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Phytochemical Screening, Isolation and Characterisation of Phytoconstituents of *Lagerstroemia lanceolata*

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The present study focuses on the phytochemical screening, isolation and characterisation of phytoconstituents from *Lagerstroemia lanceolata*, a medicinally significant species known for its diverse therapeutic applications. Preliminary phytochemical evaluation of the methanolic extract confirmed the presence of major secondary metabolites, prompting further purification. Thin layer chromatography (TLC) was employed to optimize the solvent system for separation, where the mobile phase toluene:ethyl acetate (7.5:2.5) provided superior resolution of phytoconstituents. Based on the TLC analysis, column chromatography resulted in the isolation of three pentacyclic triterpenoids, namely oleanolic acid, ursolic acid and corosolic acid. Structural elucidation and confirmation of purity were achieved using a combination of spectroscopic techniques, including IR, ^{1}H NMR, ^{13}C NMR and LC-MS.

Keywords: *Lagerstroemia lanceolata* Wall., Column chromatography, Oleanolic acid, Ursolic acid, Corosolic acid.

INTRODUCTION

Lagerstroemia lanceolata Wall. belonging to the family Lythraceae, is an ethnomedicinally important tree distributed primarily in the Western Ghats and Deccan regions of India. Traditionally known as "Nandi" or "Kadambi," the plant has been employed in indigenous systems of medicine for wound healing, skin ailments, inflammation, metabolic disorders and gastrointestinal disturbances [1,2]. Growing interest in the therapeutic potential of *L. lanceolata* stems from the broader pharmacological reputation of the genus Lagerstroemia, which includes species such as *L. speciosa*, *L. Indica*, renowned for anticarcinogenic, antidiabetic, antioxidant and anti-inflammatory bioactivities [3-7]. Despite this, *L. lanceolata* remains comparatively understudied, particularly in terms of its phytochemical composition, systematic isolation of bioactive constituents, and advanced spectroscopic characterization.

Phytochemical investigations reported for *Lagerstroemia* species indicate the presence of diverse secondary metabolites [8-10] including ellagitannins, gallotannins, triterpenoids, sterols, flavonoids and phenolic acids. Corosolic acid, ellagic acid, lagerstroemin, gallic acid, β-sitosterol, quercetin and various oleanane-type triterpenoids are considered bioactive benchmarks in the genus [11]. While *L. speciosa* has been thoroughly explored for corosolic acid and other tannins, early studies on *L. lanceolate* primarily focused on crude extracts, leaving gaps in the understanding of its phytoconstituents [12]. Phytochemicals found in plants typically exhibit potent antioxidant and anti-inflammatory properties [13], which underpin many of their additional biological activities and associated health benefits [14-21]. The absence of comprehensive chromatographic and spectral data further underscores the need for detailed phytochemical profiling.

Isolation and characterization of phytoconstituents typically involve a sequential workflow of extraction, fractionation, purification and identification [22-30]. Extraction using solvents of varied polarity (petroleum ether to methanol) allows selective isolation of metabolite classes based on solubility. Recent advances in hyphenated techniques such as LC-MS, GC-MS and HPTLC profiling have greatly improved the accuracy of phytoconstituent analysis and finger-printing. Structural elucidation is achieved using spectroscopic methods such as UV-Vis, IR, NMR (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC) and mass spectrometry [31]. NMR spectroscopy remains the benchmark standard for structural confirmation of natural products, enabling identification of complex tannins, flavonoids and triterpenoids, even in microgram quantities.

Given the biological significance of phytocompounds reported from related species and the increasing demand for novel plant-derived therapeutics, a systematic study of *L. lanceolata* is scientifically justified. Preliminary reports indicate antioxidant, antimicrobial, anti-inflammatory and antidiabetic potentials for extracts derived from its leaves, bark and stem, highlighting the likelihood of significant bioactive constituents [32]. However, the lack of comprehensive phytochemical isolation studies restricts its potential application in pharmacognosy, drug discovery and herbal standardization. Therefore, the present investigation aims to isolate and characterize the phytoconstituents of *Lagerstroemia lanceolata* through a combination of chromatographic separation and advanced spectroscopic techniques.

EXPERIMENTAL

Collection and authentication of plant material: The herbarium was prepared and the plant material was authenticated by the Botanist, Dr. S.S Hebber, Government P.U. College, Dharwad, with the help of the flora of the residency of Bombay by Theodore Cooke and http://indiabiodiversity.org/species/show/31487 voucher specimen of the same has been deposited in the Herbarium accession No. (AN/GCP/2023-24/08). The leaves of *L. lanceolata* were collected from the western ghats of Dandeli, India. The leaves were washed to eliminate dirt and other particles before drying in sunlight.

Characterization: IR spectra of the isolated compounds were obtained using FTIR-ATR (Shimadzu). ^{1}H NMR were measured using DMSO-d_6 on a Bruker Avance 400 MHz instrument. ^{13}C NMR were measured using DMSO on a Bruker AVII 100 MHz instrument. Mass spectra were acquired using Agilent Technologies (HP) 5973 mass spectrometer.

