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Research Article

Composition of *Cyperus luzulae* rhizome essential oil from Peru

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Abstract

Cyperus luzulae (L.) Rottb. ex Retz is an understudied species within the *Cyperus* genus. To the best of the authors' knowledge, the essential oil from this species has never been fully characterized. Essential oil obtained through steam distillation of *C. luzulae* rhizomes was analyzed to establish the chemical profile by GC/FID and GC/MS. The chemical profile revealed a high content of sesquiterpene hydrocarbons (36.9%) and oxygenated sesquiterpenes (34.9%). The major compounds of the oil were caryophyllene oxide (23.5%), β-selinene (12.9%), and α-copaene (8.3%). These results provide fundamental data for future investigations into the ethnobotanical and pharmacological uses of this species, broadening the scope of knowledge on the *Cyperus* genus and its potential applications.

1. Introduction

The Cyperaceae family is comprised of annual herbs and rhizomatous perennials, and covers a broad array of diversity within the herbaceous plant kingdom encompassing around 5700 unique species [1]. These species are distributed among two distinct subfamilies, which are further divided into 24 tribes and classified under 95 different genera [1, 2]. The broad geographical reach of the plant family provides a vast array of diversity in their biological traits and habits [3].

Cyperus luzulae (L.) Rottb. ex Retz, commonly known as "*Piri Piri*", is one of the members of the Cyperaceae family growing primarily in tropical and subtropical regions of America that has cespitose rhizomes, brown to reddish leaf bases, and it has been traditionally used for a variety of social and/or medicinal purposes [4, 5].

The *Cyperus luzulae* plant holds historical and Therapeutic significance across various cultures. Historically, the Shipibo-Conibo peoples utilized the plant's rhizomes as an aphrodisiac, while in Guatemala, its crushed rhizome forms the basis of the Mesoamerican drink, "atole" [5, 6]. Indigenous stories from the Huaorani group in San Francisco, Ucayali, Peru, tell of shamans crafting love potions from *C. luzulae* to attract partners [7]. Furthermore, conversations with a Shaman from the Shipibo-Konibo highlight its use in childbirth, for its pain-minimizing effects, and as a diuretic aiding individuals with fluid retention or high blood pressure [8]. In Panama, it has therapeutic applications for eye infections and complications during childbirth [9].

While this is an understudied plant species, recent research has been done to investigate botanical, ecology, and utility of this species [10–12]. Scientific

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research underscores its medicinal potential. Methanol extracts from *C. luzulae* roots have exhibited DNA intercalation, tumor inhibition, and cytotoxic effects in V79 cultures, hinting at its oncological applications [13]. Both aqueous and methanol extracts of the plant also demonstrate molluscicidal activity against *Biomphalaria glabrata*, a known host of schistosomiasis [14].

Despite the referenced studies, to the best of the authors' knowledge, the chemical composition of the essential oil of *C. luzulae* rhizomes has not been reported. This lack of knowledge limits the understanding of its potential usefulness in terms of possible applications. This study aims to determine the chemical composition of the essential oil of rhizomes from *C. luzulae* to provide fundamental data for future investigations of this species.

2. Materials and methods

Cyperus luzulae rhizomes were collected in December 2022 from cultivated populations in Coronel Portillo province, Peru (8°16'26.1" S 74°38'40.1" W). The rhizomes were washed with water to remove the soil surrounding them and were left to dry out of sunlight for 4 days. Then the rhizomes were crushed and weighed, and the extraction process was conducted. A representative voucher sample of the species is held at the Universidad Nacional de Cajamarca (Herbario Isidoro Sánchez Vega_UNC; herbarium code CPUN). The distillation was conducted in a 250 L distillation chamber (Albrigi Luigi S.R.L., Italy). Distillation was conducted by steam distillation for 4 hours. The essential oil obtained was separated by a cooled condenser, collected, filtered, and stored in sealed amber vials at room temperature (25 °C) until analysis. The essential oil yield was calculated as the ratio of the essential oil volume (mL) to the plant material mass (kg) before the distillation process.

