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Research Article

Piper robustipedunculum Yunck.: essential oil profile and chemosystematic insight of this Brazilian native species

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Abstract

The aim of this study was to analyze the chemical composition of the essential oil extracted from the leaves of the species *Piper robustipedunculum* Yunck. The essential oil was obtained through hydrodistillation and analyzed using GC-FID and GC-MS. The results showed that 93.88% of the total composition was composed of 24 compounds. The essential oil was light yellow in color, with a yield of 0.68%, and was rich in sesquiterpenes, including both oxygenated (37.09%), such as *E*-nerolidol (13.60%), and non-oxygenated sesquiterpenes (45.06%), such as δ-cadinene (10.72%), α-copaene (9.85%), and valencene (8.34%) as the major components. This essential oil has the potential for industrial and pharmaceutical applications, and can aid in future research in ecology, chemotaxonomy, and chemophenetics related to the Piperaceae species.

1. Introduction

Piperaceae family belongs to the Piperales order, which is part of the Magnoliideas group. This pantropical family encompasses approximately 4,100 plant species found worldwide, with the largest genus being *Piper* L., containing around 2,000 species [1]. In Brazil, the highest diversity of this genus is located in the Atlantic and Amazon Forests, housing 347 taxa out of the 466 present in Brazil [1]. The Northeast region of Brazil records 93 species of this genus.

Several well-known plants from the *Piper* genus, such as Black Pepper (*Piper nigrum* L.), Long Pepper (*Piper longum* L.), and Kava-Kava (*Piper methysticum* G. Forst), are commonly used as condiments and have various therapeutic purposes [4, 5]. Ethnobotanical surveys of medicinal plants emphasize the importance of the *Piper* genus for its medicinal properties, including increased gastrointestinal motility, antifungal, antibacterial, larvicidal, antinociceptive, and antihepatotoxic activities. The popularity of its medicinal use is linked to the number of *Piper* species available [6-7].

Due to their similar morphological features, species from this genus can often be found in the presence of different ethnospecies, including Jaborandi (*Piper amalago* L., *Piper crassinervium* HB & K., *Piper mollicomum* Kunth and *Piper aduncum* L.), Pariparoba (*Piper cernuum* Vell., *Piper mikanianum* (Kunth) Steudel and *Piper umbellatum* L.), White Betis (*Piper rivinoides*

Kunth., *Piper arboreum* Aubl. and *Piper tuberculatum* Jacq.), among others [8-9].

The essential oils extracted from various parts of the *Piper* genus have demonstrated remarkable biological properties, including antioxidant, antibacterial, antifungal, antiprotozoal, antiproliferative, anti-inflammatory, and neuropharmacological activities [10-12, 7, 13]. This diversity of activities can be attributed to the extensive chemical diversity present in *Piper's* essential oils, which make them valuable tools for chemophenetic and ecological characterization, particularly for new and unstudied species. The presence of specialized secondary metabolites in these oils provides important information for taxonomic descriptions and suit for defining characteristic of a particular species [14].

Piper robustipedunculum Yunck (Fig. 1) is a species native to Brazil, restricted to the Northeastern states. It has morphological similarities with *Piper amplum* Kunth, *Piper arboreum* Aubl. and *Piper ilheusense* Yunck which are plants of popular use and coexist in the same region [15-18]. However, this species has no phytochemical approach in the literature.

Figure 1: Habit of *Piper robustipedunculum* Yunck.

With all the above, this work aims to characterize the composition of the essential oil of *Piper robustipedunculum* Yunck from a natural population of the city of São Vicente Férrer, Pernambuco, Northeastern Brazil.

2. Materials and methods

2.1 Plant material and isolation of essential oil

Piper robustipedunculum Yunck leaves were collected (three samples) on October 22, 2019 at 9 am, in the municipality of São Vicente Férrer, at coordinates 7^0 36′51″ N and 35^0 30′ 53″ E, at an elevation of 352 m.d. An exsiccate was identified and deposited in the Herbarium of the Botany Department of the National Museum of the Federal University of Rio de Janeiro under voucher number 45321. This study was assigned in the Brazilian Genetic Heritage Management Council under the number AE4E953.

The hydrodistillation was the chosen extraction technique, with Clevenger apparatus type, where 100 g of fresh leaves was placed in a volumetric flask (2 L) with 700 mL of distilled water and heated for 2 h. Yielding was calculated in relation to the obtained essential oil (g) and the amount of fresh leaves (g). The essential oil was kept in sealed amber flasks under refrigeration -20 ^{o}C until analysis [12-13].