Preparation of methanolic extract: Fresh leaves (1 kg) of *L. lanceolata* wall. were collected, washed thoroughly and dried. It was made into coarse posswder and subjected for extraction with 90% ethanol using refluxing method. This process was repeated in triplicate (3 × 1 L) to give methanolic extract. The solvent was removed using a rotary vaccum evaporator, after which the resulting concentrate was reduced to a syrup-like consistency and subsequently dried (45 g).

Phytochemical screening: Literature reports indicate that only limited phytochemical studies have been conducted on *L. lanceolata*. Previous invesstigations of the leaves have identified the presence of steroids, terpenoids, alkaloids, anthocyanins, ellagic acid and tannins. Phytochemical screening of the light petroleum ether extract revealed glycosides and steroidal constituents [1], while crude ethyl acetate and methanolic extracts were reported to contain flavonoids, anthraquinones, resins, alkaloids and cardiac glycosides [8]. The present study aimed to investigate the phytochemical profile of methanolic extracts with particular emphasis on comparing with the reported ethyl acetate extract [8] for their secondary metabolite composition. The results are shown in Table-1.

Optimisation of TLC mobile phase for methanolic extract of *L. lanceolata*: TLC for the extract was adopted after examining a series of solvent systems in the interest of achieving the most effective mobile phase for the separation of phytoconstituents. The mobile phase that successfully separated the phytoconstituents was chosen to be the most effective solvent for the investigation.

TABLE-1
COMPARATIVE PHYTOCHEMICAL SCREENING DATA OF *L. lanceolate* IN DIFFERENT EXTRACTS

Secondary metabolites	Extract	
	Methanolic	Ethyl acetate [8]
Glycosides	Absent	Absent
Alkaloids	Low amount	Absent
Flavonoids	High/abundant amount	Moderate amount
Tannins	High/abundant amount	Low amount
Steroids	Low amount	Low amount
Saponins	Absent	Absent
Phenols	Moderate amount	Low amount
Carbohydrates	Low amount	Low amount
Proteins	Low amount	Low amount

Procedure to prepare the sample for performing TLC: Methanolic extract of *L. lanceolata* (10 mL) was dissolved in petroleum ether (60-80 ºC) in an Eppendorf tube and used to carry out the TLC. The sample was spotted on precoated TLC plates, which served as stationary phase. The plates were then developed in the mobile phase and subsequently visualized using anisaldehyde/sulphuric acid (ANS) as a spraying reagent, followed by heating at 105 ºC. The mobile phase that provided optimal separation was selected after evaluating various solvent systems including toluene:ethyl acetate (5:5, 7:3, 7.5:2.5, 9:1, 8:2), hexane:ethyl acetate (5:5, 6:4, 7:3, 9:1, 8:2), chloroform: ethyl acetate (7:3, 7.5:2.5) and chloroform:acetone (9:1, 8:2, 5:5).

Optimisation of TLC of extract: A superior separation of the phytoconstituents was obtained using the mobile phase of toluene:ethyl acetate (7.5:2.5).

Isolation of phytoconstituents

Column chromatography of methanolic extract: A total of 12.5 g of methanolic extract of *L. lanceolata* was dissolved in 200 mL of methanol and subsequently adsorbed onto 15 g of silica gel (60-120 mesh). The mixture was evaporated on a water bath until a dry, free-flowing powder was formed. This prepared sample was then introduced into a glass column pre-packed with 600 g of silica gel (60-120 mesh), which had been previously conditioned using petroleum ether (60-80 ºC) as the packing solvent.

The column chromatography was carried out using a gradient elution technique, beginning with 100% pet. ether (60-80 ºC) and progressively increasing the polarity of the solvent mixtures. Sequential elutions were performed using petroleum ether:chloroform in the different ratios *viz*. 99:1, 98:2, 95:5, 90:10, 85:15, 80:20, 75:25 and 50:50. This was followed by 100% chloroform and subsequently by chloroform:ethyl acetate mixtures in similar gradient proportions. Afterward, 100% ethyl acetate was used, followed by graded mixtures of ethyl acetate:methanol (99:1, 98:2, 95:5, 90:10, 85:15, 80:20, 75:25, 50:50) and finally 100% methanol. Fractions of 10 mL each were collected in conical flasks. Similar fractions were pooled based on their TLC profiles, using toluene:ethyl acetate (75:25) as the mobile phase, visualised with anisaldehyde-sulphuric acid spray reagent and heating at 110 ºC. The combined fractions were concentrated to dryness using a rotary evaporator.

Elutions carried out with $CHCl_3$:EtOAc, 75:25 and 50:50 resulted in a mixture of compounds on TLC (toluene:ethyl acetate 75:25, UV 254 nm, Visualizing agent: anisaldehyde-sulphuric acid). After removing the solvent, a mixture of light greenish white amorphous powder was obtained. It was further decolourised by using 100 mg of activated charcoal in methanol and heated on a water bath. The hot solution was filtered and the filtrate was allowed to crystallise, finally the recrystallisation process was repeated three times to obtain the purified crystals. This yielded a mixture of compounds, which was further subjected to re-chromatography to isolate individual constituents; the mixture was designated as **LAN-3** (74 mg).

