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EXPLORING KEY COMPOUNDS IN CALLICARPA LONGIFOLIA: A STUDY ON ISOLATION AND IDENTIFICATION

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ABSTRACT
Background: The present study aims to extract the bioactive phyto-components from the hydroalcoholic extract of *Callicarpa longifolia*. The study also aims to isolate and characterize the phytochemicals through quantitative and qualitative measures**. Methodology:** The mixture of Toluene, Ethyl Acetate, and Acetic Acid (8:4:0.4) was used as a solvent system with the thin-layer chromatography (TLC) technique. Twelve distinct spots were observed, indicating the presence of a variety of compounds with Rf values ranging from 0.12 to 0.89. The specific fractions were isolated using silica gel column chromatography, which was further analyzed with TLC. **Results and Discussion:** Fraction F showed consistency with standard terpenoids and was subjected to advanced analytical techniques for further evaluation. UV-visible spectroscopy identified a key absorption peak at 288 nm**,** while Fourier-transform infrared (FTIR) spectroscopy revealed functional groups such as alcohols, hydroxyls, alkanes, alkenes, and carboxylic acids**.** The compound's structure and proton environment were confirmed through ^1H Nuclear Magnetic Resonance (NMR) spectroscopy. The compound was identified as Methyl 3,11-dioxo-olean-12-en-28-oate with the molecular formula $C_{31}H_{46}O_4$, supported by mass spectrometry (M+ peak at 482.33 m/z). **Conclusion:** The significant bioactive terpenoids identified in the *C. longifolia* hydroalcoholic extract highlight its potential for developing therapeutic agents, particularly for anti-inflammatory and anticancer applications**.** The detailed phytochemical characterization provides a robust foundation for future studies exploring the pharmacological and clinical applications of *C. longifolia*. Quantitative analysis and advanced spectroscopic techniques confirmed the compound's identity and established its importance in medicinal chemistry.

INTRODUCTION

Callicarpa longifolia is a deciduous shrub from the Lamiaceae family. It is also well known as Beautyberry. It is characterized by its vibrant green, serrated, lance-shaped leaves, diminutive tubular flowers ranging from light purple to lavender, and distinctive clusters of vivid purple berries [1]. Indigenous to tropical and subtropical regions of Asia, encompassing Malaysia, India, China, Indonesia, and Thailand, *C. longifolia*

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exhibits adaptability across diverse habitats, thriving in sunlit, well-drained areas within lowland forests and hilly terrains. The plant is significant in landscaping and reforestation due to its ornamental appeal and adaptability [2]. In alternative medicine, various components of *C. longifolia*, including leaves, stems, and berries, have been traditionally utilized for their anti-inflammatory, antimicrobial, antioxidant, wound-healing [3], and analgesic properties. Approximately 80% of the world's population relies on herbal medicine as a primary source of healthcare, particularly in developing nations, emphasizing the significance of plants like *C. longifolia* in traditional and modern medicine systems [4–7]. The historical applications, such as the reduction of inflammation by topical application of extracts, wound care, and alleviation of fever and pain, have emphasized and validated the importance of medicinal value in plants [8–11].

The scientific investigation of phytochemicals in medicinal plants is a foundation for developing novel pharmaceuticals. These medicinal plants show diverse biological activities such as anticancer [12], antimicrobial [13], antioxidant, and anti-inflammatory properties [14-16]. Despite the long-standing traditional use of *C. longifolia***,** a significant knowledge gap exists in identifying and validating its bioactive compounds at a molecular level. Previous phytochemical studies on *C. longifolia* have primarily been limited to general screenings and lacked comprehensive characterization of specific bioactive compounds. These studies did not focus on advanced spectroscopic or chromatographic techniques, leaving critical gaps in structural elucidation. Moreover, the absence of data linking phytoconstituents to specific biological activities and their mechanisms of action restricts their utility in evidence-based medicine [17].

This study bridges this gap by isolating and characterizing specific phytoconstituents and linking them to therapeutic applications, providing a foundation for their inclusion in evidence-based medicine. Herbal renaissance is happening with advancements in nanotechnology and quality-by-design approaches, leading to the development of targeted drug delivery and different nano-herbal formulations for treating several diseases [18–24]. The presence of bioactive compounds and traditional applications of *C. longifolia* roots were the selections of this study. The preliminary studies have shown the presence of beneficial phytochemicals such as flavonoids, terpenoids, and phenolic acids. A detailed study of phytoconstituents of *C. longifolia* has yet to be attained.

The primary purpose of the present study is to isolate, characterize, and identify the bioactive phytoconstituents from *C. longifolia*. This will validate the traditional approach and applications of unraveling new therapeutic agents, significantly contributing to drug development and phytochemistry. Phytoconstituents are the naturally occurring chemical compounds in plants responsible for therapeutic activity.

These constituents encompass alkaloids, flavonoids, terpenoids, phenolic acids [25, 26], glycosides, and essential oils, each harboring unique biological effects such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Within medicinal chemistry and pharmacology, phytoconstituents are fundamental for formulating new drugs and therapeutic agents. Identifying potential treatments for various conditions and developing new pharmaceuticals are facilitated by examining these constituents, owing to their broad spectrum of biological activities. Globally, over 25% of prescription drugs are derived directly from plant-based compounds, reflecting the importance of medicinal plants like *C. longifolia* in modern drug discovery. Moreover, a more profound comprehension of their pharmacological effects advocates for enhancing traditional medicinal practices and their integration into modern medical treatments [27].