2.2 Essential oil analysis

The chemical characterization and quantification of *P. robustipedunculum* essential oil was made by gas chromatography coupled to mass spectrometry (GC-MS) and a gas chromatography equipped with a flame ionization detector (FID), respectively. Samples in triplicates were diluted in dichloromethane (HPLC grade, Tedia, Brazil) before analysis (1 mg/mL).

A 1 µL of the solution was injected into an HP Agilent GC 6890 coupled to Agilent MS 5973 series mass selective detectors, splitless mode, wherein the injector temperatures were set at 270°C, operating at 70 eV in positive mode. A HP-5MS (5%-phenyl-methylpolysiloxane) capillary column [Agilent J & W; GC Columns (USA)] was used with 30 m x 0.25 mm i.d. x 0.25 µm particle size. Chromatography conditions were started at 60 - 240 °C at 3 °C / min, totaling 60 min. For the separation of the constituents, helium (~99.9999%) was used as a carrier gas at 1,0 mL/ min, at a rate of 1,0 s sweeps and mass range of *m/z* 40 - 600 atomic mass unit (*u*) [12-13].

The GC-FID was achieved in a chromatograph equipped with a flame ionization detector [HP-Agilent 6890 GC-FID]. A 1 µL of the solution were injected under the same analytical conditions described above, except for the carrier gas used, which was hydrogen at flow rate of 1,0 mL/min. The retention times (Rt) of the compounds were measured in minutes and they were used to calculate their linear retention index, obtained from the injection of a homologous series of hydrocarbons (C_8-C_{25} de *n*-

alkane, Sigma-Aldrich, Brazil) under the same sample analytical condition [19-20, 12-13].

The mass spectra of the constituents were compared with those from library (NIST, 98 e WILEY 7n) and with those published in the literature [21]. Additionally, when possible, the samples were analyzed in conjunction with authentic standards (Sigma-Aldrich, St. Louis, MS, USA).

3. Results and discussion

The essential oil (EO) obtained from the leaves of *P. robustipedunculum*, a native species and endemic to Northeastern Brazil, collected in the region of the Atlantic Forest and studied by GC-MS and GC-FID are presented in Table 1. A chromatographic profile of the essential oil is also presented in Fig. 2. EOs obtained by the same methods in other *Piper* species also showed high percentage of monoterpenes and sesquiterpenes in leaves and reproductive parts in all investigation periods [14].

The EO showed light-yellow color and average yield of 0.68% (g/g). This is very important to industrial applications when compared to other commercial species [14, 22-23].

It was possible to characterize 93.88% of the total EO. Sesquiterpenes (acyclic, monocyclic, bicyclic and tricyclic) were registered as the main fraction, with a total of 82.15%, being non-oxygenated 45.09% and oxygenates 36.09%. *E*-nerolidol (13.60%), δ-cadinene (10.72%), α-copaene (9.85%), and valencene (8.34%) were identified as major compounds. For Piperaceae species, the high content of sesquiterpenes is in accordance with literature data [24, 7]. The monoterpene class represent only 2.66% of the total. These similar variations of monoterpenes and sesquiterpenes have already been reported by Santos *et al.* [24] in Brazilian Atlantic Forest for species of the same genus, including *Piper arboreum* var. *arboreum* Yunck. and *Piper goesii* Yunck.

The Piperaceae family is known for the frequent occurrence of ethnospecies, as previously mentioned. Considering taxonomic approaches, in the subclate Isophyllon [25], the taxonomic classification group that belongs to *P. robustipedunculum*, it is possible to find species that in their EO from leaves compounds produced by acetate-mevalonate or methylerythriol-4-phosphate metabolic pathways.

In 1966, Yuncker [26] pointed out the ease of errors caused by the similarity in morphology among the

Table 1. Aromatic profile of *P. robustipedunculum* essential oil from leaves (n = 3) from Pernambuco, Brazil.

