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The Chemical Study of Components of *Saussurea lappa* Root Extract

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ABSTRACT

Natural products, sourced from plants, marine organisms, and microorganisms, have been vital to human health for millennia, offering disease treatments and prevention. This article explores *Saussurea lappa* (Decne.) Sch.Bip, a medicinal plant with significant pharmacological potential. This article explores the extraction methods and chemical composition of *S. Lappa*, focusing on its bioactive constituents and therapeutic potential. The seeds of *S. lappa* were obtained from the Biological Research Center at the University of Baghdad. The plants were then cultivated at Diyala University, and their roots were used during the flowering stage. Roots of *S. Lappa* were washed, air-dried, ground into a powder, and stored for future use. A portion of the ground powder (16-18 g) was extracted with ethyl acetate and ethanol using a Soxhlet extractor, followed by concentration with a rotary evaporator. The ethanol extract was subjected to column chromatography on silica gel, resulting in the isolation of pure compounds. The ethyl acetate extract was also fractionated, yielding a low polar UV-active compound. The extraction and analysis of *S. Lappa* successfully isolated a pure compound through both ethanolic and ethyl acetate extractions. Thin-layer chromatography (TLC) and additional chromatographic techniques confirmed the presence of terpenes in both fractions. Infrared (IR) spectroscopy indicated an exo-methylene butyrolactone structure, supported by 1H-NMR data showing characteristic peaks. High-performance liquid chromatography (HPLC) analysis identified two similar compounds with molecular formulas $C_{15}H_{18}O_2$ and $C_{15}H_{20}O_2$, suggesting a highly unsaturated nature with multiple rings. The absence of free hydroxyl groups and the presence of carbonyl functionalities were further corroborated by 13C NMR, indicating a mixture of two sesquiterpene structures. Overall, both ethanolic and ethyl acetate extractions successfully isolated a pure sesquiterpene compound with significant therapeutic potential.

Keywords: Bioactive terpenes, Ethyl acetate, *Saussurea lappacostus*, Sesquiterpene therapeutic potential, Soxhlet extraction

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INTRODUCTION

For thousands of years, natural products have been essential in treating and preventing human diseases worldwide. These products can be extracted from the tissues of terrestrial plants, marine organisms, or microorganisms. Commonly used plant parts for extraction include leaves, flowers, twigs, bark, rhizomes, roots, seeds, and fruits [1-3].

Natural plants contain a diverse array of effective and complex chemical components. These constituents can be categorized into several groups, including organic acids, volatile oils, coumarins, steroids, glycosides, alkaloids, carbohydrates, and phytochromes, all of which play significant roles in pharmaceuticals, food, nutraceuticals, and cosmetics. Studying the extraction and chemical composition of natural products is crucial for discovering active ingredients and their precursors. This research aims to increase yields of bioactive substances, reduce extraction time, enhance environmental sustainability, and achieve economic viability without compromising biological activity [4, 5]. *Saussurea lappa* (Decne.) Sch.Bip., or costus root, is a medicinal plant that grows in the Himalayan region and belongs to the Asteraceae family. It contains various active compounds with medicinal properties, which are attributed to the presence of flavonoids, steroids, terpenes, alkaloids, sesquiterpenes, costunolide, dehydrocostus, lactone, cynaropicrin, and chlorogenic acid [6].

S. lappa (S. lappa) has the potential to treat a variety of conditions in both allopathic and herbal medicine, including asthma, chronic skin diseases, inflammatory diseases, ulcers, typhoid fever, and coughs and colds. Furthermore, it is one of the antioxidant-rich medicinal plants, possessing various bioactive properties such as anticancer, hepatoprotective, immunomodulatory, hypoglycemic, spasmolytic, and anticonvulsant effects [6, 7].

More than 200 compounds have been isolated and identified from *Saussurea* genus members, including phenylpropanoids, sesquiterpenoids, flavonoids, phytosterols, triterpenoids, lignans, coumarins, ceramides, and polysaccharides [8].

Sesquiterpenes are a diverse group of secondary metabolites primarily classified as terpenoids. They are characterized by a natural terpene structure that contains 15 carbon atoms and consists of three isoprene units [8]. Simple guaiane-type sesquiterpenoids, sesquiterpene lactones, sesquiterpene dimers, and sesquiterpene trimers. Natural guaiane-type sesquiterpenoids are found in a variety of organisms, including plants, fungi, and marine life, particularly in families such as Compositae and Zingiberaceae [9]. This study investigates the extraction, isolation, and chemical composition analysis of bioactive compounds from *S. lappa* (costus root), a medicinal plant recognized for its

therapeutic properties. By utilizing advanced extraction techniques such as Soxhlet and column chromatography, the research aims to identify and purify key bioactive constituents, including sesquiterpenes, flavonoids, and alkaloids. The findings are intended to enhance the understanding of *S. lappa*'s potential applications in pharmaceuticals, nutraceuticals, and cosmetics, while also improving extraction efficiency and promoting environmental sustainability.