Re-column chromatography of LAN-3: A mixture (74 mg) was dissolved in $CHCl_3$, adsorbed onto flash-grade silica gel (2 g, 240-400 mesh), evaporated to obtain a free-flowing powder and then loaded onto a column packed with flash-grade silica gel (50 g, 240-400 mesh) using $CHCl_3$. Elutions were carried out at very slow flow rate in order to separate the individual compounds. Each time 5 mL of elutes were collected in test tubes, identical elutes was combined and TLC was performed (toluene:EtOAc 75:25, UV 245 nm, visualising agent: anisaldehyde-sulphuric acid) Fractions 15-30 showed a mixture of compounds on TLC and were combined as LAN-4 (37 mg). This fraction was further purified by preparative TLC, leading to the isolation of three compounds, **LLAN-4** (8 mg), **LLAN-5** (7 mg) and **LLAN-6** (4 mg).

Spectral data

Compound LLAN-4: m.p.: 300 ºC, R_f value: 0.48. IR (KBr, ν_{max}, cm^{-1}): 3475.73 (br, -OH), 2937.59 (C-H *str*. CH_3), 1687.71 (C=O *str*., carboxylic acids), 1442.75 (C-C *str*. in ring Ar) 1274.95, 1093.64 (C-O *str*., C-OH), 918.12 (=C-H bend.). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.968 (3H, s, H-23), 0.671 (3H, s, H-24), 0.848 (3H, s, H-25), 0.714 (3H, s, H-26), 1.089 (3H, s, H-27), 0.890 (3H, s, H-29), 0.871 (3H, s, H-30), 0.871 (3H, s, H-30), 1.144-2.503 (24H, m, CH_2 and CH protons), 2.992 (1H, d, H-3), 5.152 (1H, d, H-12), 12.022 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 38.565 (C-1), 27.463 (C-2), 77.333 (C-3), 38.876 (C-4), 55.289 (C-5), 18.532 (C-6), 32.001 (C-7), 39.392 (C-8), 47.602 (C-9), 37.100 (C-10), 23.403 (C-11), 121.997 (C-12), 144.344 (C-13), 41.312 (C-14), 27.721 (C-15), 23.123 (C-16), 46.183 (C-17), 40.037 (C-18), 45.925 (C-19), 30.901 (C-20), 16.544 (C-25), 17.371 (C-26), 26.128 (C-27), 180.782 (C-28), 33.822 (C-29), 23.8 (C-30); LC-MS (*m/z*) 455.55 $[M-1]^-$. Other peaks appeared at 455.55, 306.49, 242.65, 242.48, 143.47, 83.20.

Compound LLAN-5: m.p.: 285 ºC, R_f value: 0.71. IR (KBr, ν_{max}, cm^{-1}): 3400.50 (broad, OH), 2924.09, 2326.15 (C-H *str*. in CH_3 and CH_2), 1687.71 (C=O *str*. of COOH), 1604.77 (C=C *str*.), 1365.60 cm^{-1} (C-H def. in *gem*. dimethyl), 1047.35 (C-O *str*. of *sec*. alcohol); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.713 (s, 3H, H-23), 0.768 (s, 3H, H-24), 0.891 (s, 3H, H-25), 0.859 (s, 3H, H-26), 0.879 (s, 3H, H-27), 1.005 (s, 3H, H-30), 1.057-2.975{m, 25H, (CH_2 and CH protons) H-1, 2, 5, 6, 7, 11, 12, 13, 15, 16, 18, 19, 21, 22}, 5.092 (s, 1H, H-29), 4.261 (d, 1H, H-3), 11.912 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 37.920 (C-1), 28.920 (C-2), 73.933 (C-3), 39.953 (C-4), 42.124 (C-5), 23.373 (C-6), 29.262 (C-7), 39.748 (C-8), 52.793 (C-9), 30.605 (C-10), 24.223 (C-11), 124.797 (C-12), 138.668 (C-13), 39.536 (C-14), 29.457 (C-15), 27.934 (C-16), 60.206 (C-17), 38.929 (C-18), 31.728 (C-19), 36.744 (C-20), 27.934 (C-21), 38.853 (C-22), 14.389 (C-23), 17.363 (C-24), 18.304 (C-25), 17.485 (C-26), 23.699 (C-27), 178.711 (C-28), 21.514 (C-29), 16.862 (C-30); LC-MS (*m/z*) 457.48 $[M+H]^+$. The other peaks appeared at 142.23, 143.20, 151.15, 242.48, 260.25, 306.39, 307.45.

