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ISOLATION AND CHARACTERIZATION OF CHEMICAL CONSTITUENTS OF AERIAL PARTS OF *LANTANA CAMARA*

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Abstract

Lantana camara belongs to the family Verbenaceae, is an evergreen plant found throughout India. Traditionally it has been used in treating various ailments and they were supported by scientific data's. Various literatures have reported the phytoconstituents present in the aerial parts of *Lantana camara*. In our present study we have selected to isolate compound from toluene extract of the aerial parts and analyze the major constituent present in these extract by developing a novel HPLC method. The dried aerial parts were subjected for extraction with n-hexane and toluene in soxhlet apparatus. The dried toluene extract were subjected for column chromatography for the isolation of phytoconstituent. The structural analysis of the isolated compound (LC-01) was carried out by IR, NMR, MS and qualitative estimation by HPLC method using C-18 column with SPD-M20A prominence diode array detector was used. From the retention time and percentage yield (1.02%w/w) of the isolated compound obtained from toluene extract, it was concluded that compound LC-01(pentacyclic triterpenoid) were found as major chemical constituent which have structural similarities with the **reference standard (Lupeol).**

INTRODUCTION

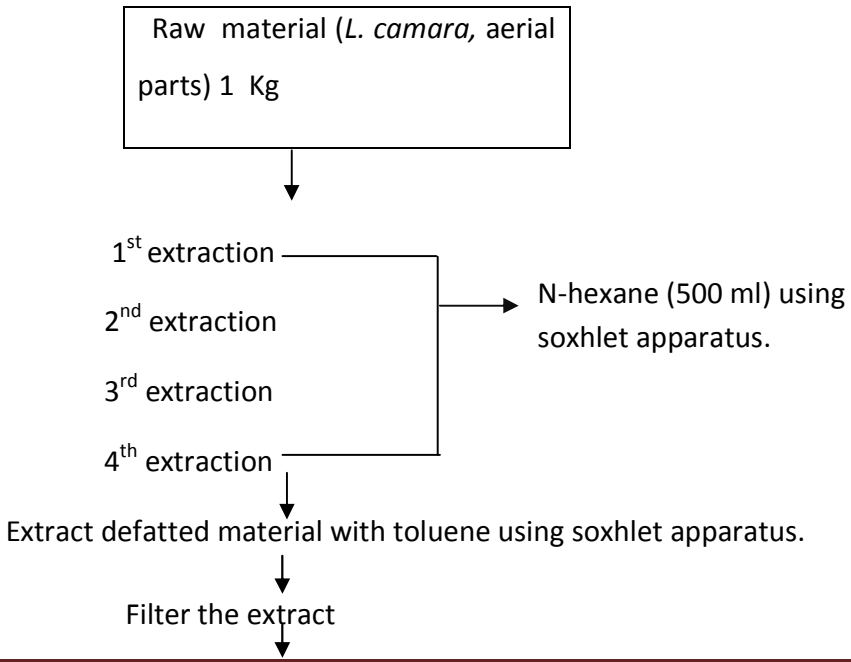
Lantana camara plant is found mostly in the south India, in Tamilnadu[1], in America[5], in Africa[6], and also found in Himachal Pradesh, Jammu-Kashmir and Uttar Pradesh[11].

Figure 1 Lantana camara plant

The different parts of plant extract were useful in various diseases like diaphoretic, tonic, wounds, swelling, rheumatism, anti-spasmodic, carminative, anti-tumor[1], anti-inflammatory[2], anti-malarial[3], anti-ulcerogenic, treatment of emotional stress and trauma[4], anti-microbial, nematicidal, insecticidal, fungicidal[5], influenza, asthma[6], antidote to snake venom, eczema[7], gastrointestinal disorders, anti-nociceptive, anti-pyretic, inhibitor of acetyl cholinesterase[8], abortifacient[9], anthelmintic, febrifuge[10], adulticidal activity, larvicidal, biological control[12].