RT	Compounds[1]	RI*	RI**	Composition (%)
9.792	limonene	1026	1024	1.05
13.440	*Z*-β-ocimene	1034	1032	1.61
22.002	δ-elemene	1323	1335	1.62
24.043	α-copaene	1374	1374	**9.85**
25.679	*E*-caryophyllene	1419	1417	4.75
26.916	α-humulene	1455	1452	1.05
27.800	*E*-cadina-1(6),4-diene	1476	1475	1.58
27.972	germacrene D	1483	1480	2.43
28.302	β-selinene	1490	1489	1.12
28.746	valencene	1500	1496	**8.34**
28.816	γ-cadinene	1515	1513	4.60
29.911	δ-cadinene	1524	1522	**10.72**
31.163	*E*-nerolidol	1560	1561	**13.60**
32.163	germacrene D-4-ol	1574	1574	5.16
32.359	gleenol	1587	1586	1.02
32.649	viridiflorol	1593	1592	1.54
33.229	1-*epi*-cubenol	1628	1627	1.94
33.362	*Z*-cadin-4-en-7-ol	1634	1635	1.04
33.752	*epi*-α-cadinol	1640	1638	2.21
33.960	*epi*-α-muurolol	1642	1640	2.17
35.010	α-muurolol	1645	1644	2.12
34.519	α-cadinol	1657	1652	5.29
35.796	tricosane	2299	2300	3.98
48.573	octacosane	2798	2800	4.09
Total identified				93.88
Compound classes (%)				
Non-oxygenated Monoterpene				2.66
Non-oxygenated Sesquiterpene				45.06
Oxigenated sesquiterpenes				37.09
Others (hydrocarbons)				8.07

[1]Compounds an in order of elution on the HP-5MS column; RT = Retention time in minutes; RI*= Experimentally determined retention indices for C_8 - C_{20} n-alkanes on the HP-5MS RI column; RI**= Literature Retention Indices [21], %= average compound concentration in the three samples obtained.

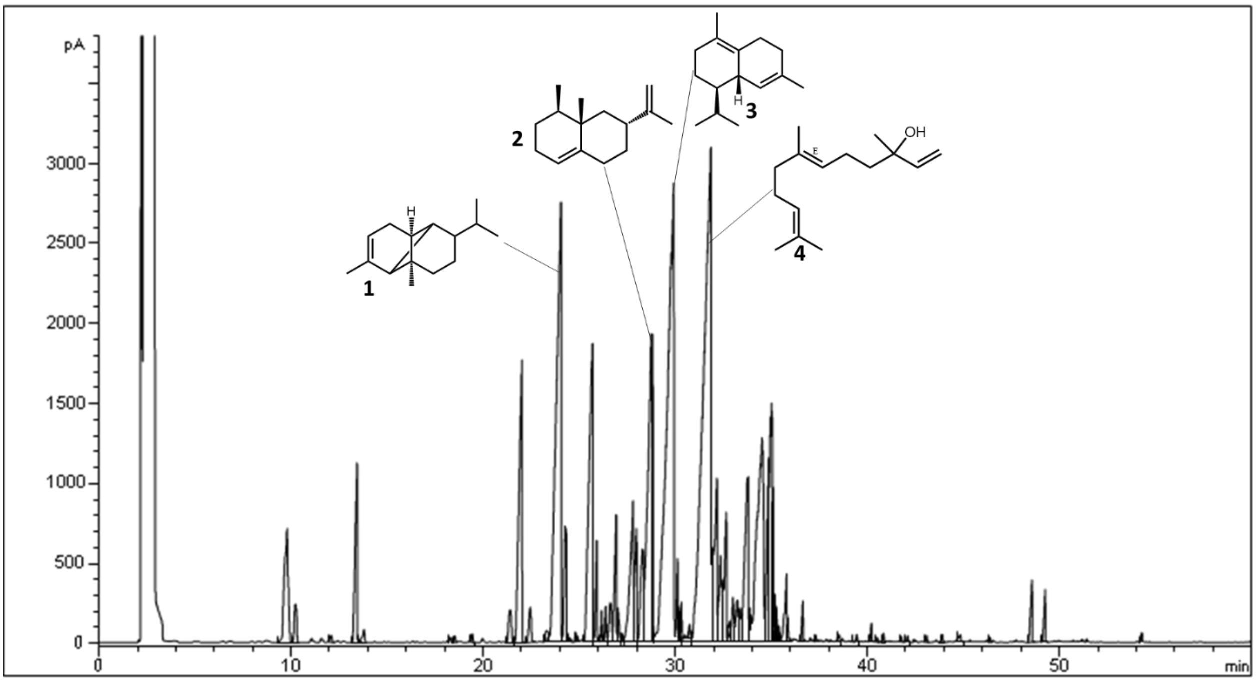

Figure. 1. Chromatographic profile of the flame ionization detector (CG-FID) of the essential oil of *P. robustipedunculum* from Pernambuco, Brazil. (See compound 1 - α-copaene; 2 – valencene; 3 - δ-cadinene; and 4 - *E*-nerolidol)