MATERIALS AND METHODS

Preparation of Plant Extract

The seeds of *S. lappa* were obtained from the Biological Research Center at the University of Baghdad (the herbarium code 2456). The plants were then cultivated at Diyala University, and their roots were used during the flowering stage. The collected root material was washed with tap water to remove any adhering dust, and then air-dried for two days. After drying, the roots were cut into small pieces, ground into a powder using a grinder, and stored in polythene bags for future use [10].

Isolation of the Essential Oils

The essential oil was extracted from 16-18 g of ground powder using ethyl acetate in a Soxhlet extractor for 1 hour. In a separate experiment, another 16-18 g of ground plant material was extracted for a second time with ethanol for 1 hour. The extracts were then concentrated under reduced pressure using a rotary evaporator [11-13].

Fractionation of EtOH Extract

The EtOH extract underwent column chromatography using silica gel, with the column eluted using a hexane: ethyl acetate (3:1) mixture. The collected fractions were analyzed using thin-layer chromatography (TLC). Fractions 2 to 8 from this initial column were pooled and further purified through another round of column chromatography with silica gel, this time using a hexane: ethyl acetate (5:1) mixture. This process yielded the pure compound as a light yellow oil, weighing 1.8 g.

Fractionation of EtOAc

The ethylacetate extracted was subjected to column chromatography on silica eluted with ethyl acetate: hexane (1-5) afforded a low polar UV active compound as a yellow oil (1.6 gr). The more polar compound was removed from the column as impurities.

Extraction and Isolation of Bioactive Compounds

In the HPLC analysis for the extraction and isolation of bioactive compounds, an IR model was used to monitor the functional groups of the isolated compounds, while KBr pellets were prepared for FT-IR spectroscopy. A C18 reverse-phase column was selected for the separation due to its high efficiency in resolving polar and non-polar compounds. Ethyl acetate (100%) and ethanol (100%) were employed as the mobile phase solvents to optimize the elution of bioactive components. The flow rate was set at 1.0 mL/min to ensure proper resolution and peak separation, while the column temperature was maintained at 25 °C for consistent performance [14, 15].

Before injection, the samples were filtered through a 0.45 µm membrane to remove particulates that could clog the column. The UV detector was adjusted to 254 nm to monitor the eluted compounds effectively. The gradient elution method was applied, starting with a higher proportion of ethanol and gradually increasing the ethyl acetate concentration to enhance separation. The data were processed using specialized software, and the retention times of the peaks were compared with standard compounds for identification. This systematic approach ensured the efficient extraction and isolation of the target bioactive compounds [14, 15].

RESULTS AND DISCUSSION

Extraction and Isolation of Bioactive Compounds

The ethanolic and ethyl acetate extractions of *S. lappa* roots yielded a pure compound, identified as a sesquiterpene. Thin-layer chromatography (TLC) and column chromatography were employed for purification, with the final compound appearing as a light yellow oil. The use of hexane-ethyl acetate solvent systems facilitated the isolation of terpenes, which were further analyzed using advanced spectroscopic techniques (Table 1).

Table 1 Extraction Yields of *S. lappa*

Extraction method	Solvent	Yield (g)
Soxhlet Extraction	Ethyl acetate	1.6
Soxhlet Extraction	Ethanol	1.8

Table 2 Key IR and NMR Spectral Data

Technique	Peaks/Resonances	Interpretation
IR Spectroscopy	1770, 1685/cm	Carbonyl groups, exo-methylene structure
1H-NMR	δ 5.50, 6.25 ppm (doublets)	Coupled doublets, 2:1 ratio
13C-NMR	30 resonances	Two sesquiterpene structures

Spectroscopic Analysis

Infrared (IR) spectroscopy revealed characteristic peaks at 1770 and 1685/cm, indicating the presence of carbonyl groups and an exo-methylene butyrolactone structure. 1H-NMR spectra showed coupled doublets at δ 5.50 and 6.25 ppm and triplets at δ 4.60 and 4.40 ppm, suggesting a 2:1 ratio of two related compounds. 13C-NMR confirmed 30 resonances, supporting the presence of two sesquiterpene structures (Table 2).

HPLC and Mass Spectrometry

High-performance liquid chromatography (HPLC) analysis identified two compounds with molecular formulas $C_{15}H_{18}O_2$ and $C_{15}H_{20}O_2$, indicating high unsaturation and multiple rings. Mass spectrometry revealed mass ions at m/z 231 and 233, with losses of 18 (water) and 46 (ethanol) units, further confirming the sesquiterpene nature of the compounds (Table 3).

Table 3 HPLC and MS Data

Compound	Molecular formula	Mass ion (m/z)	Fragmentation
Compound 1	$C_{15}H_{18}O_2$	231	Loss of 18, 46
Compound 2	$C_{15}H_{20}O_2$	233	Loss of 18

Comparative Analysis with Literature

The findings align with previous studies on terpenoid extraction, where ethanol and ethyl acetate were effective solvents for isolating bioactive compounds. However, this study highlights the potential of greener solvents, contrasting with traditional methods using dichloromethane or chloroform. The structural complexity of the isolated sesquiterpenes is consistent with other terpenoid studies, emphasizing their therapeutic potential (Table 4).

