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Chemical composition of essential oil from cultivated *Saussurea costus* Clarke (Kuth) in Kashmir Himalayas

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Abstract
Saussurea costus, a critically endangered plant species endemic to the Himalayas, is recognized for its medicinal properties in traditional systems of medicine. This study aimed to analyze the chemical composition of the essential oil derived from *S. costus* rhizomes collected from different forest divisions in the Kashmir region. Hydrodistillation and gas chromatography-mass spectrometry (GC/MS) analysis were conducted to identify and quantify the chemical constituents. The essential oil content percent was estimated to be 0.90%. The analysis revealed twenty-four compounds, with dehydrocostus lactone and costunolide as major components. These sesquiterpene lactones have exhibited diverse pharmacological activities, including anti-inflammatory, anti-tumor, and hepatoprotective effects. The findings underscore the potential therapeutic applications of *S. costus* essential oil and provide insights for further research and development in natural medicine. Moreover, the observation of a unique chemical profile in the *S. costus* samples from the Kashmir division suggests the presence of region-specific chemical constituents. Future investigations can explore the specific therapeutic properties and applications of these chemicals, contributing to the development of region-specific medicinal interventions. Overall, this research enhances our understanding of *S. costus* and its medicinal potential, emphasizing the importance of conservation efforts and sustainable utilization of this endangered plant species.

Keywords: *Saussurea costus*, essential oil, chemical composition, GC/MS analysis, sesquiterpene and pharmacological activities

Introduction
Saussurea costus, also known as *Saussurea lappa*, belongs to the Asteraceae family, which comprises approximately 1,000 genera and 30,000 species distributed worldwide. However, India is home to numerous species within this family [1]. This species is endemic to a specific region of the Himalayas and thrives on damp slopes at elevations ranging from 2600 to 4000 meters above sea level [2, 3]. In India, *S. costus* can be found naturally in the Suru Valley, Kishenganga, and the upper regions of the Chenab valleys in Jammu and Kashmir. It may also sporadically occur in adjacent areas of the Kashmir Valleys [4, 5].
Saussurea costus is classified as a critically endangered species, leading the Government of India to impose a ban on the export of 29 medicinal and aromatic plants, including *S. costus* [6]. The species is highly regarded for its medicinal properties in traditional systems of medicine such as Ayurvedic, Chinese, and Tibetan practices. The roots of *S. costus* possess a bitter taste and a strong aromatic odor, and they are believed to exhibit various beneficial properties in humans, including anti-inflammatory, antimicrobial, analgesic, anti-ulcer, anti-cancer, and hepatoprotective effects. The major constituents of this plant hold potential for the development of bioactive molecules. Notably, costus oil extracted from *S. costus* roots has been traditionally used in treating leprosy [7]. Furthermore, the roots of *Saussurea costus* are employed in the management of several other medical conditions such as chronic gastritis, stomach ulcers, rheumatoid arthritis, asthma, bronchitis, and inflammation-related diseases according to traditional medicine practices. The species is widely utilized in various indigenous systems of medicine [8]. However, it is worth noting that the use of costus oil in perfumery, due to its rich content of sesquiterpene lactones, has been associated with numerous cases of allergic contact dermatitis [9]. The planning commission of India has reported a requirement of 0.43 tons of *S. costus* annually. Despite its importance, there is a lack of systematic research on the essential oil compositions of *S. costus* from the Kashmir valley.

Material and Methods

Collection of plant material

Rhizomes of *Saussurea costus* were collected from three different forest divisions *viz*., Sindh Forest division, Langate forest division and Lidder forest division during sep to oct 2019. The collected rhizomes were visually inspected for health and any visible damage or contamination before further processing. The collected rhizomes were transferred to a nursery with controlled environmental conditions. The nursery provided suitable temperature, light, and watering to facilitate the growth of the rhizomes. The rhizomes were allowed to grow for a period of two years under these conditions. After the two-year growth period, mature rhizomes were harvested from the nursery. The harvested rhizomes were carefully cleaned to remove any dirt or debris. Subsequently, they were dried in a well-ventilated area at a controlled temperature of 40-50 °C until they reached a completely dry and brittle state. The dried rhizomes were then ground into a fine powder using a mixer grinder.

Hydrodistillation

Approximately 250 grams of powdered rhizomes were subjected to hydrodistillation using a Clevenger-type apparatus for 6 hours. The aim was to extract the essential oil from the dried rhizomes and estimate its oil content (%).

GC/MS Analysis

The gas chromatography-mass spectroscopy (GC-MS) analysis of the oil was performed out on a Perkin Elmer mass spectrometer (Model Claurus 500) coupled to a Perkin Elmer Clarus 500 GC with a 60 m × 0.32 mm × 0.2 µm film thickness column (RtX5). The sample was injected directly into the column. Helium was used as the carrier gas (flow rate 1 ml/min). The oven temperature was programmed from 60 °C to 220 °C at 3 °C/min. Other conditions were the same as described under GC. The mass spectrum was taken with a mass range of 40–600 Daltons

Identification and Quantification

The chemical constituents present in the essential oil were identified by comparing their retention times and mass spectra with those of known standards or reference databases. Quantification of the identified compounds was performed using appropriate calibration curves or internal standards.

Data Analysis

The GC/MS data was analyzed to determine the composition and relative abundance of the chemical constituents in the essential oil. The percentage composition of each compound present in the essential oil was calculated.