MATERIALS AND METHODS

EXTRACTION PROCESS

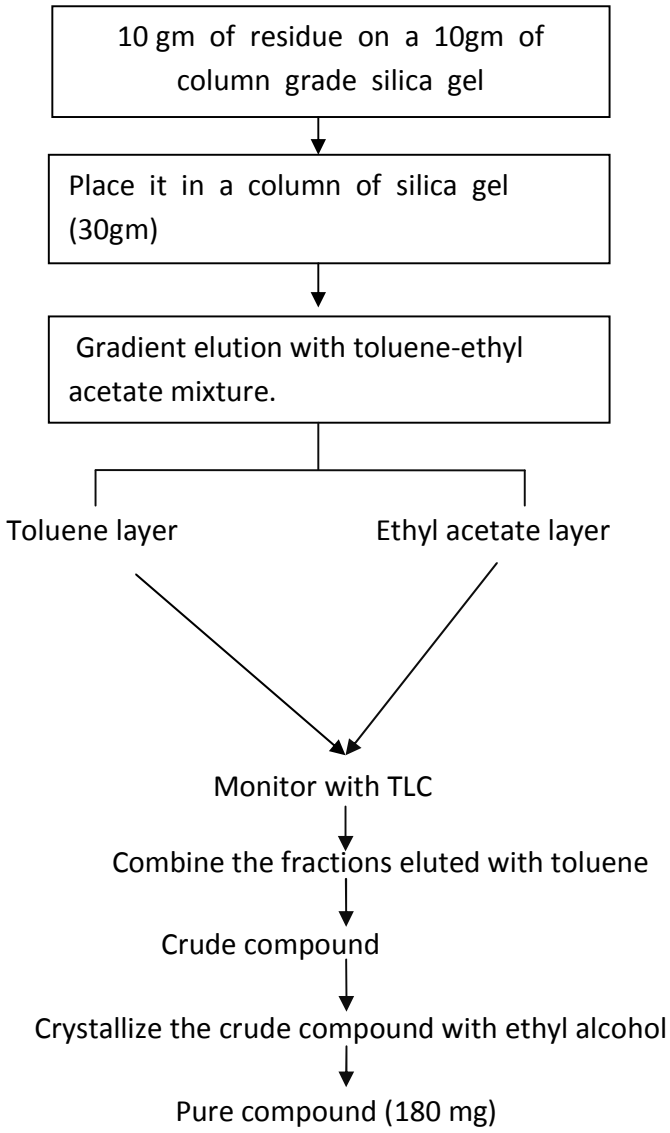


Remove solvent under pressure

↓

Yield the residue (16 gm)

Isolation process: 10 gm of the residue of ***L. camara*** toluene extract was partitioned with different solvents- toluene, ethyl acetate, ethanol as shown below:-



Mobile phase: n-hexane: methanol: acetic acid (4.5:5.5:0.2)

Procedure: The extract was dissolved in methanol and then spotted on the silica gel G 254 plates with the help of

capillary tubes. TLC plates were developed and scanned at 254nm. The Anisaldehyde sulphuric acid (ANS) reagent was sprayed and the chromatogram was observed for separation.

HPLC ANALYSIS OF LC-01 (AGILENT-1100).

Requirements:

Column: phenomenex, C_{18}, 5µm, 250×4.6 mm

Mobile Phase: Pump A (Acetic acid (0.5%))

Pump B (water (90 ml): Acetonitrile (10 ml)).

Elution: gradient.

Flow rate: 1ml/min.

Injection volume: 20 µl.

Detector: SPD-M20A prominence diode array detector at 242 nm.

Results and Discussions:

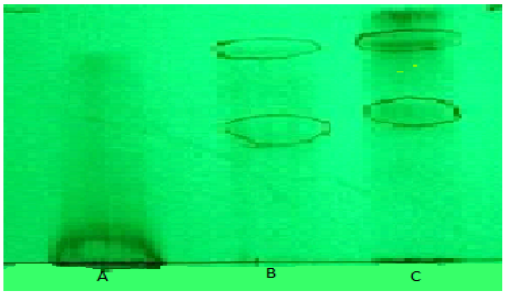


Figure 2 TLC studies of chloroform, petroleum ether, n-hexane extract at 254 nm, n-hexane was selected due to good spots and Rf value.

A- Chloroform extract

B- Petroleum ether extract

C- n-hexane extract

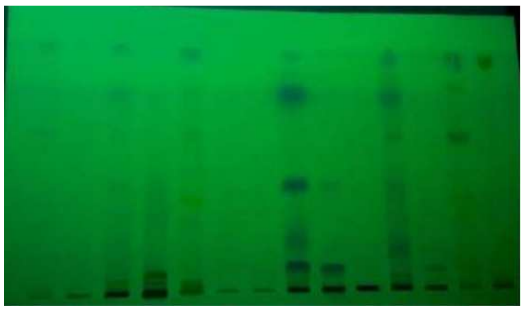
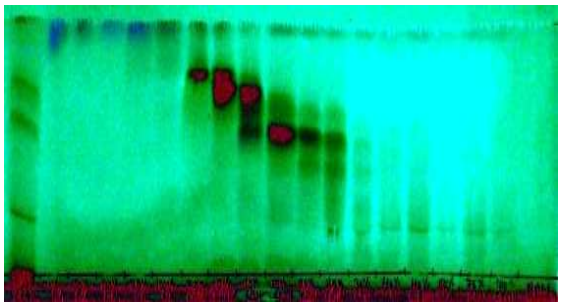


Figure 3 TLC for all fractions of CC-1 at 254 nm.