Regardless of existing research on *C. longifolia*, some areas related to bioactive compounds are still to be explored. The study not only focuses on isolating key bioactive compounds but also investigates their mechanism of action to validate traditional use and establish safety and toxicity profiles. This fills critical gaps in understanding their potential therapeutic roles and ensures their safe application in pharmacology. A detailed understanding of the mechanism of action of bioactive compounds validates the traditional use of plants and further pertains to their safety and toxicity [28]. The study also helps identify and characterize inherent phytoconstituents, filling the knowledge gaps. The study aims to elucidate biological mechanisms, validate traditional applications, and develop formulations [29]. The study's primary objective was to isolate various phytoconstituents. The chemical and structural properties of *C. longifolia* were analyzed using extraction methods and spectroscopic and chromatographic techniques

[30]. The identified phytoconstituents were subjected to various evaluations such as antioxidant, anti-inflammatory, and antimicrobial activity to ascertain their therapeutic and biological potential. The main goal is to understand the phytochemical and biological profile of *C. longifolia* and explore its potential applications in medicine and pharmacology [31].

MATERIALS AND METHODS

Chemicals and Reagents: Toluene and Silica Gel H were procured from Merck Ltd., Mumbai. Meanwhile, ethyl acetate and KBr were from Sigma Ltd. Mumbai and Rankem Ltd. Haryana, respectively.

Collection of plant material

Healthy plants of *C. longifolia* were procured from the local Hyderabad market. After collection, the leaves were washed thoroughly and shade-dried. After drying, the leaves were powdered finely using a high-speed laboratory mixer grinder for five minutes. The powder was sieved through a number 85 sieve to ensure uniformity. The powder was stored in an airtight container for further use.

Preparation of plant extract (Soxhlet extraction)

The dried and powdered leaves of *C. longifolia* were initially defatted with petroleum ether before being subjected to extraction using a Soxhlet apparatus. The extraction was carried out using a hydroalcoholic solvent maintained at 40 and 60 °C for 8 and 10 hours. After extraction, the sample was filtered and concentrated until dry. The resulting extracts were then stored in an airtight container [32].

Preliminary Thin Layer Chromatography

Thin-layer chromatography (TLC) is a solid-liquid adsorption technique in which plates with 0.2 mm silica gel 60 F254 are used as the stationary phase. The solvent system, also known as the mobile phase, ascends the stationary phase, and samples are manually applied to the plates using a capillary tube.

After air drying, the plates are developed in a TLC chamber at room temperature with the selected solvent system. As the solvent moves up the plate by capillary action, it carries the applied mixture upwards at different rates, separating the analytes based on their polarity, solid phase, and solvent [33]. In the preliminary TLC for the hydroalcoholic extract of *C. longifolia*, the maximum spots were observed using a mobile phase of Toluene: Ethyl acetate: Acetic acid (8:4:0.4) with a standard terpenoid. Therefore, this solvent system (Toluene: Ethyl acetate: Acetic acid in a ratio of 8:4:0.4) was chosen as the mobile phase for column chromatography.

Column chromatography

To isolate terpenoids, the hydroalcoholic extract of *C. longifolia* underwent silica gel column chromatography. A borosilicate glass column was cleaned with acetone and thoroughly dried before being packed using the wet packing method with silica gel (60-120 mesh). A toluene slurry was prepared and poured into the column, adding 1 gram of the extract. The column was eluted using a gradient solvent system of Toluene, Ethyl Acetate, and Acetic acid in an 8:4:0.4 ratio. TLC collected, concentrated, and analyzed multiple fractions to identify individual compounds [34].

Spectroscopic characterization

UV-visible Spectroscopy

The isolated fraction (F) from the *C. longifolia* extract was analyzed using a Shimadzu UV-1700 UV-visible spectrophotometer across a wavelength range of 200 to 800 nm. The resulting characteristic peaks were detected and documented [35].

FT-IR

The functional groups of the isolated fraction were identified using FTIR spectroscopy. The dried sample was ground with KBr pellets and analyzed using a Thermo Nicolet 6700 spectrometer. A disk was created by combining 2% of the dried sample with 200 mg of KBr, and the infrared spectra were recorded over a range of 400 to 4000 cm^{-1} [36, 37].

NMR Spectroscopy

The compound was elucidated structurally using NMR spectroscopy with a JEOL RESONANCE Fourier Transform Nuclear Magnetic Resonance (FT-NMR) spectrometer [38].

Mass Spectroscopy

Mass spectrometry, a technique that converts molecules into ions for separation and sorting by mass and charge, was used to determine the molecular weight of the isolated fraction (F) from the *C. longifolia* extract. This analysis used a micro TOF-Q 228888.10348 mass spectrometer [39,40].

RESULTS AND DISCUSSIONS

Preliminary Thin layer chromatography: The thin-layer chromatography (TLC) analysis for the hydroalcoholic extract of *C. longifolia* was conducted using silica gel 60 F254 pre-coated plates with a 0.2 mm layer thickness. Various solvent systems were evaluated to achieve optimal separation. Among these, the Toluene: Ethyl Acetate: Acetic Acid (8:4:0.4) solvent system demonstrated the most distinct separation, showing a maximum number of visible spots. The mobile phase traveled upwards through the stationary phase via capillary action, effectively separating the analyte applied at the bottom of the plate. For the preliminary TLC analysis, the Toluene: Ethyl Acetate: Acetic Acid (8:4:0.4) solvent system was selected based on the visibility of the spots when viewed under UV light at 365 nm and 254 nm. The results revealed 12 distinct spots with varying colors and fluorescence intensities.

The extract's Rf values ranged from 0.28 to 0.87, with the standard terpenoid showing a prominent spot at an Rf value of 0.59 (Table 1).

Table 1: TLC of *Callicarpa longifolia* Hydro alcoholic extract.