Results and Discussion

Chemical constituents of the essential oil

The essential oil content percent was estimated and it was found to be 0.90%. Gas chromatographic analysis of the essential oil obtained from *S. costus* are presented in Table 1 revealed that the constituents found in the oil were twenty four in number *viz*., α-Pinene (2.35), β-Pinene (1.54), α –Selinene (2.04), α-Curcumene (0.62), 1-Cetene (0.25), Elema-1,3,11(13)-trien-12-ol (3.56), Dihydro-α-ionone (2.22), Carvacrol (0.75), Elemol (2.15), γ-Eudesmol (1.01), β –Eudesmol (0.90), α- Eudesmol (1.14), 1,3- Cyclododecane (4.05), Cyclododecene 12- methyl-1-(1-propynyl) (0.70), 8-Cedren-13-ol (3.60), β-Costol (0.29), δ-Terpineol (0.18), 4-Terpineol (1.04), α-Terpineol (0.23), Alpha-Guaiene (1.03), β-Caryophyllene (0.90), α-Ionene (2.07), Costunolide (9.35), Dehydrocostus lactone (36.33). The chemical composition of the essential oil of *S. costus* essential oil was similar to that reported in other studies. Liu *et al.* (2012) reported that the main compounds in *Saussurea lappa* essential oil were dehydrocostus lactone, costunolide, 8-cedren-13-ol, and α-Curcumene [10]. Hu *et al.* (2022) identified thirteen different compounds, including seven sesquiterpenes, representing 95.84% of the total oil composition, through GC-MS analysis of *S. costus* essential oil [11]. Bagheri *et al.* (2018) also reported thirty-one compounds in the essential oil extracted from the root, with dehydrocostus lactone (17.73%) and 1,3-cyclooctadiene (16.10%) being the predominant components [12]. Gwari *et al.* (2013) identified a total of forty-one components, accounting for 92.81% of the total oil composition [13].

Previous studies have consistently identified dehydrocostus lactone and costunolide as the main components in the essential oil of *S. costus* roots, which is in line with the findings of the present study. These two sesquiterpenes have been recognized as major active constituents of *Saussurea costus* essential oil in studies conducted by Zahara *et al.* (2014) [14], Kraker *et al.* (2001) [15], Gwari *et al.*, (2013) [13] and Liu *et al.* (2012) [10]. Sesquiterpene lactones are colorless, bitter, relatively stable, and lipophilic compounds that are commonly found in the Asteraceae family. However, they can also occur in other flowering plant families such as Umbelliferae, Magnoliaceae, Lauraceae, Winteraceae, Illiciaceae, Aristolochiaceae, Menispermaceae, Curtiariaceae, and Acanthaceae [16]. Dehydrocostus lactone and costunolide, in particular, have been associated with various pharmacological activities, including anti-tumor, anti-inflammatory, immunomodulatory, hepatoprotective, anti-ulcer, and anti-viral effects, as confirmed by studies conducted by Zahara *et al.* (2014) [14] and Pandey *et al.* (2007) [1].

Table 1: Chemical constituents of *Saussurea costus* essential oil

S. No.	Identified compounds	Chemical formula	Percentage
1	α-Pinene	$C_{10}H_{16}$	2.35
2	β-Pinene	$C_{10}H_{16}$	1.54
3	α –Selinene	$C_{10}H_{16}$	2.04
4	α-Curcumene	$C_{15}H_{22}$	0.62
5	1-Cetene	$C_{16}H_{32}$	0.25
6	Elema-1,3,11 (13)-trien-12-ol	$C_{15}H_{24}O$	3.56
7	Dihydro-α-ionone	$C_{13}H_{22}0$	2.22
8	Carvacrol	$C_{10}H_{14}O$	0.75
9	Elemol	$C_{15}H_{26}O$	2.15
10	γ-Eudesmol	$C_{15}H_{26}0$	1.01
11	β –Eudesmol	$C_{15}H_{26}O$	0.90
12	α- Eudesmol	$C_{15}H_{26}0$	1.14
13	1,3- Cyclododecane	$C_{12}H_{24}$	4.05
14	Cyclododecene, 12- methyl-1-(1-propynyl)	$C_{13}H_{26}$	0.70
15	8-Cedren-13-ol	$C_{15}H_{24}O$	3.60
16	β-Costol	$C_{15}H_{24}O$	0.29
17	δ-Terpineol	$C_{10}H_{18}O$	0.18
18	4-Terpineol	$C_{10}H_{18}O$	1.04
19	α-Terpineol	$C_{10}H_{18}O$	0.23
20	Alpha-Guaiene	$C_{13}H_{22}O$	1.03
21	β-Caryophyllene	$C_{15}H_{24}$	0.90
22	α-Ionene	$C_{13}H_{20}O$	2.07
23	Costunolide	$C_{15}H_{20}0_2$	9.35
24	Dehydrocostus lactone	$C_{15}H_{18}O_2$	36.33

Conclusion

In conclusion, the comprehensive analysis of *Saussurea costus* essential oil from the Kashmir division identified several important chemical constituents, including dehydrocostus lactone, costunolide, and other compounds. These findings highlight the unique chemical profile of *Saussurea costus* from the Kashmir region. The presence of these specific chemical constituents further enhances the medicinal potential of *Saussurea costus* from this specific geographic location. Future research can focus on exploring the specific therapeutic properties and applications of these chemicals derived from the Kashmir division, contributing to the development of region-specific medicinal interventions.

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