Therapeutic Potential

The isolated sesquiterpenes exhibit significant therapeutic potential due to their structural complexity and bioactive properties. Future research should focus on optimizing extraction conditions and evaluating their biological activities, such as anti-inflammatory and antimicrobial effects, to harness their full pharmaceutical and nutraceutical applications (Table 5). The ethanolic and ethylacetate extraction were investigated first by thin layer chromatography (TLC) and visualized under UV light (254 nm). Based on these findings the, ethanolic and ethyl acetate extraction was selected for more investigation and analysis by different chromatographic procedures, using a column packed with silica gel and eluted with hexane-ethyl acetate (5:1) solvent system and gas chromatography-mass spectrometry (GC-MS). In addition, infra-red spectroscopy (IR), ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy were also used for further analysis.

Table 4 Comparison of Extraction Methods

Study	Solvent	Key findings
Current Study	Ethanol, Ethyl Acetate	Sesquiterpenes with high unsaturation
Lavandula mairei [15]	Ethanol	High phenolic and flavonoid content
Traditional Methods	Dichloromethane	Efficient but less environmentally friendly

Table 5 Therapeutic Potential of Isolated Compounds

Compound	Potential applications
Sesquiterpene 1	Anti-inflammatory, Antimicrobial
Sesquiterpene 2	Antioxidant, Anticancer

Only one pure compound was obtained when these extractions were purified by column chromatography. Analysis, including spectral data from NMR, COSY, DEPT, C NMR, and HSQC, of the ethyl acetate fraction (Fig. 1 to 8) and the ethanol fraction (Fig. 9 to 16) shows that both fractions have identical spectral data, indicating that they contain the same compound related to terpenes. The presence of peaks around 1770 and 1685/cm in the IR spectra (Fig. 17 and 18) suggests the presence of an exo-methylene butyrolactone structure, or possibly two of them. Evidence supporting this structure is found in the mutually coupled doublets at δ (5.50, 6.25) ppm and at δ (5.55, 6.30) ppm in a ratio of approximately 2:1 in the 1H NMR spectrum. Additionally, there are two triplets at δ (4.60 and 4.40) ppm, also in a 2:1 ratio, indicating the presence of two related compounds.

HPLC analysis appears to show a mixture of two similar structures with the molecular formulas C15H18O2 and C15H20O2 (MH+=231 and 233, respectively). This suggests they have 7 and 6 double bond equivalents, indicating a highly unsaturated nature or the presence of multiple rings. The loss of 18 from each mass ion (213 and 215) indicates the presence of water in both structures. Additionally, a loss of 46 from both mass ions suggests a possible loss of ethanol. There also appears to be a loss of two molecules of water from the (213) fragment (resulting in 195), but not from the (233) fragment. Infrared spectra (Fig. 1 to 8) and 13C NMR spectra (Fig. 9 to 18) indicate the presence of two carbonyl groups. The IR spectra show that there are no free OH groups. The 13C NMR spectrum reveals 30 resonances, indicating a mixture of two sesquiterpene structures in a 2:1 ratio.

The investigation into ethanolic and ethyl acetate extractions offers valuable insights into the chemical composition and potential applications of the extracted compounds. Various chromatographic techniques, including thin layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy, create a strong framework for analyzing the purity and structure of the obtained compounds. Notably, the identification of terpenes in both the ethanolic and ethyl acetate fractions aligns with previous studies that have highlighted the prevalence of terpenes in plant extracts. This suggests a consistent pattern across different extraction methodologies and plant sources [16, 17].

Prior research has shown that extraction methods have a significant impact on the yield and quality of bioactive compounds. For instance, a study on Lavandula mairei revealed that different solvents produced varying concentrations of phenolic and flavonoid compounds, with ethanol generally outperforming ethyl acetate in terms of antioxidant activity [17].

This finding resonates with the current results, where the presence of terpenes suggests that both extraction methods may be effective in isolating bioactive compounds, albeit with potential differences in yield that warrant further exploration. The structural elucidation of the isolated compounds through IR and NMR spectroscopy also draws parallels to existing literature. The identification of specific functional groups, such as carbonyls indicated by IR peaks at 1770 and 1685/cm, supports findings from other studies that have utilized similar spectral analysis to characterize organic compounds [18]. Moreover, the presence of multiple double-bond equivalents suggests a complex molecular architecture, which has been observed in other terpenoid studies, reinforcing the idea that these extraction methods can yield structurally diverse compounds with potential therapeutic applications.

