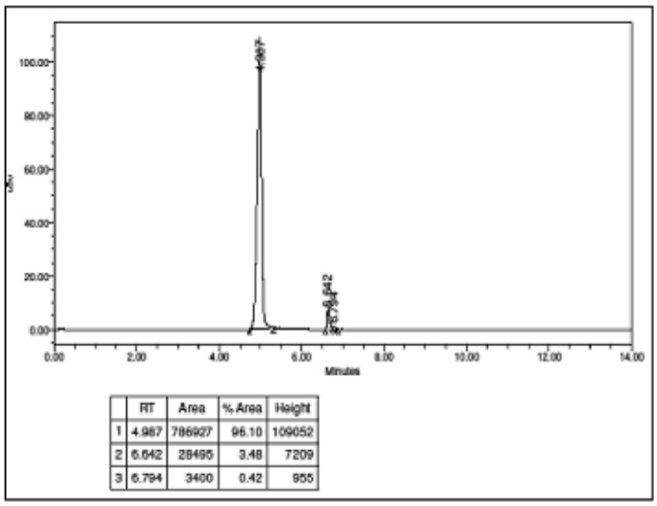
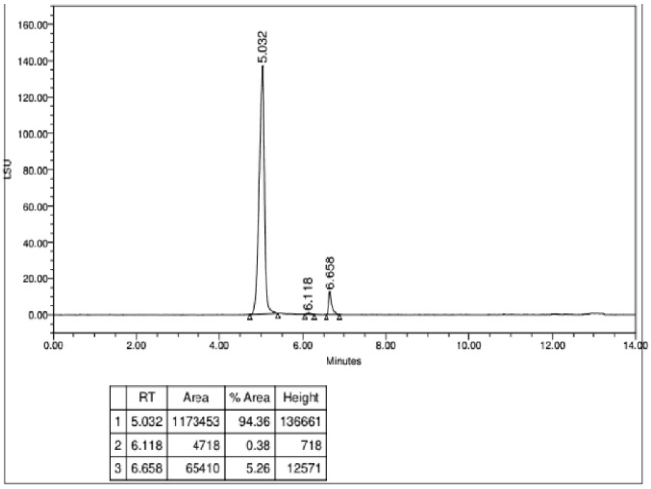


Figure 5 Ethyl acetate extract shows peak at 4.98 minutes



RESULTS AND DISCUSSIONS :

Extraction of powdered stems (*Lantana camara Linn.*) by alcoholic reflux method and column chromatography gives low yield of Lantadene (0.00013%w/w) (figure 3&7). Further there is very little difference in physicochemical properties of pentacyclic triterpenoids gives difficulty in getting pure form of Lantadene.
Standard Lantadene (figure 3) showed retention time at 4.98

minutes and gives above 99% purity by HPLC method using ELSD. Ethylacetate (figure5) and aqueous extract (figure 4) showed same retention time as that of standard, which indicates presence of Lantadene in the extract further. n-Butanol extract (figure 6) gives peak at the retention time 5.032 minutes. This is different from the retention time 4.98 minutes (figure 3). Ethylacetate was used to extract Lantadene from the methanol-water mixture was not able to extract Lantadene completely from the mixture. This can be overcome by selecting proper organic solvents for the reflux, extraction and fractional crystallisation may improve yield of Lantadene.

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