Solvent system	No. of spots	Colour of spots at Wavelength (365nm)	Colour of spots at Wavelength (254)	Rf value (Extract)	Rf value (Std. Terpenoid)
Toluene: Ethyl Acetate: Acetic acid (8:4:0.4)	12	Dark Blue (Std.)	Green (Std.)	-	0.59
		Blue (CL)	Dark Green (CL)	0.28	
		Florescence	Light Green	0.32	
		Florescence	Green	0.44	
		Florescence	Light Green	0.48	
		Florescence (White)	-	0.51	
		Florescence	Green	0.54	
		Light blue	Dark Green	0.59	
		Florescence	Light Green	0.67	
		Florescence	Light Green	0.74	
		Florescence	Green	0.82	
		Light Florescence	Light Green	0.87	

The colors observed under UV light at 365 nm included dark blue, fluorescence (white), light blue, light fluorescence, and several shades of green, corresponding to both the extract & the standard terpenoid. Under UV light at 254 nm, the colors were primarily fluorescence and various shades of green, Figure 1

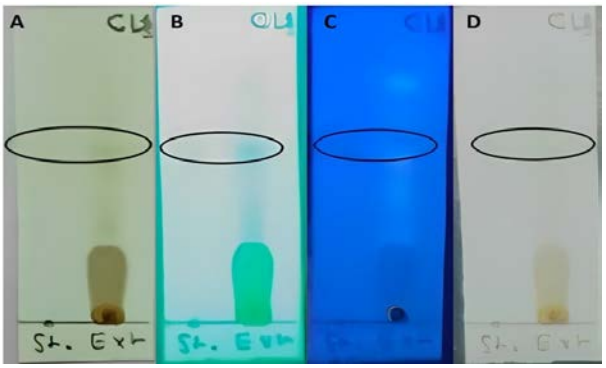

Figure 1: Thin Layer Chromatography estimation of *C. longifolia* against standard under UV radiation. TLC of A) *C. longifolia* B) Under Short-UV (254 nm) C) Under Long-UV (254 nm) D) Under Visible light (Std.= Standard, CL = *C. longifolia*)

The TLC analysis confirmed the presence of terpenoids in the *C. longifolia* extract, as indicated by the matching Rf value of 0.59 for both the extract and the standard terpenoid. This solvent system was, therefore, deemed suitable for further separation and analysis using column chromatography.

Column chromatography: To isolate terpenoids, the hydroalcoholic extract of *C. longifolia* underwent silica gel column chromatography. A borosilicate glass column was cleaned with acetone and dried before being packed with silica gel (60–120 mesh) using the wet packing method. A toluene slurry was prepared and added to the column, followed by 1 gram of the extract placed on top. The column was eluted using a gradient solvent system of toluene, ethyl acetate, and acetic

acid in an 8:4:0.4 ratio, yielding several fractions. The fractions were concentrated and analyzed using TLC. Each collected fraction was further subjected to UV spectrum analysis. The TLC analysis revealed various fractions with distinct colorations, indicating the presence of different phytocompounds (Table 2).

Table 2: Fraction collected from Column Chromatography of *C. longifolia* Hydro alcoholic extract

Eluent composition	Fraction collected	Remarks
Toluene: Ethyl Acetate: Acetic acid (8:4:0.4)	01-2 (A),(A1, A2)	White colored mixture of compound
	03 (B)	Creamy colored mixture of compound
	04 (C)	Light Greenish colored mixture of compound
	05 (D)	Creamy colored mixture of compound
	06 (E)	White colored mixture of compound
	07 (F)	Light Greenish colored mixture of compound
	08 (G)	Very Light Greenish colored mixture of compound
	09 (H)	White colored mixture of compound
	10 (I)	Light Greenish colored mixture of compound
	11 (J)	White colored mixture of compound
	12 (K)	Creamy colored mixture of compound
	13 (L)	White colored mixture of compound

Table 3: Rf values of all collected fractions of CL after column chromatography.

SNo	Fraction	Solvent system	No. of spots	Color of spots at 365 nm	Color of spots at 254 nm	Rf value (Extract)	Rf value (Std. Terpenoid)
1	A1	Toluene: Ethyl Acetate: Acetic acid (8:4:0.4)	-	-	-	-	0.61
2	A2		-	-	-	-	
3	B		01	Fluorescence	Light Green	0.89	
4	C		01	Fluorescence	Light Green	0.84	
5	D		01	Fluorescence	Light Green	0.78	
6	E		01	Fluorescence	Light Green	0.63	
7	F		01	Blue	Green	0.61	
8	G		02	Fluorescence Fluorescence	Light Green Light green	0.58 0.56	
9	H		02	Fluorescence Fluorescence	Light Green Light green	0.48 0.51	
10	I		02	Blue Fluorescence	Green Light Green	0.38 0.42	
11	J		01	Blue	Dark green	0.27	
12	K		01	Blue	Dark green	0.09	
13	L		-	-	-		

The initial fractions (A1, A2) were white, followed by a creamy-colored fraction (B). Subsequent fractions included light greenish (C), creamy (D), and white-colored mixtures (E). Light greenish fractions (F, G, I) and very light greenish mixtures (G) were also observed, along with additional white (H, J, L) and creamy-colored fractions (K) (Figure 2).

This detailed fractionation and subsequent analysis confirmed the successful isolation of various phytocompounds, including terpenoids, from the hydroalcoholic extract of *C. longifolia*. The TLC analysis of the fractions collected from the silica gel column chromatography of *C. longifolia* hydroalcoholic extract revealed distinct phytocompounds (Table 3).

Figure 2: Isolation of components by column chromatography

This detailed fractionation and subsequent analysis confirmed the successful isolation of various phytocompounds, including terpenoids, from the hydroalcoholic extract of *C. longifolia*. The TLC analysis of the fractions collected from the silica gel column chromatography of *C. longifolia* hydroalcoholic extract revealed distinct phytocompounds (Table 3). Using the solvent system Toluene: Ethyl Acetate: Acetic Acid (8:4:0.4), various fractions exhibited different Rf values and spot characteristics at wavelengths of 365 nm and 254 nm. Fraction B showed an Rf value of 0.89 with fluorescence, while fractions C, D, and E had Rf values of 0.84, 0.78, and 0.63, respectively, also displaying fluorescence. Fraction F, which matched the standard terpenoid with an Rf value of 0.61, exhibited a blue spot at 365 nm and green at 254 nm. Fractions G, H, and I had multiple spots with Rf values ranging from 0.38 to 0.58, showing fluorescence and light green colors. Fractions J and K displayed blue spots with Rf values of 0.27 and 0.09, respectively (Figure 3).