Compound LLAN-6: m.p.: 243-245 ºC, R_f value: 0.40. IR (KBr, ν_{max}, cm^{-1}): 3400.50 (br. OH), 2924.09 (C-H *str*. in CH_3), 2856.58 (C-H *str*. in CH_2), 2326.15 (C=C *str*.); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.043 (s, 3H, H-23), 0.917 (s, 3H, H-24), 0.822 (s, 3H, H-25), 0.952 (s, 3H, H-26), 0.974 (s, 3H, H-27), 0.705 (s, 3H, H-29), 1.005 (s, 3H, H-30), 1.126-3.506 {m, 24H, (CH_2 and CH protons) H-1, 2, 3, 5, 6, 7, 9,11, 15, 16, 18, 19, 21, 22}, 5.139 (t, 1H, H-12), 2.106 (t, 1H, COOH, H-20); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 40.583 (C-1), 67.559 (C-2), 80.436 (C-3), 39.331 (C-4), 60.206 (C-5), 18.304 (C-6), 31.728 (C-7), 39.536 (C-8), 47.246 (C-9), 37.920 (C-10), 23.373 (C-11), 124.797 (C-12), 138.668 (C-13), 40.371 (C-14), 27.934 (C-15), 24.223 (C-16), 42.124 (C-17), 52.793 (C-18), 38.929 (C-19), 38.853 (C-20), 30.840 (C-21), 36.744 (C-22), 28.920 (C-23), 17.614 (C-24), 16.862 (C-25), 17.485 (C-26), 23.697 (C-27), 178.711 (C-28), 21.514 (C-29), 17.363 (C-30); LC-MS (*m/z*) 471.62 $[M-H]^-$. The other peaks appeared at 419.54, 319.39, 252.46, 143.20, 83.23.

RESULTS AND DISCUSSION

The present investigation resulted in the isolation of three pentacyclic triterpenoids from the leaves of *L. lanceolata*, identified as oleanolic acid, ursolic acid and corosolic acid through IR, ^{1}H NMR, ^{13}C NMR and LC-MS analyses. The spectral data confirmed the presence of hydroxyl, olefinic and carboxylic functionalities consistent with oleanane- and ursane-type triterpenoids. The occurrence of these compounds agrees with phytochemical patterns reported for the genus *Lagerstroemia*, indicating their chemotaxonomic significance. Their co-existence also suggests a conserved triterpenoid biosynthetic pathway in *L. lanceolata*, highlighting the plant as a potential source of biologically important triterpenoids for further pharmacological studies.

The chemotaxonomic relevance of isolation of oleanolic acid, ursolic acid and corosolic acid was of high importance on the leaves of *L. lanceolata*. Oleanane and ursane type pentacyclic triterpenoid pentacyclic triterpenoids are common reported secondary metabolites of the genus *Lagerstroemia* and also the family Lythraceae [33-35]. These structurally related triterpenoids have also been verified in *L. lanceolata* which is also consistent with the phytochemical profiles of species in the genus and therefore validates the taxonomic position of the plant and makes these compounds reliable chemotaxonomic biomarker candidates.

Spectral characteristics of compound LLAN-4: The IR spectrum (Fig. 1) displayed absorption bands at 2937.59 cm^{-1} C-H stretching in CH_3, the presence of carbonyl group was confirmed at 1687.71 cm^{-1}. Further, the IR spectrum confirmed the presence of C-C stretch in aromatic ring 1442.75 cm^{-1}, C-O stretch at 1274.95 cm^{-1}, 1093.64 cm^{-1} and

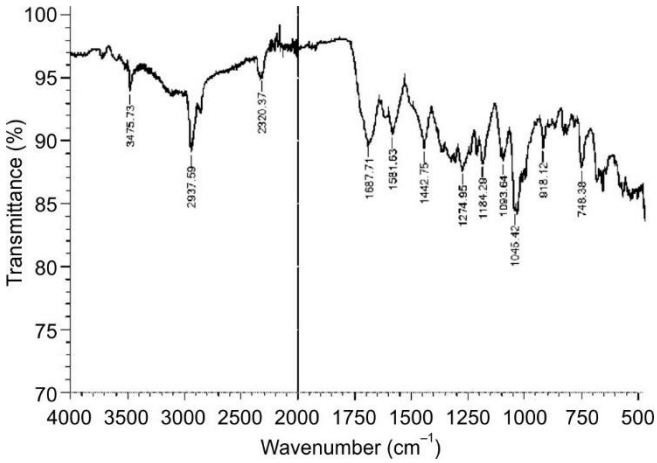

Fig. 1. IR spectrum of **LLAN-4**

=C-H bend at 918.12 cm^{-1}. The ^{1}H NMR spectrum (Fig. 2) showed multiplet at δ 0.978-1.089 ppm indicating the presence of seven tertiary methyl groups on oleanane skeleton. One OH proton at δ 2.992 ppm (1H, d, H-3) showed that the compound has at least one hydroxyl group. The multiplet at δ 1.144-2.503 ppm indicated the CH and CH_2. The olifinic protons appeared as a doublet in the range of δ 5.152 ppm at H-12. The protons of a carboxylic acid group at C-28 was observed at δ 12.022 ppm. ^{13}C NMR spectrum (Fig. 3), the signal corresponding to the carboxylic C-28 appeared at δ 180.782 ppm Also, it confirmed the presence of thirty carbon consisting of eight quaternary, five tertiary, ten secondary carbons and seven methyl groups. The molecular ion peak at *m/z* 455.55 [M-1]$^-$ was indicative of molecular formula $C_{30}H_{48}O_3$ and molecular weight 456.711 g/mol (Fig. 4). From the physical state and spectral analysis data, the compound **LLAN-4** is identified as oleanolic acid (3β)-3-hydroxy-olean-12-en-28-oic acid.

