Investigation of antimicrobial potency of sesquiterpenoid dominant essential oil of *Curcuma inodora* species

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Abstract:

An Ultra-sound assisted solvent extraction of *Curcuma inodora* rhizomes was carried out using ethyl acetate and characterized with the GC/ GC-HRMS and GC-FID techniques revealed a total of 24 components. The key components observed in the extract were - α- Bisabolol (31.20%), β- Bisabolene (16.67%), β – Phellandrene (10.49 %), β -Pinene (7.08%) and Pregn-14-en-2-ol (6.23%) that constituted- 50.75% sesquiterpenoids, as dominant part, 25.82% monoterpenoids and 23.33% other compounds with 0.52% yield. Out of four tested bacteria (two gram +ve and two gram –ve), the oil showed excellent antibacterial activity versus *P. aeruginosa* with Ampicillin as the standard drug while excellent antifungal potency was found against *C. albicans* with Griseofulvin as the standard drug. This report reveals the chemical composition of essential oil of this underexplored species along with its antimicrobial efficacy. It also highlights the geographical and environmental impact on the constituents of the oil.

Keywords: α -Bisabolol, essential oil, Bisabolene, antibacterial activities, antifungal activities.

1. Introduction:

Plants, the richest source of primary medicines for humans are being valued in many developing countries for primary diseases. Being the principal medicinal plant producer country, India, with its characteristics flora have the potential to formulate new antibiotics. Furthermore, India is having a long history of Ayurvedic formulations. (1).

The present pandemic urges the devising of antimicrobial drugs to deal with recently evolving microbes. So, plant products can fulfill this necessity (1, 2, 3).

The Zingiberaceae family having the genus *Curcuma, Hedychium, Zingiber, Amomum,* and *Alpinia* are valuable families with the presence of essential oils, useful to ward off many infections. These oils containing a single or cluster of compounds with potent medicinal value are being part of many pharmaceutical formulations since earlier age (4,5).

The species of the genus *Curcuma* exhibit medicinal properties and as a part of this genus, *C. longa, C. amada, C. aromatica, C. caesia, C. zeodaria* etc. have important medicinal applications (6). In contrast, one of the species from this genus - *C. inodora*, is being used as ornamental plants on a large scale and its medicinal properties are yet to be explored. This species is reported mostly from south India and Malaysia but this is vastly present in the Konkan region too, during the monsoon season (7, 8,9).

The chemical structures of oils are greatly influenced by geographic and environmental factors (10,11). Therefore, an attempt has been made to investigate this effect on C. *inodora* collected from the Konkan region of Maharashtra-India.

2. Materials and methods:

2.1 Plant material: The species was chosen from the forest area located on Lonere-Shriwardhan road, in the month of September 2019. The plant species was authenticated by Botanical Survey of India, Pune.

2.2 Extraction: Fresh rhizomes were collected, washed, and dried. The solvent extraction was furnished using ethyl acetate as solvent. The rhizomes were soaked in solvent and the beaker was enclosed with Al foil. For half an hour, the same was exposed to ultrasonic irradiation (5.5-liter capacity,230 V, 50 Hz, and max. temp. 70^{0}C). Then, centrifuged the mixture and finally, the solvent was removed by a rotary evaporator (12). After the dehydration process with Anhy. Sod. Sulfate, the oil was kept in the freezer till further use. The percentage yield was calculated by volume-to-weight ratio (%v/w).

2.3 Essential oil analysis: GC/GC-HRMS and GC-FID analysis were conducted by using Agilent technologies 7890 Gas chromatograph fortified with JEOL The Accu ToF GCV JMS-T100 GCV MS detector. The carrier gas Helium was used with flow rate 1.0ml/min. The EB5 column (30m length x 0.25mm diameter x 0.25µm thickness) was used during the analysis. The column temperature 60˚-280˚C was programmed at 3̊C/min and the scanning range was set between 45-650amu. The 0.1µL sample prepared in acetone, was injected in injector at 250̊ c with split ratio 1:10. The mass spectra was recorded at 70ev (EI) and the compositions of the oil were determined by Retention index, Library MS search (NIST) and by comparing MS with literature data (13).

2.4. In vitro Antibacterial Study: Broth dilution method was employed to know MIC of extracted rhizome oils (14). DMSO was used as diluent as it has no significant effect against selected bacteria. Two gram positive bacteria - *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus* (MTCC 442), and two gram negative bacteria- *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 1688) were tested against rhizome oil. Ampicillin and Ciprofloxacin were used as standard reference drug.

2.5. In vitro Antifungal Study: Agar dilution protocol was employed to test rhizome oils against some selected fungal strains viz. *Candida albicans*(MTCC227), *Aspergillus niger* (MTCC 282) and *Aspergillus clavatus* (MTCC 1323) (15).

The dilution which showed 99% inhibition was considered as MIC. Griseofulvin and Nystatin were the reference drugs used in the experiment and triplicate analysis were performed to minimize errors.

3. Results and Discussion:

3.1 Chemical composition :

The solvent extraction yielded brown-colored oil with 0.52% yield (0.4 ml from 130g rhizome) constituting 24 components, comprising of 99.9% oil. The key components of rhizome oil were- α- Bisabolol (31.20%), β-Bisabolene (16.67%), β –Phellandrene (10.49 %), β -Pinene (7.08%) and Pregn-14-en-2-ol (6.23%). The essential oil constitutes 50.75% sesquiterpenoids, 25.82.% monoterpenoids and 23.33% other compounds.