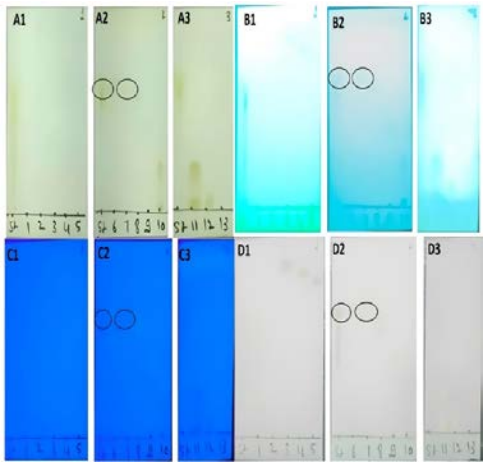

Figure 3: Thin Layer Chromatography estimation for fractions of *C. longifolia* after column chromatography under UV radiation. A1, A2and A3) *C. longifolia* B1, B2 and B3) Short-UV (254 nm), C1, C2 and C3) Long-UV (365 nm), D1, D2 and D3) visible light. (Std.= Standard)

This comprehensive analysis confirmed the presence of active constituents, particularly terpenoids, in the *C. longifolia* extract, validating the successful isolation and identification process.

Spectroscopic characterization:

Active constitutes estimation by UV-spectrophotometer: The isolated fraction (F) of the *C. longifolia* hydroalcoholic extract was analyzed using a UV-visible spectrophotometer (Shimadzu UV-1700) over a scanning range of 200 to 800 nm. The UV spectra were recorded using Toluene: Ethyl Acetate: Acetic Acid (8:4:0.4) as the solvent and blank. The analysis revealed a characteristic absorption peak at a wavelength of 288 nm, indicating the λmax of the isolated compound. This UV spectrophotometric analysis confirms the presence of specific phytocompounds in fraction F, further supporting the successful isolation and identification of active constituents from the *C. longifolia* extract.

Active constitutes estimation by FTIR.

Determination of the functional groups in the isolated fraction (F) from the hydroalcoholic extract of *C. longifolia*, FT-IR spectroscopy was conducted. The sample was dried and ground with KBr pellets. A disk containing 200 mg of KBr and 2% of the dried sample was prepared and analyzed using a Thermo Nicolet 6700 FT-IR spectrometer. Spectra were recorded in the 400 to 4000 cm^{-1} range. The IR spectra of the isolated fraction (F) indicated the presence of several functional groups (Figure 4).

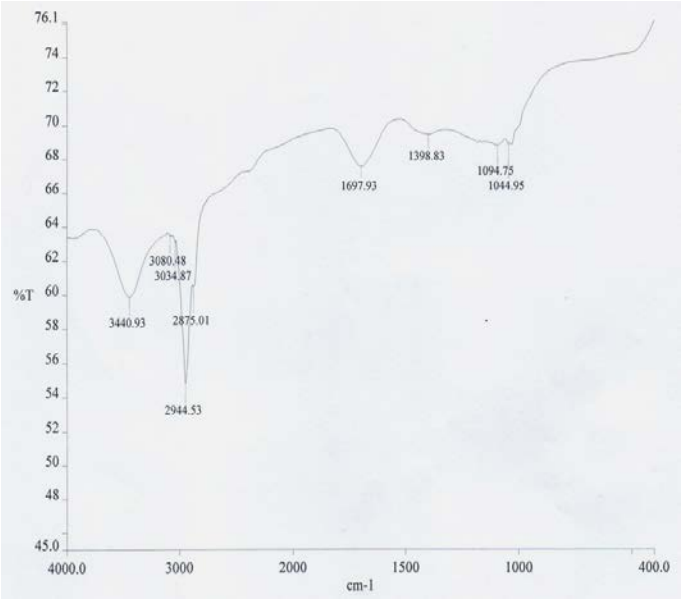

Figure 4: IR spectra of the isolated fraction (F) of *C. longifolia* Hydro alcoholic extract

A strong, broad peak at 3440.93 cm^{-1} corresponded to O-H stretching, indicating hydroxyl groups. Medium peaks at 3080.48 cm^{-1}, 2944.53 cm^{-1}, and 2875.01 cm^{-1} were attributed to C-H stretching in alkenes and alkanes. A strong peak at 1697.93 cm^{-1} indicated C=O stretching, characteristic of acids, while a medium peak at 1398.83 cm^{-1} was due to O-H bending in carboxylic acids. A strong peak at 1094.75 cm^{-1} was observed for C-O stretching, confirming the presence of alcohols (Table 4).

Table 4: FTIR- Spectrum Frequency Range of the isolated fraction (F) of CL Hydro alcoholic extract.

Fraction	Frequency Range (cm^{-1})	Group Absorption (cm-1)	Appearance	Group	Compound Class
F	3550-3200	3440.93	Strong, Broad	O-H stretching	Hydroxyl Group
	3100-3000	3080.48	Medium	C-H stretching	Alkene
	3000-2840	2944.53	Medium	C-H stretching	Alkane
	3000-2840	2875.01	Medium	C-H stretching	Alkane
	1710-1680	1697.93	Strong	C=O stretching	Acid
	1440-1395	1398.83	Medium	O-H bending	Carboxylic acid
	1124-1087	1094.75	Strong	C-O stretching	Alcohol

The FTIR analysis of Methyl 3,11-dioxo-olean-12-en-28-oate identifies key functional groups, including hydroxyl, alkene, and carbonyl stretches, aligning with similar triterpenoids. These findings corroborate the successful isolation and identification of functional groups in the fraction (F) of the *C. longifolia* extract, supporting its characterization and potential bioactivity.

1H NMR: To determine the structure of the compound present in the isolated fraction (F) of the *C. longifolia* hydroalcoholic extract, NMR spectroscopy was performed. Tetramethylsilane was used as an internal standard, with the signals denoted as s, d, t, and m for singlet, doublet, triplet, and multiplets, respectively (Figure 5).