Spectral characteristics of compound LLAN-5: The IR spectrum (Fig. 5) displayed characteristic absorption band for hydroxyl group at 3400.50 cm^{-1}. The absorption peak at 1687.71 cm^{-1} indicated the presence of carbonyl of the carboxyl group (COOH). The absorption band at 1047.35 cm^{-1} indicated the -C-OH band and another peak at 2924.09 cm^{-1} indicated the presence of aliphatic C-H stretching. The ^{1}H NMR spectrum (Fig. 6) revealed the presence of terminal

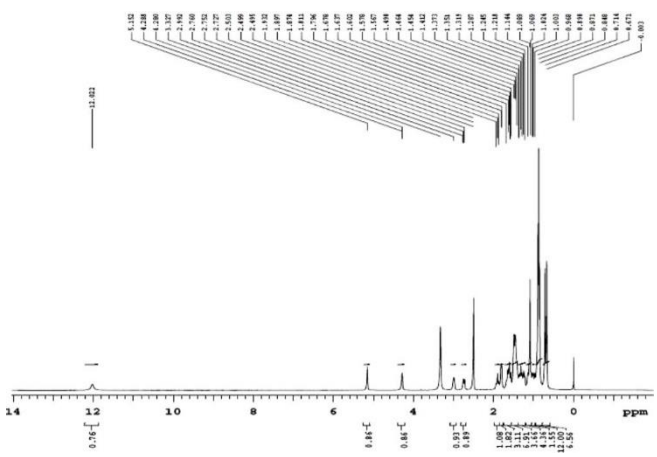

Fig. 2. ^{1}H NMR spectrum of **LLAN-4**

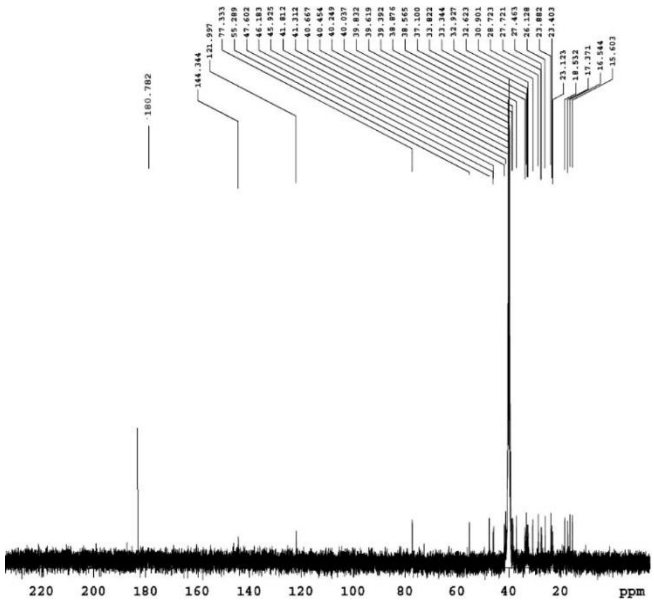

Fig. 3. ^{13}C NMR spectrum of **LLAN-4**

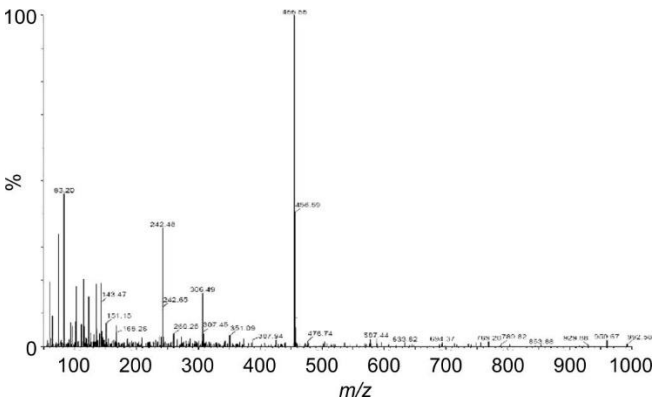

Fig. 4. Mass spectrum of **LLAN-4**

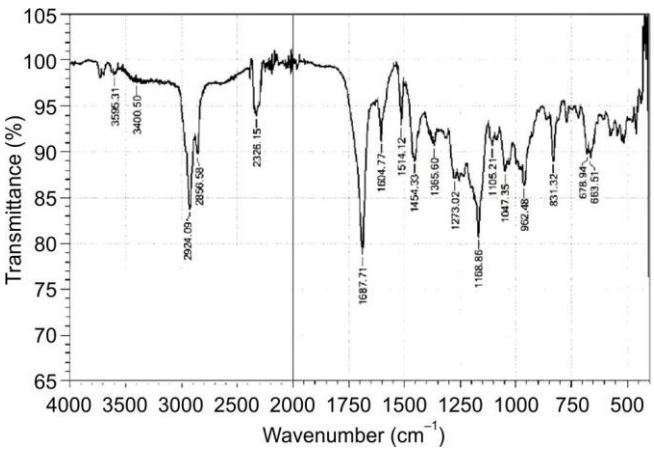

Fig. 5. IR spectrum of **LLAN-5**

methyl groups at δ 1.057-2.975 ppm. The olefinic protons appeared in the range of δ 11.912 ppm at H-12, which is coupled to the proton at H-11. One proton doublet at δ 4.261 ppm was assigned to α-oriented carbinol H-3 proton. A doublet at δ 2.132 ppm and multiplet at δ 1.192 ppm indicated that the protons at H-18 and H-19 are *trans* to one another. This