Sub fractionation of fraction 12-17 of Column-1(CC-1).

Figure 4 TLC of all the fractions collected from column-2 at 254 nm.



Sub fractionation of fractions 12 & 13 of Column-2(CC-2).

12 fractions were collected from column-3 using fraction CC-2/F-12 and F-13 as starting material. These fractions were concentrated under vacuum. The fraction 6 from CC-3 yields 4gm, which was further purified with ethyl alcohol, an amorphous powder with a single spot on TLC having the R_f value 0.93 and the code given was **LC-01**.

HPTLC of the Fr-7 collected from column-3 was shown in the Figure 6

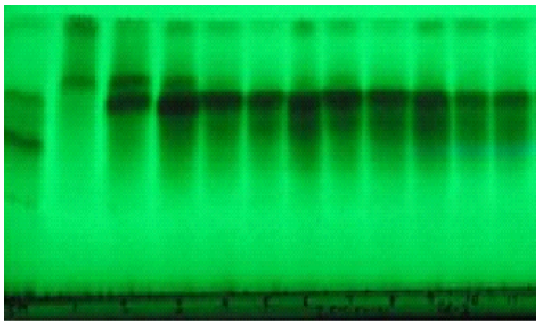


Figure 5 TLC of the all fractions of CC-3 at UV 254 nm.

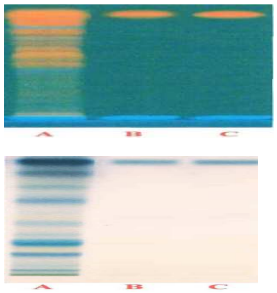


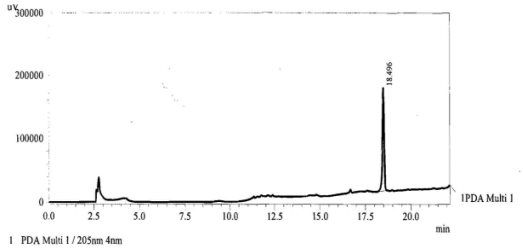
Figure 6 HPTLC of Isolated compound (LC-01) at 366 nm and with anisaldehyde sulphuric acid

A- Lantana camara extract

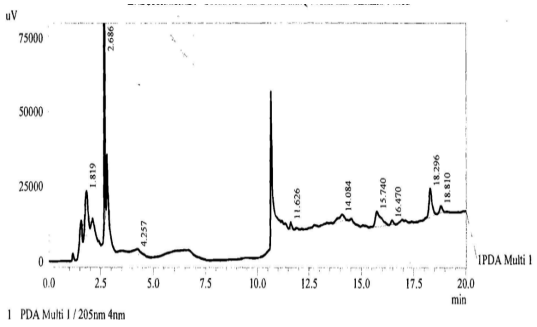
B- Isolated compound (LC-01)

C- Reference standard

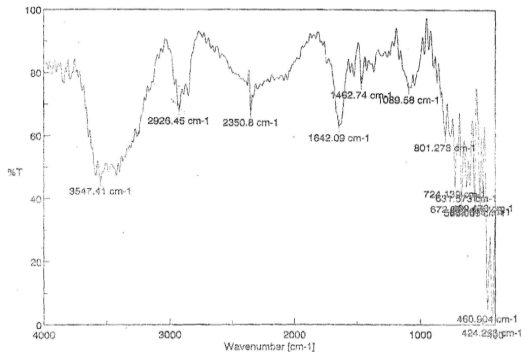
- HPLC graph of isolated compound **LC-01**



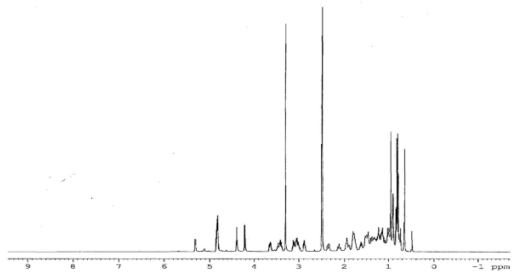
- HPLC graph of the *L. camara* extract



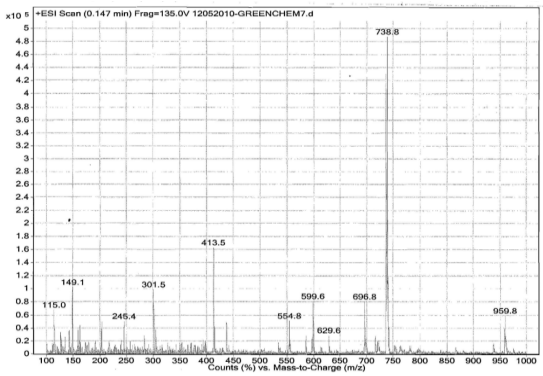
I.R. spectrum of Lc-01



N.M.R spectrum of Lc-01



Mass spectrum of LC-01



Quantitative estimation of LC-01 in toluene extract by HPLC Method.