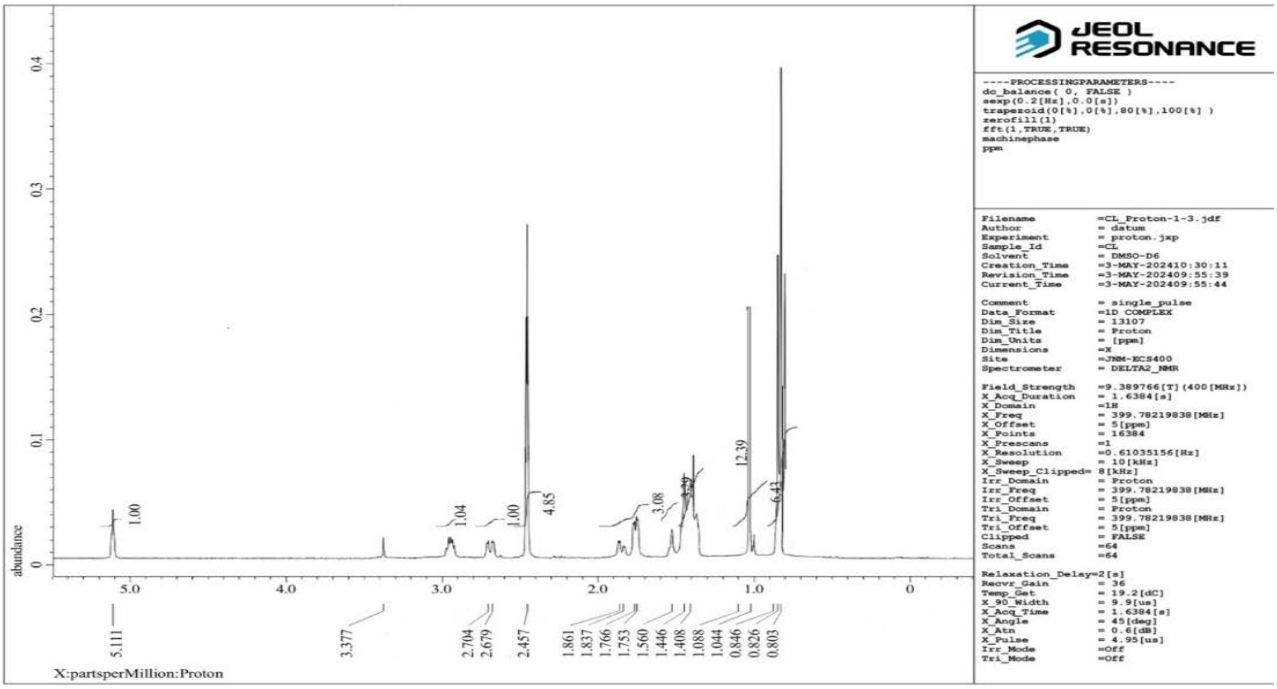

Figure 5: l H-NMR spectra of the isolated compound Fraction (F) of *C. longifolia*

The [1]HNMR spectra of the isolated fraction (F) revealed several distinct proton signals. In the 1H NMR spectrum, coupling constants (J values) of 7–8 Hz confirm the vicinal relationship of protons, aiding in the structural assignment. The 1HNMR-12 protons appeared in the 0.88-1.03 ppm range, with specific singlets at 0.80, 0.82, and 0.84 ppm. H-6 protons were observed

as a doublet of doublets (dd) at 1.04 ppm, and H-3 protons also appeared as a (dd) at 1.08 ppm. H-17 protons showed multiple signals between 1.50 and 1.94 ppm, including ddd (doublet of doublet of doublets), dddd (doublet of doublet of doublet of doublets), and (dd) peaks. Specific peaks for H-17 included signals at 1.40, 1.44, 1.56, 1.75, 1.76, 1.83, and 1.86 ppm. H-2 protons appeared as a (ddd) at 2.45 ppm, while H-1 protons exhibited doublets at 2.67 and 2.70 ppm. Additionally, H-3 protons presented a singlet at 3.37 ppm, and a distinct singlet for H-1 protons was observed at 5.11 ppm. These ^{1}HNMR spectral data provided crucial insights into the structure of the compound present in the isolated fraction (F), confirming the presence of various proton environments and aiding in the structural elucidation of the phytoconstituents from *C. longifolia* extract.

Mass Spectroscopy: The molecular weight and composition of the isolated fraction (F) were determined using a Bruker micrOTOF-Q mass spectrometer. This technique involves converting molecules into ions, which are then separated and sorted based on their mass-to-charge (m/z) ratios (Figure 6).