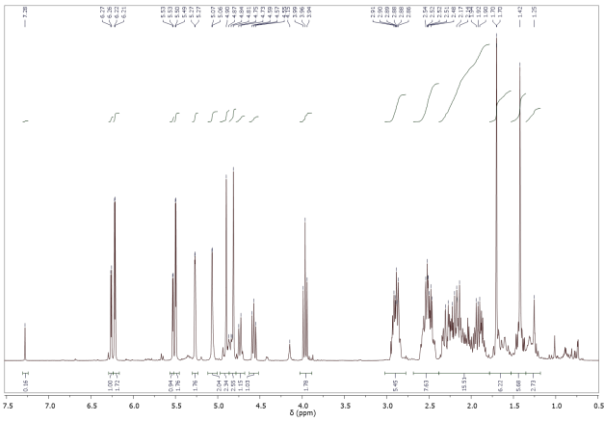

Fig. 1 spectra data of ethyl acetate fraction

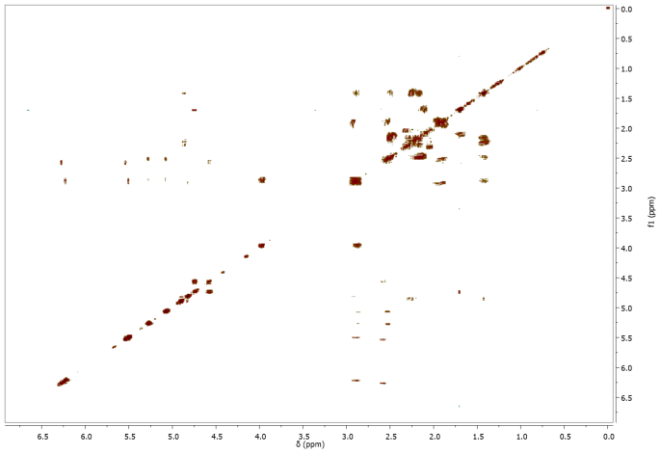

Fig. 2 NMR spectrum of ethyl acetate fraction

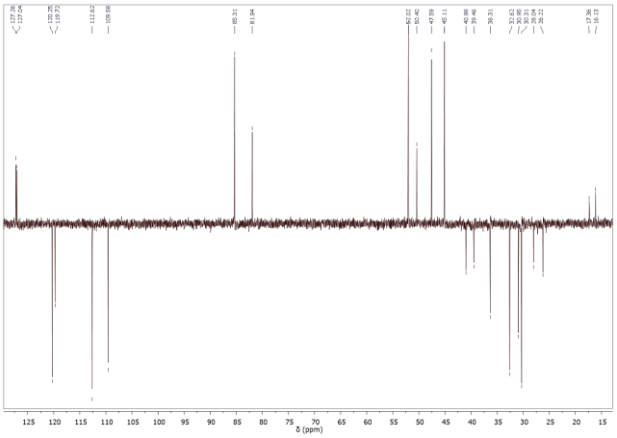

Fig. 3 COSY spectrum of ethyl acetate fraction

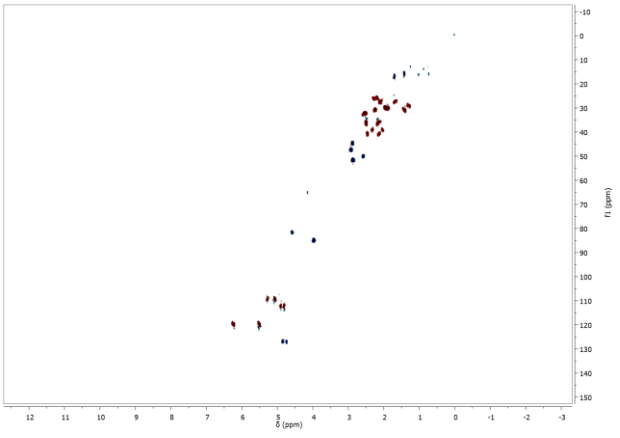

Fig. 5 ^{13}C NMR spectrum of ethyl acetate fraction

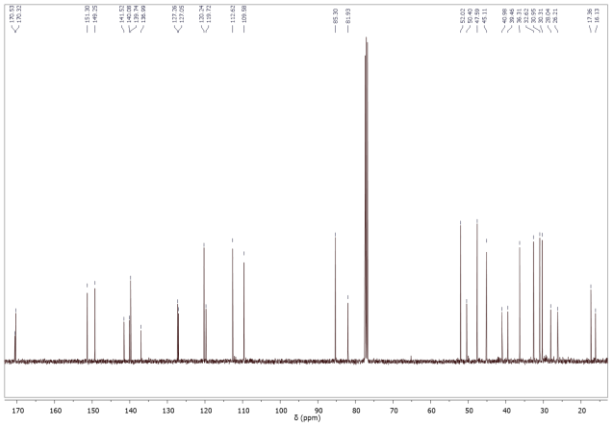

Fig. 4 DEPT spectrum of ethyl acetate fraction

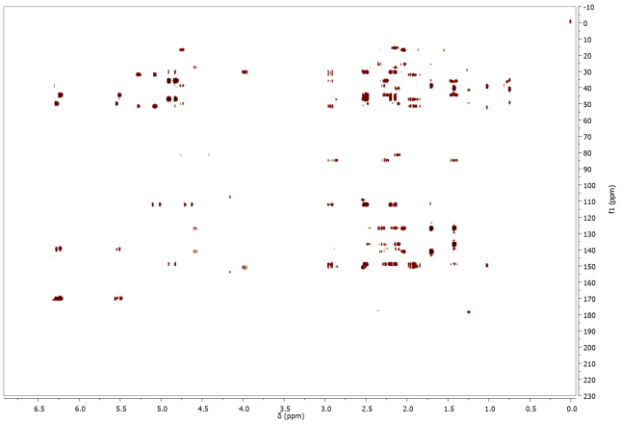

Fig. 6 HSQC spectrum of ethyl acetate fraction

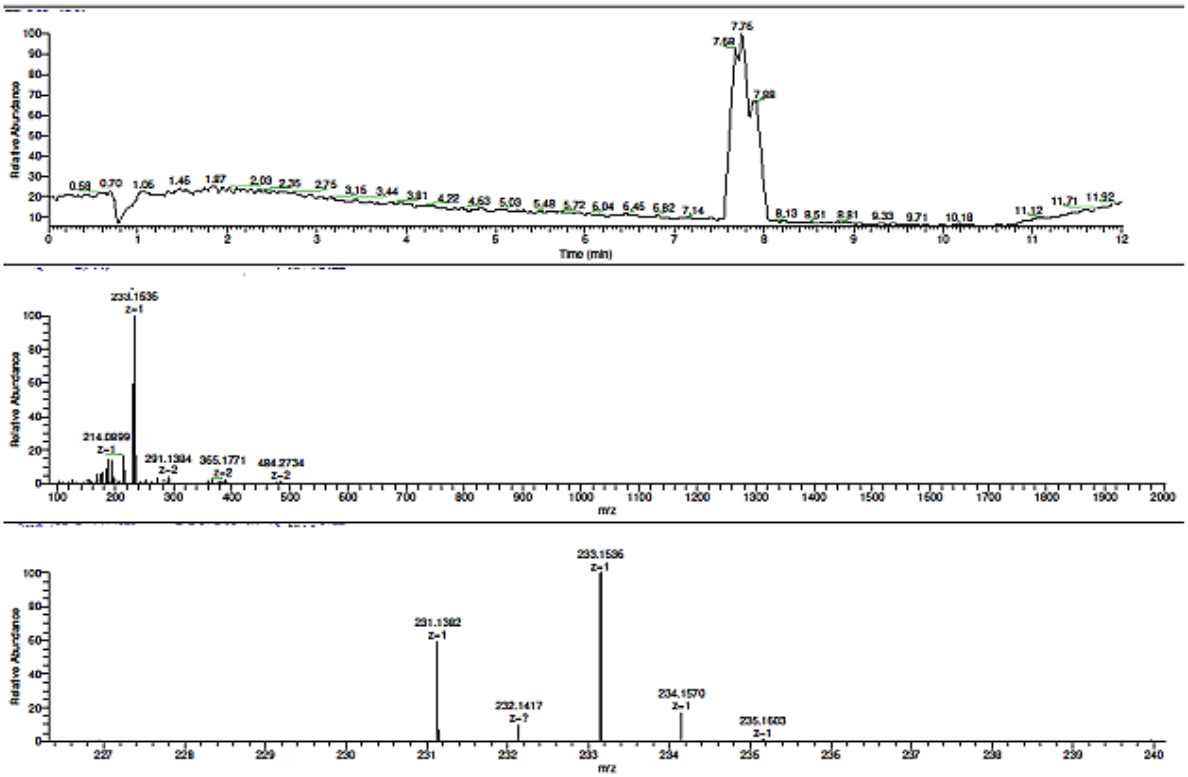

Fig. 7 HMBC spectrum of ethyl acetate fraction