The essential oil compounds were analyzed and identified by GC/MS using Agilent7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J&W DB-5, 0.25 mm x 60m, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of the sample (20% soln. for essential oils in ethanol), 100:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 5 min., oven ramp rate of 4.5 °C per min. to 310 °C with a hold time of 5 min. The electron ionization energy was 70 eV, scan range 35-650 amu, scan rate2.4 scans per sec., source temp. 230 °C, and quadrupole temp. 150 °C. Volatile compounds were identified using the Adams volatile oil library [15] using Chemstation library search in conjunction with retention indices. Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent7890B GC and Agilent J&W DB-5, 0.25 mm x 60 m, 0.25μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of sample (20% soln. for essential oil in ethanol, 1% for reference compounds in ethanol, 0.1% soln. for C7-C30 alkanes in hexane), 25:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 2 min., oven ramp rate of 3.0 °C per min. to 250 °C with a hold time of 3 min. The essential oil sample was analyzed in triplicate by GC/FID to ensure repeatability (standard deviation < 1 for all compounds). Compounds were assigned using retention indices coupled with the retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, MO, USA).

3. Results and discussion

The essential oil yield from rhizomes of *Cyperus luzulae* was 2.1 mL/kg, and the chemical profile is detailed in Table 1, revealing that this essential oil is rich in sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

Twenty-seven compounds of *Cyperus luzulae* essential oil were identified in this study. The primary monoterpene hydrocarbons were α-pinene (1.1%) and β-pinene (1.8%). The major oxygenated monoterpenes were (E)-pinocarveol (1.8%) and myrtenol (2.0%). The principal sesquiterpene hydrocarbons were β-selinene (12.9%), α-copaene (8.3%), cyperene (5.9%), and α-selinene (2.1%). The most abundant oxygenated sesquiterpenes were caryophyllene oxide (23.5%), α-cyperone (5.2%), humulene epoxide II (3.4%), and longiverbenone (2.8%). Fig. 1 is provided for a more intuitive visual representation.

Comparing our results with species of the same genus, *Cyperus rotundus*, a commonly studied species in the same genus with rhizome-extracted essential oil, shows a high concentration of cyperene, α-cyperone, rotundene, α-selinene, β-selinene [16-19]. The essential oil of *Cyperus distans* is characterized by prominent compounds such as cyperene, caryophyllene oxide, and β-pinene [20]. Additionally,

Table 1. Chemical profile of *Cyperus luzulae* rhizome essential oil determined by GC/FID.

KI	Compound Name	Area percentage (%)
932	α-Pinene	1.1
974	β-Pinene	1.8
1020	p-Cymene	0.2
1024	Limonene	0.6
1026	1,8-cineole	0.8
1135	Nopinone	0.2
1135	(E)- Pinocarveol	1.8
1174	Terpinen-4-ol	0.2
1186	α-Terpineol	0.4
1194	Myrtenol	2.0
1204	Verbenone	0.3
1369	Cyclosativene	1.2
1374	α-Copaene	8.3
1389	β-Elemene	0.4
1390	Sativene	0.2
1398	Cyperene	5.9
1457	Rotundene	1.3
1473*	Unknown	1.0
1478	γ-Muurolene	0.6
1489	β-Selinene	12.9
1498	α-Selinene	2.1
1506*	Unknown	1.1
1528	cis-Calamene	1.1
1539*	Unknown	0.5
1544	α-Calacorene	1.2
1564	β-Calacorene	1.9
1570*	Unknown	0.6
1582	Caryophyllene oxide	23.5
1608	Humulene epoxide II	3.4
1620*	Unknown	0.5
1623*	Unknown	1.7
1635*	Unknown	1.7
1638*	Unknown	0.7
1643*	Unknown	1.5
1646*	Unknown	0.6
1649	Longiverbenone	2.8
1650*	Unknown	1.3
1663*	Unknown	0.9
1666*	Unknown	1.1
1673*	Unknown	0.6
1695*	α-Cyperone	5.2
	Other	0.2
	Total (%)	81.2
Compound Classes		
Monoterpene hydrocarbons		3.7
Oxygenated monoterpenes		5.5
Sesquiterpene hydrocarbons		36.9

Table 1. (*Continued*)

Compound Classes	
Oxygenated sesquiterpenes	34.9

Note: Essential oil sample was analyzed in triplicate to ensure repeatability (standard deviation < 1 for all values). Unidentified compounds of less than 0.5% are not included. KI is the Kovat's Index previously calculated by Robert Adams using a linear calculation on a DB-5 column [15]. *KI not previously calculated [15] and manual calculations performed using alkane standards.