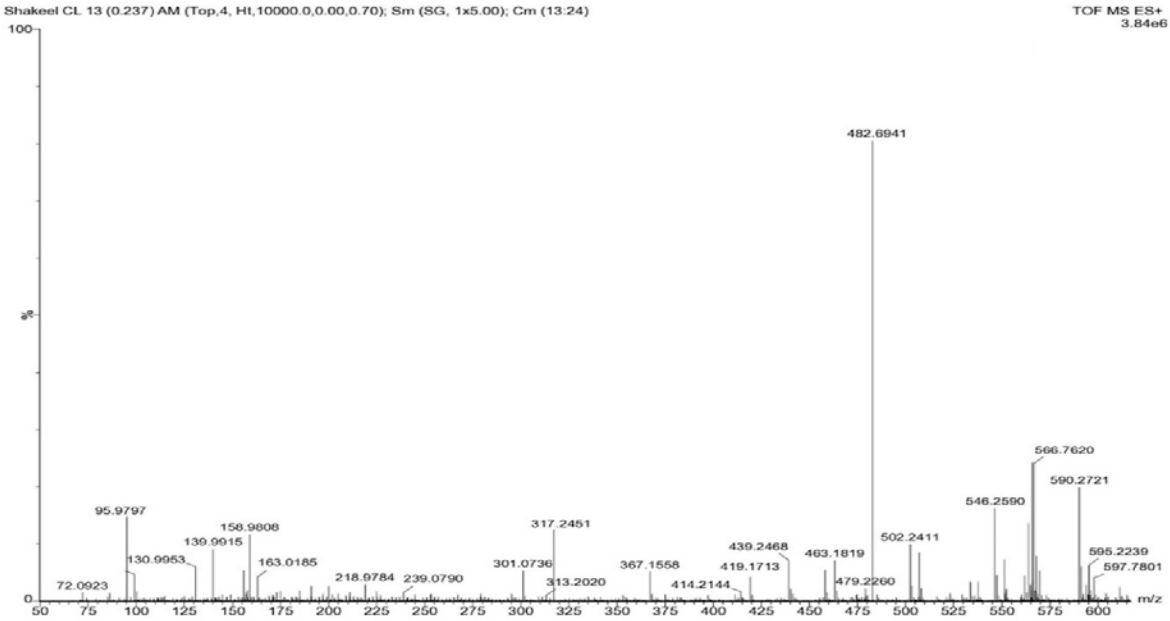

Figure 6: Mass spectra of the isolated Fraction (F) of *C. longifolia*

The isolated fraction (F) mass spectrum revealed a prominent molecular ion [M+] peak at m/z 482.6941. Analysis of the elemental composition indicated the presence of 31 carbon atoms, 46 hydrogen atoms, and 4 oxygen atoms, corresponding to the molecular formula $C_{31}H_{46}O_4$. This data identified the compound as Methyl 3, 11-dioxo-olean-12-en-28-oate, as deduced from its fragmentation pattern. The mass spectrum reveals a molecular ion peak at m/z 482, with fragmentation at m/z 437 and m/z 405, supporting the loss of methoxy and keto groups, consistent with fragmentation patterns in oleanane triterpenoids. This mass spectrometric analysis confirmed the molecular weight and structure of the isolated phytoconstituents, further validating the successful isolation process from the *C. longifolia* extract.

CONCLUSION

This study undertook comprehensive isolation, identification, and characterization of bioactive phytochemicals from the hydroalcoholic extract of *C. longifolia*. The selection of petroleum ether for defatting effectively removes nonpolar components, enhancing the subsequent hydroalcoholic extraction of polar and moderately polar phytochemicals such as flavonoids and phenolic. The hydroalcoholic solvent was chosen for its broad extraction range, combining ethanol's ability to dissolve moderately polar compounds and water's affinity for highly polar constituents. Soxhlet extraction at 40–60°C ensures optimal recovery while protecting thermolabile compounds, with an 8–10-hour duration allowing exhaustive extraction without degradation. Advanced techniques like HPTLC, HPLC, and GC-MS provide precise identification and quantification of bioactive compounds, ensuring a comprehensive phytochemical profile of *C. longifolia*.

Initially, thin-layer chromatography (TLC) was employed to detect and separate the phytoconstituents present in the extract.

Using the solvent system Toluene: Ethyl Acetate: Acetic Acid (8:4:0.4), twelve distinct spots were identified, indicating a range of compounds, including a fraction with an Rf value that matched standard terpenoids. Following TLC, silica gel column chromatography was utilized to isolate specific extract fractions further. The fractions were subsequently analyzed using TLC to confirm the presence and purity of the phytochemicals. Fraction F, in particular, showed properties consistent with standard terpenoids. A suite of analytical techniques was employed to delve deeper into the composition of fraction F. UV-visible spectrophotometry revealed a characteristic absorption peak at 288 nm, indicative of specific phytocompounds. Fourier transform infrared (FT-IR) spectroscopy identified different functional groups, such as hydroxyl, alkene, alkane, acid, and alcohol groups, with strong and distinct absorption peaks. The structure of the isolated compound was further elucidated using 1H Nuclear magnetic resonance (NMR) spectroscopy. The 1H NMR spectra detailed the proton environments, revealing multiple signals corresponding to the expected chemical shifts of the compound's protons. Finally, mass spectrometry provided a precise molecular weight and compositional analysis, confirming the molecular formula as C31H46O4. The primary compound identified was Methyl 3,11-dioxo-olean-12-en-28-oate, deduced at m/z 482.6941 from the molecular ion peak (Figure 7).

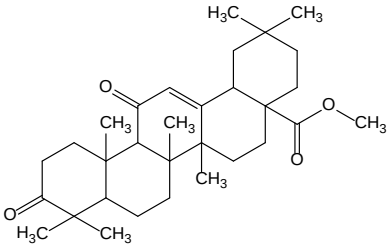

Figure 7: Methyl 3, 11-dioxo-olean-12-en -28-oate

The above study highlights the importance of isolating and identifying phytoconstituents from herbal drugs, which contributes to formulating novel therapeutic agents. This integrated approach effectively isolated and identified key bioactive compounds from the *C. longifolia* extract. The findings underscore the presence of significant phytochemicals, particularly terpenoids, which hold potential medicinal and pharmacological applications. The methodologies applied in this study, encompassing chromatography, UV-visible spectrophotometry, FT-IR, 1H NMR, and mass spectrometry, collectively facilitated a thorough characterization of the extract's bioactive constituents.

Methyl 3,11-dioxo-olean-12-en-28-oate, an oleanane-type triterpenoid, offers promising applications in anti-inflammatory, anticancer, and antioxidant therapies, given its structural alignment with bioactive triterpenoids used in pharmaceuticals. It could address conditions like chronic inflammation, oxidative stress disorders, and cancer, among others. However, the study is limited by variability in compound yields due to environmental and methodological factors, a lack of biological validation through in vitro or in vivo assays, and unexplored pharmacokinetics and toxicity profiles. Addressing these limitations through standardized extraction methods, comprehensive bioactivity testing, and pharmacological studies is essential for its advancement into therapeutic applications. These results not only enhance the understanding of the phytochemical profile of *C. longifolia* but also pave the way for future research into its therapeutic properties. Future investigations should focus on exploring the bioactivity of other fractions obtained during the chromatographic separation, as these may harbor additional compounds with significant pharmacological potential. Furthermore, in vivo and in vitro studies are essential to validate the therapeutic efficacy and safety of the identified compounds.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Md. Shakeel Alam carried out data collection, drafted the article, and wrote the article. Nidhi Shrivastava conceptualized, designed the work and critically revised the article. All the authors have read and approved the manuscript, and each author has agreed to its publication.