The comparative study of the percentage of chemical constituents of rhizome oil of species is reported in table no.1.Out of the two available reports, the report of Santoshkumar & A. Yusuf from south India varies from the report by Malek et al. from Malaysia while present results too vary with respect to reported articles in qualitative and quantitative aspects. Only two components viz. β –Pinene and β- Caryophyllene are found common in the three reports. The α -Bisabolol, an excellent antifungal and the anti-inflammatory agent is reported first time as major component in this study (16,17). It has been reported that the α –Bisabolol is applied in skin care which smoothens inflammation, stimulates skin healing, disinfects the skin, moisturizes in-depth skin, and provides a natural scent. As *C. inodora* contains a major portion of α –Bisabolol in its essential oil, it can be a good choice in skin care treatment (18) while β- Bisabolene, an excellent antibacterial agent can enhance bactericidal efficacy(19).

Phellandrene is the second-largest component found in oil. α- Phellandrene has major applications in fragrances due to their pleasing aroma. β – Phellandrene has been described by pepper minty and slightly citrusy. It is widely used in flavor industry (20).

Table 1: Percentage composition and comparison with literature of *C. inodora* Essential oils.

Sr. No.	Components	RI^1	RI^2	Test Sample (%)	By Santoshkumar & A.Yusuf (2019)	By Malek et al. (2006)
1.	α - Bisabolol	1683	1683	31.20	-	-
2.	β- Bisabolene	1500	1500	16.67	7.7	-
3.	β - Phellandrene	1031	1031	10.49	-	-
4	4-terpinol	1161	1158	0.37	-	-
5.	β- farnesene	1440	1448	1.39	-	0.6
6.	α -Pinene	926	927	3.23	-	0.4
7.	β -Pinene	963	965	7.08	11.3	0.7
8.	α- Phellandrene	997	1035	1.19	-	-
9	β - Myrcene	979	983	1.89	-	0.4
10	m-Cymene	1042	1043	3.04	-	0.4

11.	Cyclohexene-1-methyl-5-(1-methylethenyl)	1018	1027	1.67	-	-
12.	Eucalyptol	1023	1023	1.94	-	5.3
13.	Cis-5,8,11,14-Eicosatetraenoic acid,picolinyl ester	2975	2974	1.90	-	-
14.	7,8-Epoxylanostan-11-ol, 3-acetoxy	3145	3324	1.26	-	-
15.	β- Caryophyllene	1421	1418	1.05	1.3	1.1
16.	Pentanoic acid ,5-hydroxy-2,4-di-t-butylphenyl ester	2255	2250	0.85	-	-
17.	β -Sesquiphellandrene	1516	1543	0.44	-	2.7
18.	5,6,7-Trimehoxy-1-indanone	1785	1780	0.30	-	-
19.	Pregn-14-en-2-ol,(5α ,20S)	2102	2105	6.23	-	-
20.	2-Pentanoic acid ,3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)	2254	2250	2.17	-	-
21.	Phthalic acid,diisobutyl ester	1908	1908	0.43	-	-
22.	Cycloartenol acetate	2956	2958	0.64	-	-
23.	4,22-Stigmastadiene-3-one	2722	2730	2.70	-	-
24.	Trilinolein	6173	6180	1.77	-	-
	Total Chemical Components	-	-	24 (99.9%)	NA	55 (89.3%)

- Compounds not detected.

RI[1]- Retention index values detected on EB5 column.

RI[2]- Retention index values from literature (Adams 2007).

NA- Not Available.

3.2. In-vitro antibacterial activities:

This was conducted by Broth dilution method. Table 2 shows the antibacterial activities against two gram-negative bacteria- *E. coli* (MTCC-443), *P. aeruginosa* (MTCC-1688), and two gram-positive bacteria-, *S. aureus* (MTCC-96), *S. pyogenus* (MTCC-442).

The oil exhibited excellent antibacterial activities against gram-negative bacteria i.e. *P. aeruginosa and E. coli* with Ampicillin as the reference drug. This might be due to the presence of bisabolene and monoterpenes which enhanced the antibacterial activities versus gram -ve bacteria (19,20).

Table-2: MICs of essential oil against bacterial pathogens

Test Pathogens	MICs of Test Sample (µg/ml)	MICs (µg/ml) of Ampicillin (Standard)	MICs (µg/ml) of Ciprofloxacin (Standard)
E. coli (MTCC-443)	100	100	25
P. aeruginosa (MTCC-1688)	50	100	25
S. aureus (MTCC-96)	250	250	50
S. pyogenus (MTCC-442)	250	100	50

3.3. In-vitro antifungal activities: An in-vitro efficacy of rhizome oil against *C. albicans (MTCC-227), A. niger (MTCC-282)* and *A. clavatus* (MTCC-1323) strains is shown in table 3. Out of three fungal strains, the essential oil exhibited excellent antifungal activity against *C. albicans (MTCC-227)* with respect to reference drug

Griseofulvin. This can be attributed to the presence of excellent antifungal agents like α - Bisabolol and Bisabolene (21).

Table-3: MICs of Rhizome oil against fungal pathogens

Test pathogens	MICs of Test Sample (μg/ml)	MICs (μg/ml) of Greseofulvin (Standard)	MICs (μg/ml) of Nystatin (Standard)
C. albicans (MTCC-227)	250	500	100
A. niger (MTCC-282)	500	100	100
A. Clavatus (MTCC-1323)	500	100	100

4.0. Conclusion: This study reveals that the α – Bisabolol, an important skin healing agent, and β – Phellandrene, an important essence agent, are the major components of the essential oil of this undervalued species. The present results do vary significantly from the reported literature in qualitative and quantitative aspects. Moreover, the essential oil exhibited excellent antibacterial and antifungal efficacy against *P. aeruginosa and C. albicans* respectively. Therefore, it may be used in the skincare treatment process, after a detailed study. Further, this study highlights the geographical and environmental impact on the chemical compositions of *Curcuma inodora* rhizome oil.

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