Peaks were observed with similar retention time (Rt) peaks of 18.496 min & 18.296 min were observed for the LC-01 and identified LC-01 in toluene extract. It would be confirmed the presence of isolated compound (LC-01) present in the toluene extract of *Lantana camara*. In the HPLC chromatogram of the toluene extract, the area of LC-01 was found to be 98476. Therefore the LC-01 present in the toluene extract was found to be 1.02 % w/w calculated by using the formula =

$$\frac{\text{Std. weight} \times \text{Sample Area} \times \% \text{ Assay}}{\text{Sample weight} \times \text{Std. Area}}$$

Spectroscopy Studies:

❖ Isolated Compound **LC-01**

- IR (KBr) λ cm^{-1}: The I.R. spectrum showed characteristic absorption bands of hydroxyl group at 3547.41 cm^{-1}(str.) and keto group at 1642.09 cm^{-1}(str.) Also C-H stretching band showed at 2926.45 cm^{-1} and ester group showed band at 1089 cm^{-1}(str).
- ^{1}H NMR δ(ppm), (DMSO): δ1.80(2H,t,a), δ1.55(2H,d,a), δ1.39(2H,t,b), δ1.49(2H,t,c), δ1.24(2H,s,c), δ1.52(2H,t,d), δ1.27(2H,d,d), δ1.40(1H,t,e), δ1.43(1H,t,f), δ1.55(1H,t,g), δ1.30(1H,s,g), δ1.63(1H,t,h), δ1.38(1H,d,h), δ2.18(1H,d,i), δ1.71(3H,s,j), δ1.16(3H,s,k), δ1.11(3H,s,l), δ2.53(2H,d,m), δ2.28(2H,s,m), δ1.44(2H,t,n), δ1.29(2H,t,o), δ2.1(1H,d,p-OH), δ1.33(2H,q,q), δ0.96(3H,t,r), δ4.88(1H,d,s), δ4.63(1H,d,t).
- The ^{1}H NMR of the compound exhibited aliphatic chain of carbons at δ 0.5 to

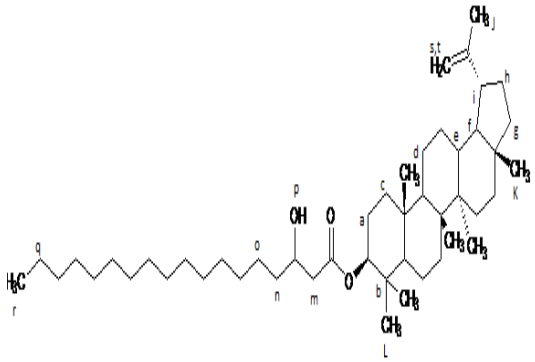
1.28 and a hydroxyl proton exhibits at δ2.1.

- Mass: The mass spectrum of compound displayed a molecular ion peak at m/z 738.8 corresponding to the molecular formula of $C_{50}H_{88}O_3$.

CONCLUSION

- As the isolated compound were present in sufficient quantity in the toluene fractions, it gives positive test for triterpenoids, so pentacyclic triterpenoid were found to be called as Major chemical constituent from the aerial parts of *Lantana camara.*

➢ **Probable structure of compound LC-01**



❖ Based on the structural interpretation of IR, ^{1}H NMR and Mass spectroscopy. Therefore it was concluded that compound LC-01 having molecular weight- 737.231920 g/mol and molecular formula- $C_{50}H_{88}O_3$.

❖ Based on the HPTLC finger print, identification test, I.R., ^{1}HNMR, MASS spectroscopy, the **isolated compound (LC-01)** have structural similarities with the **reference standard (Lupeol).**

Table 1

HPLC protocol for LC-01 by gradient system

Time	A (concentration %)	B (concentration %)
0.01	100	0.0
1.7	98	2.0
10	72	28
20	60	40
40	40	60

Table 2

Physical properties of isolated compound (LC-01)

State	Crystalline Solid
Colour	White
Solubility	Soluble in glacial acetic acid, chloroform and warm water
Melting point	287^0C
Molecular weight	737.23
Retention factor	0.93

Table 3

Quantitative estimation of LC-01 in toluene extract by analytical HPLC

Compound code	Retention time	Area	Concentration
LC-01	18.296	98476	100%

Table 4

Yield of isolated compound (LC-01)

Compound code	Yield
LC-01	1.2 %W/W

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