REFERENCES
[1] Gao C, Weibang S. The role of botanical gardens in scientific research, conservation, and citizen science. *Plant Diversity*, **40(4)**, 181-188 (2018) https://doi.org/10.1016/j.pld.2018.07.006
[2] Wang WY, Ma P, Xu LJ, Peng Y, Xiao PG. Chemical constituents and biological activities of plants from the genus Neolitsea. *Chemistry & Biodiversity*, **11(1)**, 55-72 (2014) https://doi.org/10.1002/cbdv.201200092
[3] Zhang XG, Li XM, Zhou XX, Wang Y, Lai WY, Liu Y, Luo YC, Zhang JQ. The Wound Healing Effect of Callicarpa nudiflora in Scalded Rats. *Evidence-Based Complementary and Alternative*

Medicine, **2019(1)**, 1860680 (2019) https://doi.org/10.1155/2019/1860680

[4] Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. *Journal of Traditional and Complementary Medicine*, **7(2)**, 234-244, (2017) http://dx.doi.org/10.1016/j.jtcme.2016.05.006

[5] Van Herten LM, Van de Water HP. New global Health for All targets. *BMJ*, **319 (7211)** 700-703 (1999) https://doi.org/10.1136/bmj.319.7211.700

[6] Sen S, Chakraborty R. Toward the integration and advancement of herbal medicine: a focus on traditional Indian medicine. *Botanics: Targets and Therapy*, **2015(5)**, 33-44 (2015) https://doi.org/10.2147/BTAT.S66308

[7] Sen S, Chakraborty R, De B. Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. *Journal of Herbal Medicine*, **1(3–4)**, 67-75 (2011) https://doi.org/10.1016/j.hermed.2011.11.001

[8] Manginstar CO, Tallei TE, Niode NJ, Salaki CL, Hessel SS. Therapeutic potential of propolis in alleviating inflammatory response and promoting wound healing in skin burn. *Phytotherapy Research*, **38(2)**, 856-879, (2024) https://doi.org/10.1002/ptr.8092

[9] Recio MC, Andujar I, Rios JL. Anti-inflammatory agents from plants: progress and potential. *Current Medicinal Chemistry*, **19(14)**, 2088-2103 (2012) http://dx.doi.org/10.2174/092986712800229069

[10] Jain P, Taleuzzaman M, Kala C, Gupta DK, Ali A, Aslam M. Quality by design (Qbd) assisted development of phytosomal gel of aloe vera extract for topical delivery. *Journal of Liposome Research*, **31(4**), 381-388 (2021) https://doi.org/10.1080/08982104.2020.1849279

[11] Taleuzzaman M, Sartaj A, Gupta DK, Gilani SJ, Mirza MA. Phytosomal gel of Manjistha extract (MJE) formulated and optimized with central composite design of Quality by Design (QbD). *Journal of Dispersion Science and Technology*, **44(2)**, 236-244 (2023) https://doi.org/10.1080/01932691.2021.1942036

[12] Ugwu CE, Suru SM. Medicinal plants with hepatoprotective potentials against carbon tetrachloride-induced toxicity: a review. *Egyptian Liver Journal*, **11**, 88 (2021) https://doi.org/10.1186/s43066-021-00161-0

[13] Pham-Huy LA, He H, Pham-Huyc C. Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science*, **4(2)**, 89-96 (2008) http://dx.doi.org/10.59566/IJBS.2008.4089

[14] Sherif AE, Alam R, Asif M, Khan KU, Ur Rehman MS. Evaluation of the anti-inflammatory, antinociceptive, and antipyretic potential of ethanolic extract of Aristida depressa Retz through in vitro, in vivo, and in silico models. *Frontiers in Pharmacology*, **10**, 15:13264822024 (2024) https://doi.org/10.3389/fphar.2024.1326482

[15] Taleuzzaman M, Jain P, Verma R, Iqbal Z, Mirza M. Eugenol as a potential drug candidate: a review. *Current topics in medicinal chemistry*, **21(20)**, 1804-1815 (2021) https://doi.org/10.2174/1568026621666210701141433

[16] Sharma N, Samarakoon KW, Gyawali R, Park YH, Lee SJ, Oh SJ, Lee TH, Jeong DK. Evaluation of the antioxidant, anti-inflammatory, and anticancer activities of Euphorbia hirta ethanolic extract. *Molecules*, **19(9)** 14567-14581 (2014) https://doi.org/10.3390/molecules190914567

[17] Ma Y, Guo L, Hou W, Zhuo J, Zhao M, Liu Y. Phytochemistry, bioactivities, and future prospects of *Callicarpa nudiflora*: A review. *Medicinal Plant Biology*, **1:5** (2022) https://doi.org/10.48130/MPB-2022-0005

[18] Sindhu RK, Gupta R, Wadhera G, Kumar P. Modern herbal nanogels: formulation, delivery methods, and applications. *Gels*, ***8*(2)**, 97 (2022) https://doi.org/10.3390/gels8020097

[19] Alexander A, Patel RJ, Saraf S, Saraf, S. Recent expansion of pharmaceutical nanotechnologies and targeting strategies in the field of phytopharmaceuticals for the delivery of herbal extracts and bioactive. *Journal of controlled release*, **241**, 110-124 (2016) https://doi.org/10.1016/j.jconrel.2016.09.017