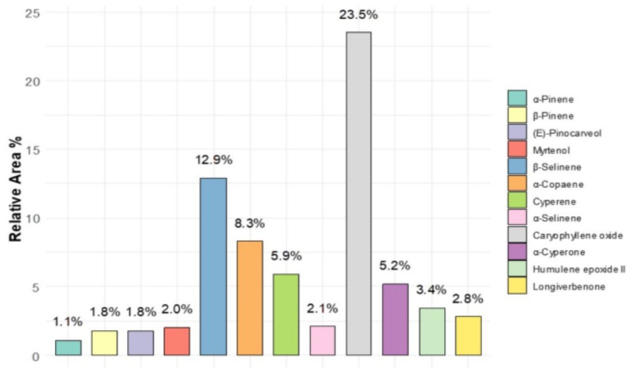

Figure 1. Comparison of compound concentrations in the essential oil of *Cyperus luzulae*.

Cyperus esculantus essential oil has been documented to possess a significant concentration of caryophyllene oxide while *Cyperus spapyrus* essential oil has cyperene and copaene as principal compounds [21]. It is noteworthy that all these compounds, known to be present in these related species' essential oils, are also found within the *C. luzulae* essential oil obtained from the current study. Despite these similarities, it's essential to highlight that the unique combinations and concentrations of these compounds in *C. luzulae* may confer this species with a unique set of properties. Further investigations are required to explore these potential applications.

The principal chemical constituents of essential oils generally dictate their bioactivities [22, 23]. Caryophyllene oxide (Fig 2) is the major compound present in the essential oil of rhizomes of *Cyperus luzulae* from this study. Literature reported caryophyllene oxide affects the growth of tumor cells, and may be used against cancer of the prostate, human breast, cancer cells of human osteosarcoma MG-63, lung cancer cells, and gastric cancer cells [24-29], which makes *C. luzulae* essential oil interesting for future studies against cancer cells.

Using the available mass spectral libraries (NIST 2020) and Adams library for this study, we were only able to identify 81.2% of *Cyperus luzulae* essential oil from the rhizomes. The limitations of the analytical

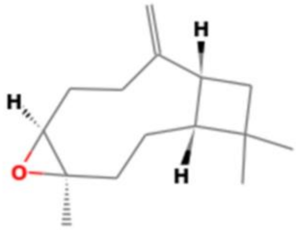

Figure 2. Caryophyllene oxide chemical structure. Obtained from NIST [30]

techniques may have hindered certain compound identification. Advanced analytical techniques such as two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS), nuclear magnetic resonance spectroscopy (NMR), and high-resolution mass spectrometry (HRMS) could improve identification [31]. Additionally, the complexity of the essential oil matrix might have contributed to the non-identification and some unidentified compounds are uncommon, suggesting potential for future discoveries.

4. Conclusions

This study, to the best of the authors' knowledge, is the first to elucidate the chemical composition of *Cyperus luzulae* essential oil derived from rhizomes, with caryophyllene oxide, β-selinene, and α-copaene identified as the predominant compounds. Despite the growing body of literature on the chemical composition of essential oils within the *Cyperus* genus, there has been limited analytical research specifically on this species. This research not only fills a gap in our understanding but also highlights the potential applications based on its unique chemical profile. Looking ahead, it would be beneficial to delve deeper into the properties and applications of this essential oil, possibly exploring its therapeutic or industrial potential. Future research should also prioritize employing advanced analytical methods and developing comprehensive databases for reference standards. Such endeavors will not only facilitate the identification of unknown compounds but will also pave the way for a more holistic understanding of this essential oil's properties and applications.

Authors' contributions

Conceptualization, C.P.; Methodology, C.P. and A.A.; Software, C.P., A.A., T.M.W., and T.O.; Validation, C.P.; Formal Analysis (GC/MS, GC/FID), C.P., A.A, T.M.W., and T.O.; Investigation, C.P. and A.A.; Resources, C.P., E.C.; Data Curation, C.P. and A.A.; Writing – Original Draft, C.P. and A.A.; Writing – Review & Editing, C.P., A.A., T.M.W., T.O., E.C., and O.P.

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Conflicts of interest

The authors declare no conflict of interest. The funding entity had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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