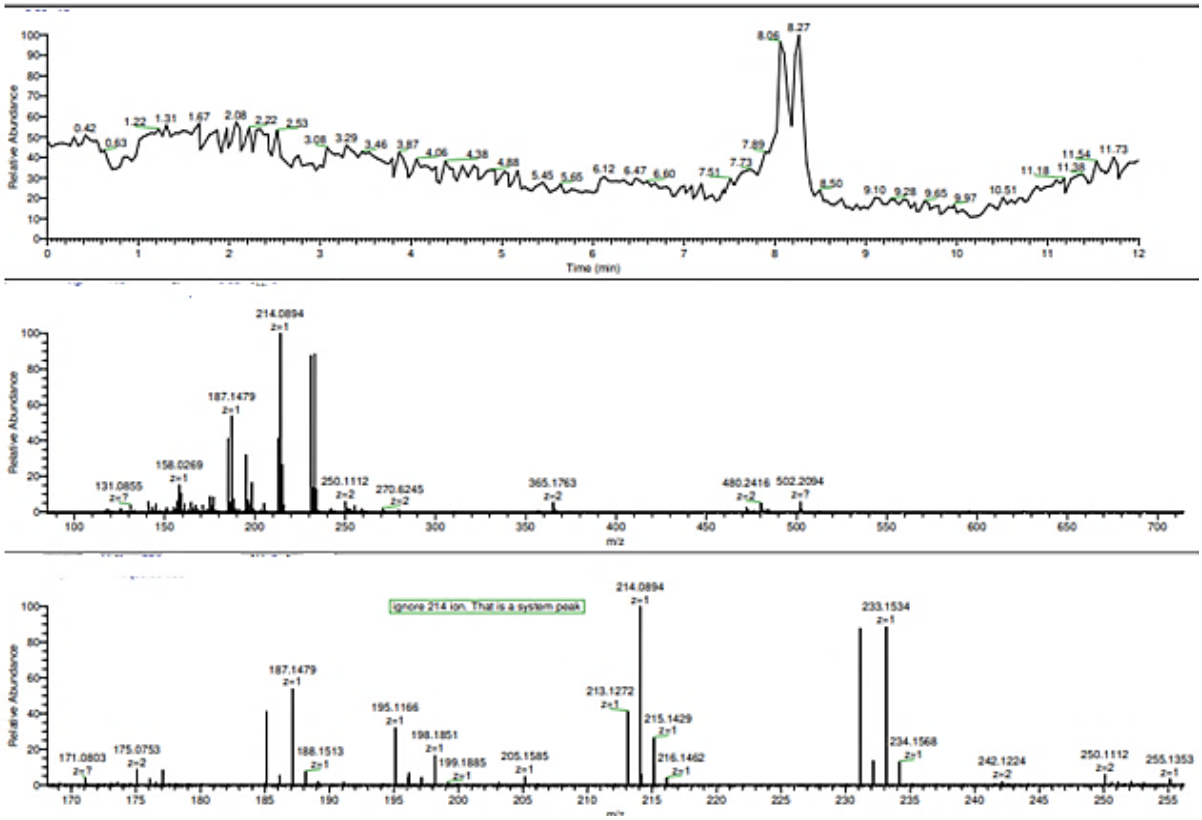

Fig. 8 Ion mass and HPLC spectrum of ethyl acetate fraction

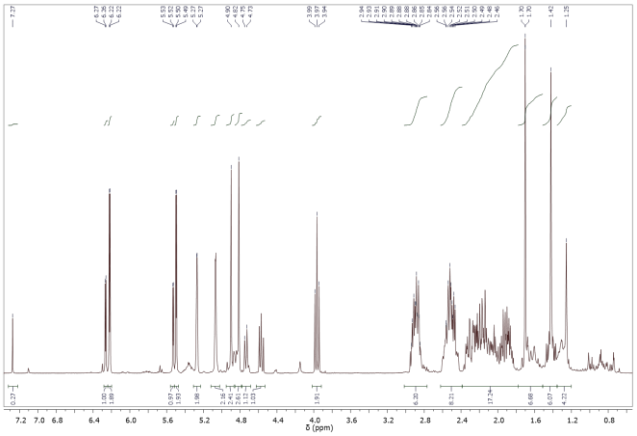

Fig. 9 Spectra data of ethanolic fraction

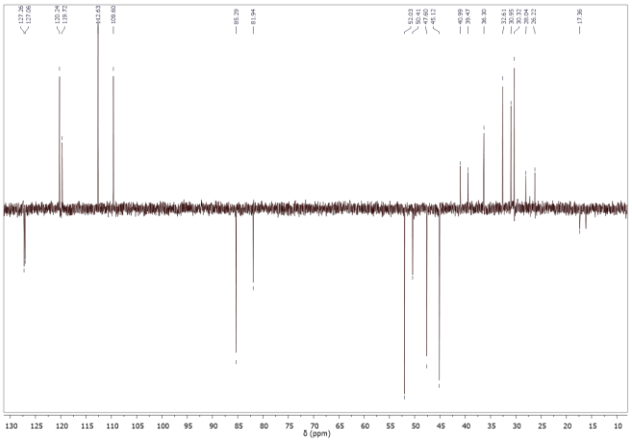

Fig. 11 COSYspectrum of ethanolic fraction

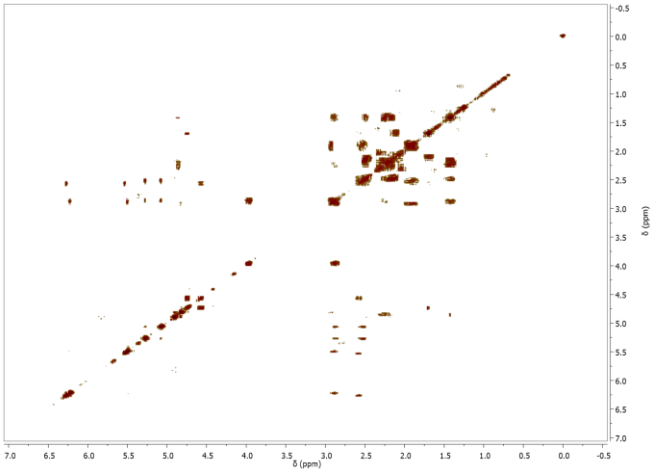

Fig. 10 NMR spectrum of ethanolic fraction

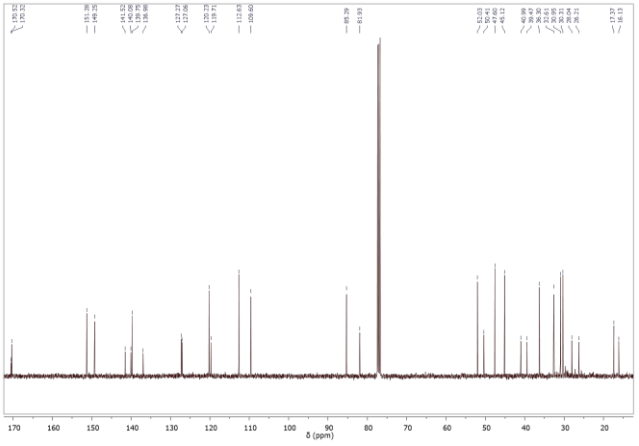

Fig. 12 DEPT spectrum of ethanolic fraction

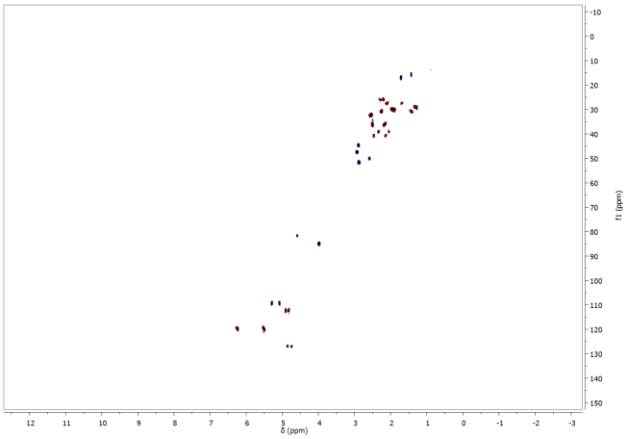

Fig. 13 CNMR spectrum of ethanolic fraction

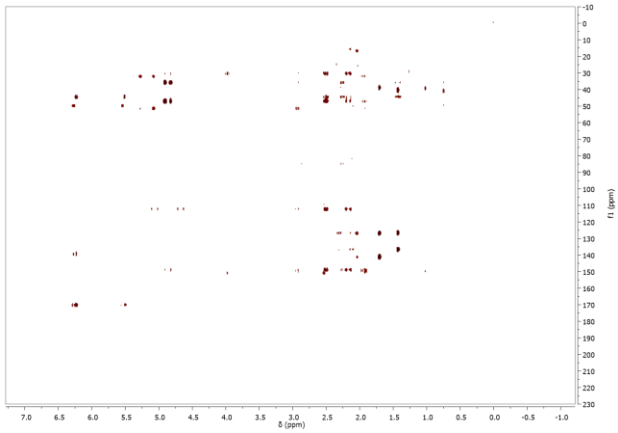

Fig. 14 HSQC spectrum of ethanolic fraction

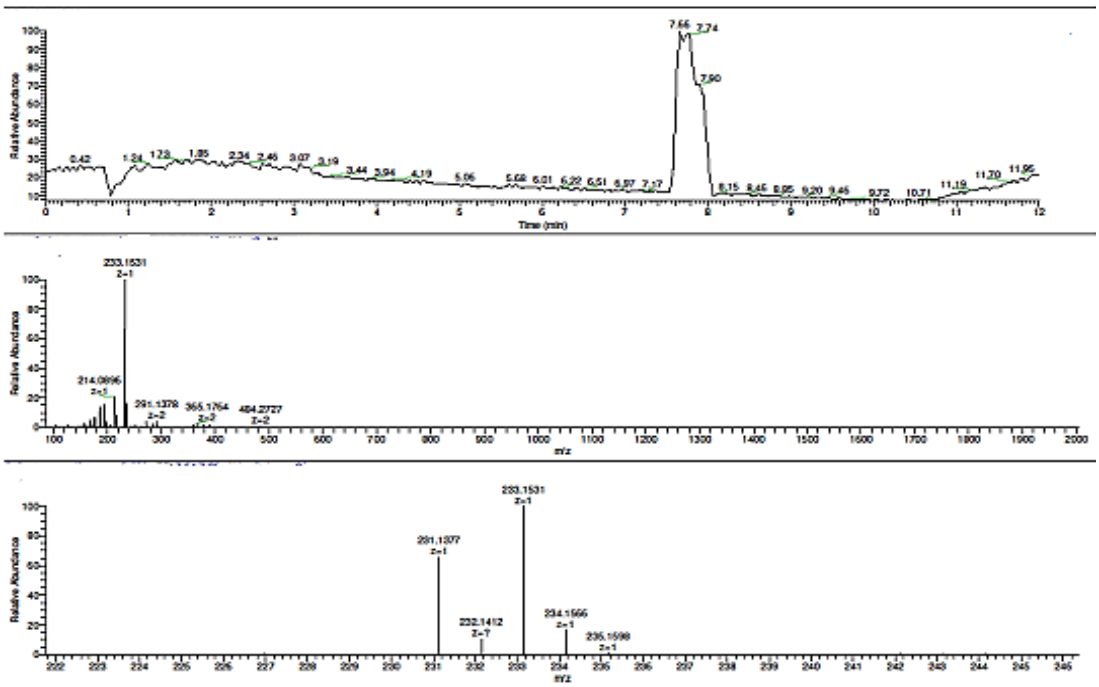