species within the Isophyllon. Specifically, the medicinal plants *Piper amplum* Kunth, *Piper arboretum* Aubl., *Piper ilheusense* Yunck *and Piper vicosanum* Yunck are often confused with *P. robustipedunculum* due to their similar appearance, which can lead to accidental or intentional mislabeling in Northeast Brazil. This can be a problem for quality control and the correct use of these plants [9, 18].

Chemotaxonomically allow for the observation of variations in chemical compositions among species within this group across different studies in the literature. For example, for the EO of *Piper ilheuense* Yunck it was registered the compounds patchouli alcohol (11.10%), *E*-caryophyllene (11.80%), gleenol (7.50%) and δ-cadinene (6.90%) [16]. Pereira *et al.* [27] describe the chemical composition of EO from different species of *Piper*, in which, *Piper amplum* contained the iso-leptospermone (26.44%) and α-eudesmol (11.57%), while *Piper vicosanum* contained α-eudesmol (17.35%) and α-copaene (12.34%). *Piper arboreum* var. *arboreum* and *Piper arboretum* var. *hirtelum* had a major *E*-caryophyllene (13.71% and 13.22%) in common, but a significant difference (>5%) in the relative percentage of bicyclogermacrene (18.50% and 5.32%) and α-cadinol (2.40% and 8.89%), respectively. Studies such as the one presented in this manuscript serve as a means to prevent adulteration and to describe the different chemophenetic phenomena of this species.

The majority of studies on EOs with a high content of *E*-nerolidol can be found in the Brazilian Atlantic Forest. For example, *P. aduncum* L. (80.6 – 82.5% content) [28, 29]; *P. claussenianum* (Miq.) C. DC. (81.4 – 83.3% content) [30]; *Piper diospyrifolium* Kunth (10.0% content) [27] and *P. gaudichaudianum* Kunth (22.1% - 22.4% content) [31-32, 14]. Chan and co-workers [33] state that *E*-nerolidol is widely used in the industry, including in the production of cosmetics, food, and pharmaceutical products. *Piper* species that are abundant in *E*-nerolidol have already been demonstrated to exhibit a variety of biological functions, such as antileishmanial (promastigotes of *Leishmania amazonensis*, IC_{50} 30.24 µg/ mL) and antifungal (*Candida albicans*, MIC 0.2 –1.26%) for *P. claussenianum* [30, 34-35]; and cytotoxic (V79 Chinese hamster lung cells, IC_{50} 4.0 µg/ mL) for *P. gaudichaudianum* [31].

The compounds δ-cadinene, α-copaene, and valencene are described in the literature for their diverse biological activities when present in the essential oils of *Piper* [4-5, 7, 11, 14, 17]. These activities include antimicrobial, anti-inflammatory, antioxidant, and anticancer properties. Some studies also suggest that these compounds may possess analgesic and sedative properties, in addition to their use in the fragrance and flavor industry due to their pleasant aroma [14, 17, 22-23].

4. Conclusions

The essential oil of the *P. robustipedunculum* leaf can be a source of vegetable raw material for future employability in the pharmaceutical, cosmetic, food and pesticide industries, since it is sesquiterpene-rich. Also, the chemical composition of this endemic species from the Atlantic Forest of Northeast Brazil, a biome high threatened by human action, can assist in studying to understand ecological, chemotaxonomics and chemophenetics issues considering Piperaceae species.

Authors' contributions

Conceptualization, G.A. Q. and Y. J. R., Methodology, Y. J. R., E. F. G. and D. L. M.; Formal Analysis, G.A. Q., D. B. M., C. C.-O. and J. R. S. F.; Investigation, Y. J. R., G.A. Q. and I.C.F.; Resources, Y. J. R., D. B. M., C. C.-O., J. R. S. F., G.A. Q. and I.C.F..; Writing – Original Draft Preparation, Y. J. R. and I.C.F.; Writing – Review & Editing, Y. J. R., I.C.F. and D.L.M.

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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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