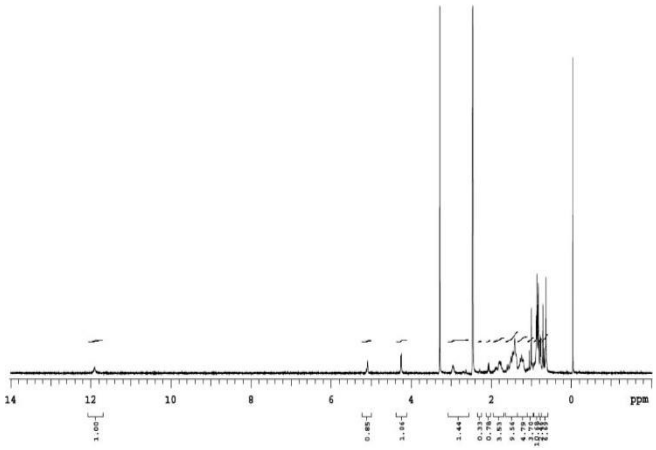

Fig. 6. ^{1}H NMR spectrum of **LLAN-5**

appears only if the compound is α-type since the two groups attached to C-19 are hydrogen and methyl. The coupling between H-18 and H-19 could resultin a doublet. On the other hand in β-type (whereby only 2 protons are attached to H-19), the coupling between the protons at H-18 and H-19 would give a quartet. The ^{13}C NMR spectrum (Fig. 7) of compound displayed 30 carbon atoms and important signals appeared for carboxyl group at δ 178.711 (C-28). The chemical shift at C-28 124.797 and C-28 138.668 represented

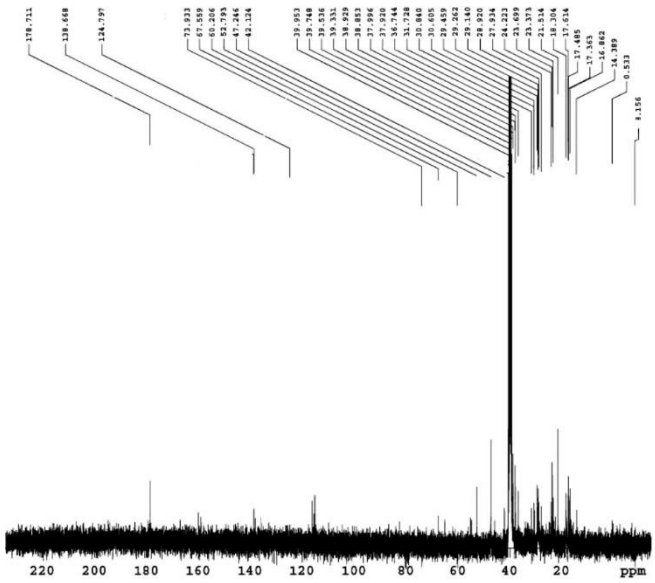

Fig. 7. ^{13}C NMR spectrum of **LLAN-5**

carbons of alkene conjugated *i.e.* C-12 and C-13 carbon, respectively. The chemical shift at δ 73.933 ppm represented carbinol carbon at C-3. The signals at δ 14.389, 17.363, 18.304, 17.485, 23.699, 21.514 and 16.862 ppm corresponds to the carbons of methyl groups attached to cyclic ring at C-23, C-24, C-25, C-26, C-27, C-29 and C-30, respectively. The LC-MS spectrum (Fig. 8) showed a molecular ion peak at *m/z* 457.48 $[M+H]^{+}$ corresponding to the molecular formula $C_{30}H_{48}O_3$. From melting point, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data, compound **LLAN-5** was identified as ursolic acid (3-β-3-hydroxy-urs-12-ene-28-oic-acid).