[20] Bonifacio B V, Da Silva P B, Ramos MA D S, Negri KMS, Bauab TM, Chorilli M. Nanotechnology-based drug delivery systems and herbal medicines: a review. *International Journal of Nanomedicine*, **2014:9(1)**, 1-15 (2014) https://doi.org/10.2147/IJN.S52634

[21] Ahmed HM, Nabavi S, Behzad S. Herbal drugs and natural products in the light of nanotechnology and nanomedicine for developing drug formulations. *Mini Reviews in Medicinal Chemistry*, **21(3)**, 302-313 (2021) https://doi.org/10.2174/1389557520666200916143240

[22] Devi VK, Jain N, Valli KS. Importance of novel drug delivery systems in herbal medicines. *Pharmacognosy Reviews*, **4(7)**, 27-31 (2010) https://doi.org/10.4103/0973-7847.65322

[23] Ansari SH, Islam F, Sameem M. Influence of nanotechnology on herbal drugs: A Review. *Journal of Advanced Pharmaceutical Technology & Research*, **3(3)**, 142-146 (2012) https://doi.org/10.4103/2231-4040.101006

[24] Yang S, Liu L, Han J, Tang Y. Encapsulating plant ingredients for dermo cosmetic application: An updated review of delivery systems and characterization techniques. *International journal of cosmetic science*, **42(1),** 16-28 (2020) https://doi.org/10.1111/ics.12592

[25] Han F, Ma GQ, Yang M, Yan L, Xiong W, Shu JC, Zhao ZD, Xu HL. Chemical composition and antioxidant activities of essential oils from different parts of the oregano. *Journal of Zhejiang*

University-Science B, **18(1)** 79-84 (2017) https://doi.org/10.1631/jzus.b1600377

[26] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, **2013(1),** 62750 (2013) http://dx.doi.org/10.1155/2013/162750

[27] Cai H, Xie Z, Liu G, Sun X, Peng G, Lin B, Liao Q. Isolation, identification and activities of natural antioxidants from *Callicarpa kwangtungensis* Chun. *PLoS One*, **9(3)** e93000 (2014) https://doi.org/10.1371/journal.pone.0093000

[28] Alara OR, Abdurahman NH, Ukaegbu C I, Kabbashi NA. Extraction and characterization of bioactive compounds in Vernonia amygdalina leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. *Journal of Taibah University for Science*, **13(1)**, 414-422 (2019) https://doi.org/10.1080/16583655.2019.1582460

[29] Arya A, Dubey SK, Lauthani A, Bahuguna A, Kumar R, Prakash O, Rawat DS. Phytochemical profile and antioxidant potential of extracts of Callicarpa macrophylla Vahl. from Uttarakhand Himalayan region. *International Journal of Plant Based Pharmaceuticals*, **3(2)**, 131-140 (2023) https://doi.org/10.29228/ijpbp.25

[30] Lin YC, Lin JJ, Chen SR, Hwang TL, Fang SY, Korinek M, Chen CY, Lin YS, Wu TY, Yen MH, Wang CH, Cheng YB. Clerodane Diterpenoids from *Callicarpa hypoleucophylla* and Their Anti-Inflammatory Activity. *Molecules*, **25(10)**, 2288 (2020) https://doi.org/10.3390/molecules25102288

[31] Rahim A, Gahlot G, Jakhmola V, Gairola N. Pharmacological and Traditional Insights into *Callicarpa macrophylla*: A Comprehensive Review. *Journal of Natural Remedies*, **25(1)**, 19-32 (2025) https://doi.org/10.18311/jnr/2025/46366

[32] Shaikh SS, Bawazir AS, Yahya BA. Phytochemical, histochemical and in vitro antimicrobial study of various solvent extracts of costus speciosus (j. koenig) sm. and costus pictus d. don. *Turkish Journal of Pharmaceutical Sciences*, **19(2)**, 145-152 (2022) https://doi.org/10.4274/tjps.galenos.2021.02058

[33] Coskun O. Separation techniques: chromatography. *Northern Clinics of Istanbul*, **3(2)**, 156-160 (2016) https://doi.org/10.14744/nci.2016.32757

[34] Srivastava N, Singh A, Kumari P, Nishad JH, Gautam VS, Yadav M, Kharwar RN. Advances in extraction technologies: Isolation and purification of bioactive compounds from biological materials. *Natural bioactive compounds*, Academic Press. 409-433 (2021) https://doi.org/10.1016/B978-0-12-820655-3.00021-5

[35] Mandru A, Mane J, Mandapati R. A Review on UV-visible spectroscopy. *Journal of Pharma Insights and Research*, **1(2)**, 091-096 (2023) https://jopir.in/index.php/journals/article/view/42

[36] Moraes LGP, Rocha RSF, Menegazzo LM, Araujo EBD, Yukimito K, Moraes JCS. Infrared spectroscopy: a tool for determination of the degree of conversion in dental composites. *Journal of Applied Oral Science*, **16(2)**, 145-149 (2008) https://doi.org/10.1590/S1678-77572008000200012

[37] Ribaudo G. Natural Products Chemistry: Advances in Synthetic, Analytical and Bioactivity Studies. *Molecules*, **28(14)**, 5577 (2023) https://doi.org/10.3390/molecules28145577

[38] Zia K, Siddiqui T, Ali S, Farooq I, Zafar MS, Khurshid Z. Nuclear magnetic resonance spectroscopy for medical and dental applications: a comprehensive review. *European Journal of Dentistry*, **13(01)**, 124-128 (2019) https://doi.org/10.1055/s-0039-1688654

[39] Arevalo Jr R, Ni Z, Danell RM. Mass spectrometry and planetary exploration: A brief review and future projection. *Journal of Mass Spectrometry*, **55(1)**, e4454 (2020) https://doi.org/10.1002/jms.4454

[40] Yan S, Bhawal R, Yin Z, Thannhauser TW, Zhang S. Recent advances in proteomics and metabolomics in plants. *Molecular Horticulture*, **2(17)**, (2022) https://doi.org/10.1186/s43897-022-00038-9