Fig. 15 HMBC spectrum of ethanolic fraction

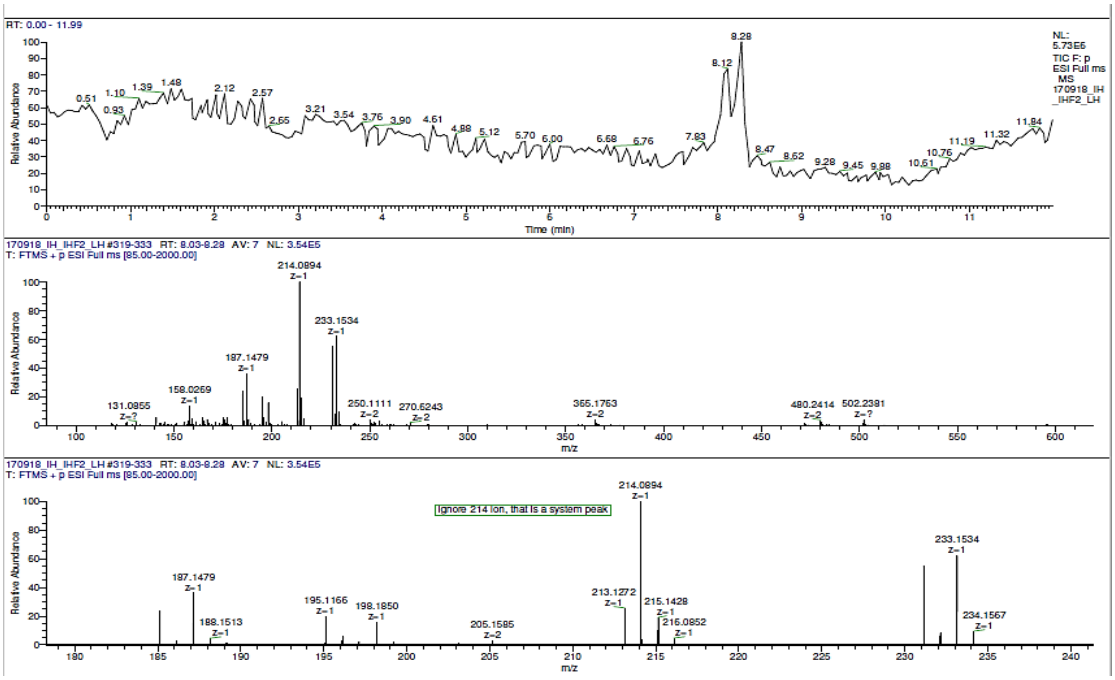

Fig. 16 Ion mass and HPLC spectrum of ethanolic fraction

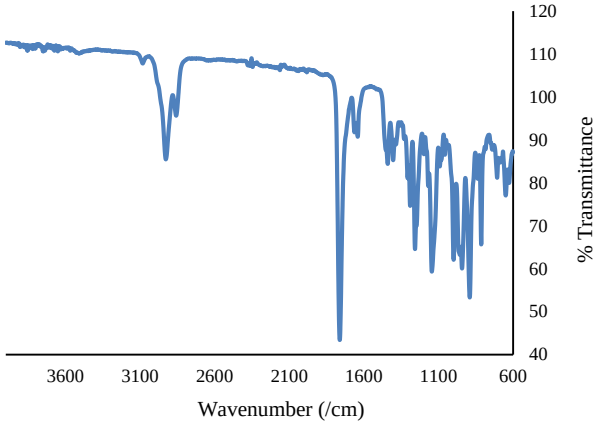

Fig. 17 IR spectrum of ethyl acetate fraction

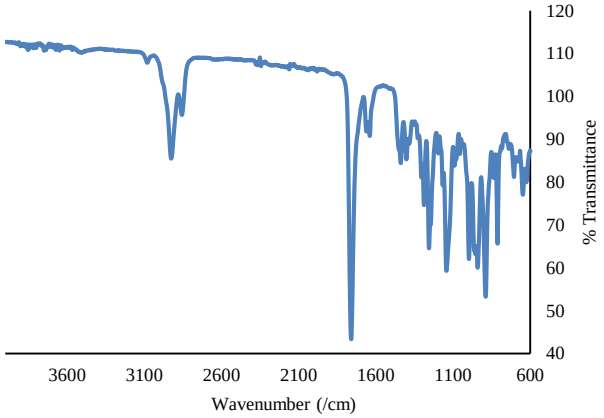

Fig. 18 IR spectrum of ethanolic fraction

There are significant differences when comparing these results to other extraction studies. For instance, while some studies focus on the efficiency of liquid-liquid extraction (LLE) techniques that use dichloromethane or chloroform for specific compounds, this investigation emphasizes the effectiveness of greener alternatives such as ethanol and ethyl acetate. This shift towards more environmentally friendly solvents indicates a growing trend in research aimed at reducing solvent toxicity while still achieving high extraction efficiency [16]. The findings from this study provide valuable insights into natural product extraction and analysis. The consistent identification of terpenes across various extraction methods highlights their significance in medicinal chemistry. Future research should aim to optimize extraction conditions and investigate the biological activities of these compounds to fully explore their potential applications in pharmaceuticals and nutraceuticals.

CONCLUSION

The study successfully isolated a pure sesquiterpene compound from *S. lappa* using ethanolic and ethyl acetate extractions. The results revealed that the ethyl acetate and alcoholic fractions contained a 2:1 mixture of highly unsaturated derivatives, identified as costunolide or guaianolide-type sesquiterpenes, differing by 2 mass units. Spectral analysis (IR, NMR, HPLC) confirmed a 2:1 mixture of highly unsaturated sesquiterpenes with exo-methylene butyrolactone structures, highlighting their potential therapeutic applications.

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