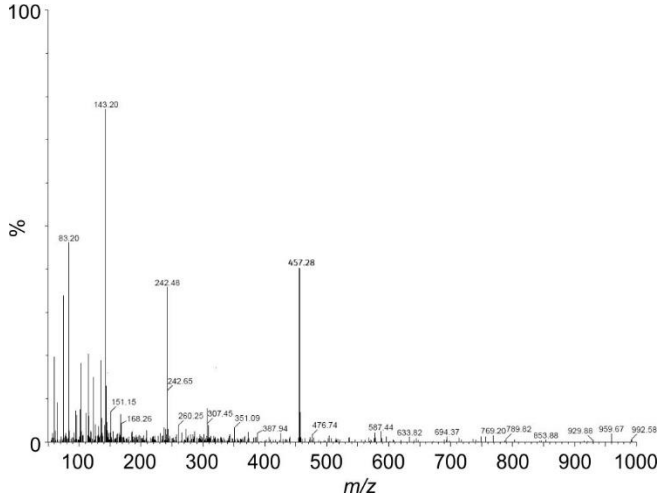

Fig. 8. Mass spectrum of **LLAN-5**

Spectral characteristics of compound LLAN-6: The IR spectrum (Fig. 9) of the compound exhibited a broad peak at 3400.50 cm^{-1} indicating the presence of hydroxyl group. The peaks at 2924.09 cm^{-1} and 2856.58 cm^{-1} indicated C-H stretching in CH_3 and CH_2, respectively. The ^{1}H NMR spectrum (Fig. 10) exhibited the singlets at δ 1.043, 0.917, 0.822, 0.952, 0.974, 0.705, 1.005 ppm at H-23, H-24, H-25, H-26, H-27, H-29 and H-30, indicating the tertiary methyl protons. The olefinic protons appeared as δ 5.139 ppm at H-12. The triplet at δ 2.106 ppm indicated proton at COOH. The multiplet at δ 1.126-3.506 ppm indicated methylene and methine protons. The ^{13}C NMR spectrum (Fig. 11) exhibited the presence of 30 carbon atom signals for the pentacyclic triterpenoid of the lupane skeleton which include seven methyl groups at δ 28.920 (C-23); δ 17.614 (C-24); δ 16.862 (C-25); δ 17.485 (C-26); δ 23.697 (C-27); δ 21.514 (C-29) and δ 17.363 (C-30), respectively. Eight methylene, eight methine, seven quaternary and one carbonyl carbon atoms. The signals at δ 124.797 ppm (C-12) and δ 138.668 ppm (C-13) were deshielded due to an olefinic bond between them. The signals at δ 80.436 and δ 178.711 ppm were deshielded due to the OH and COOH group at C-3 and C-28, respectively. The mass spectrum (Fig. 12) exhibited a molecular ion $[M-1]^{-}$ peak at 471.62 *m/z* corresponding to the molecular formula $C_{30}H_{48}O_4$. The other peaks appeared at 419.54, 319.39, 252.46, 143.20, 83.23.

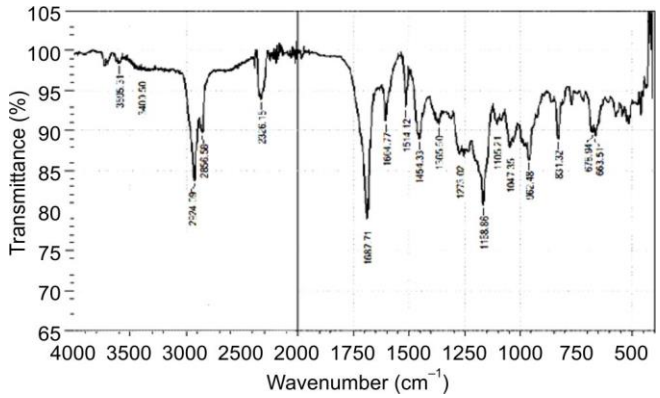

Fig. 9. IR spectrum of **LLAN-6**

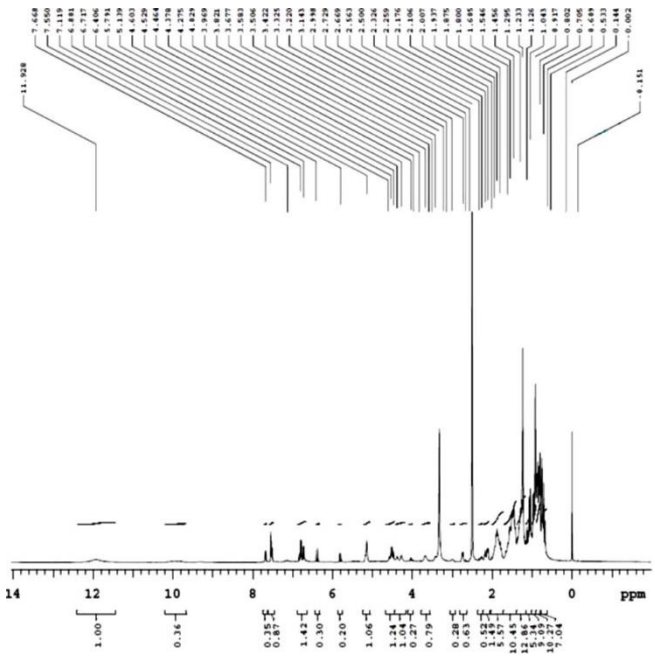

Fig. 10. ^{1}H NMR spectrum of **LLAN-6**

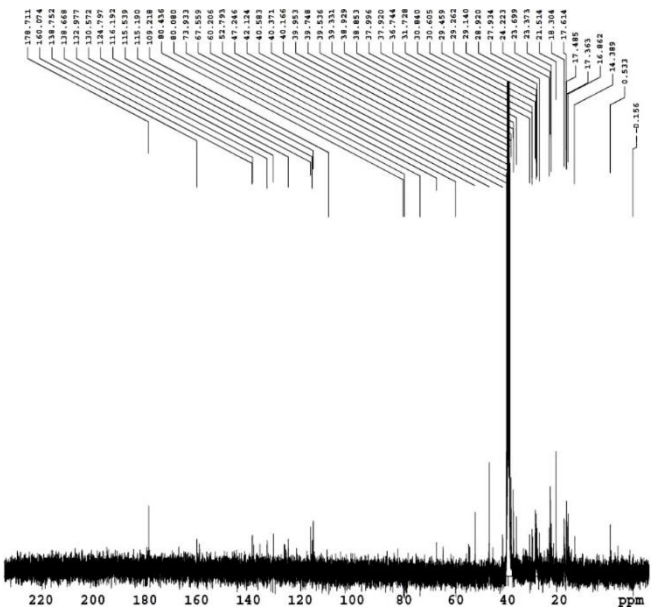

Fig. 11. ^{13}C NMR spectrum of **LLAN-6**

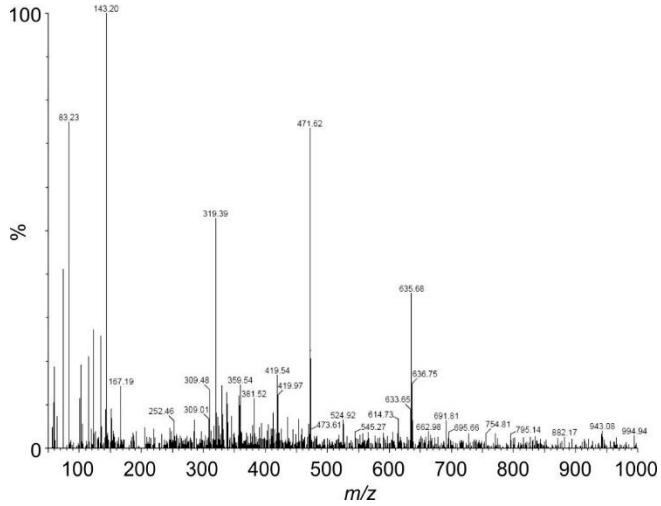

Fig. 12. Mass spectrum of **LLAN-6**

Based on the comprehensive spectral data, the structures of all the isolated compounds were elucidated and their chemical structures are presented in Fig. 13.

Conclusion

Phytochemical screening of *Lagerstroemia lanceolata* revealed the presence of diverse secondary metabolites, with the methanolic extract showing a richer composition than the ethyl acetate extract. The methanolic extract exhibited strong presence of flavonoids, tannins and phenols along with detectable levels of alkaloids, steroids, carbohydrates and proteins, whereas the ethyl acetate extract contained moderate amounts of polyphenolic compounds and lacked alkaloids. Glycosides and saponins were absent in both extracts, indicating limited occurrence or extractability under the selected conditions. These results suggest that *L. lanceolata* is particularly rich in polyphenolic constituents, especially in polar solvent extracts. The column chromatography followed by spectral analysis led to the isolation and identification of pentacyclic triterpenoids, namely oleanolic acid, ursolic acid and corosolic acid. Structural confirmation was achieved using IR, ^{1}H NMR, ^{13}C NMR and LC-MS techniques. The optimized chromatographic procedures enabled efficient separation and purification of bioactive constituents, providing a reproducible phytochemical profile of the plant. These findings expand the phytochemical knowledge of *L. lanceolata*, highlight its chemo-

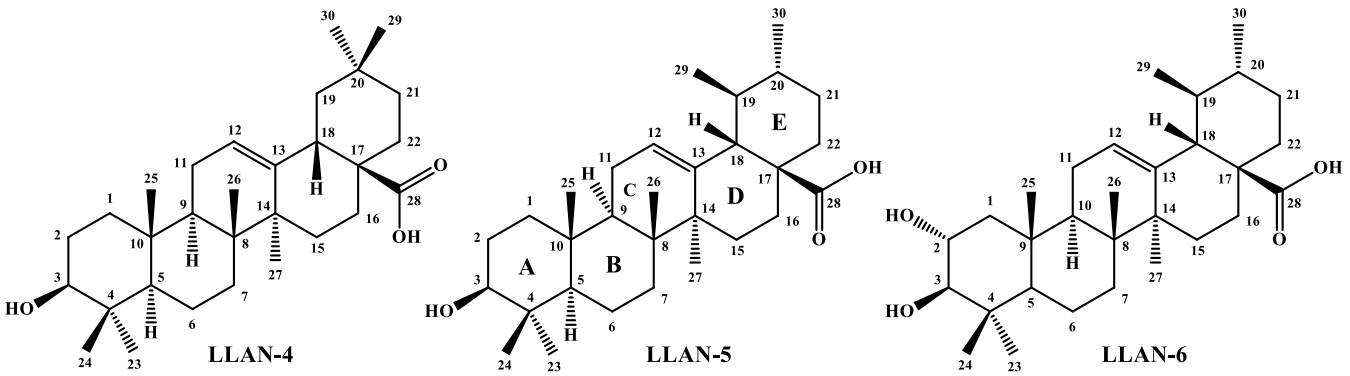

Fig. 13. Chemical structures of **LLAN-4**, **LLAN-5** and **LLAN-6** compounds isolated from methanolic extracts of *L. lanceolata*

taxonomic relevance and provide a foundation for further pharmacological studies, as well as quality control and standardization of herbal formulations derived from this species.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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