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RESEARCH ARTICLE

Bioefficacy of α-Bisabolol-rich essential oil separated from *Curcuma inodora* rhizome of Konkan region, India

Pravinkumar Nagore[1], Popat Lokhande[2], Haniph Mujawar[2], Amol Ghoti[1], Amol Salve[1] & Kanchan Mane[1*]

[1]Mangaon Taluka Education Society's Doshi Vakil Arts College and GCUB Science & Commerce College, Goregaon, Raigad 402 103, Maharashtra, India
[2]Dr Babasaheb Ambedkar Technological University, Lonere, Raigad 402 103, Maharashtra, India

*Correspondence email - kanchanamne13@gmail.com

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Abstract

Curcuma inodora Blatt., an ornate species from the Zingiberaceae family native to the peninsular region, holds unexploited medicinal potential. This study presents the first comprehensive report on the rhizome oil of *C. inodora*, integrating *in-vitro*, *in-silico* and *in-vivo* approaches. The essential oil was extracted using a relatively novel ultrasound-assisted solvent extraction method, employing ethanol as the solvent, with a yield of 0.69 %. GC/HRMS analysis identified α-Bisabolol (45.64 %), β-Thujene (17.25 %) and β-Bisabolene (14.24 %) as the major components. *In-vitro* antimicrobial activity was assessed using broth and agar dilution methods, while antioxidant potential was evaluated via 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) radical scavenging assays. Anti-inflammatory properties were tested using three distinct protein inhibition-based methods. These evaluations reflect the plant's traditional use in folk medicine for treating microbial infections, inflammation and oxidative stress. SwissADME server was used for *in-silico* ADMET profiling. Acute oral toxicity was assessed in Wistar rats following OECD 423 guidelines, including histopathological analysis. The α-Bisabolol-rich oil exhibited potent antibacterial activity against all tested strains, strong antifungal action against *Candida albicans*, significant antioxidant activity in the NO assay and notable anti-inflammatory effects. The ADMET analysis indicated high gastrointestinal absorption, favorable bioavailability and no Lipinski rule violations. Toxicity studies showed an LD_{50} > 2000 mg/kg, confirming safety. These significant bioactivities are primarily attributed to α-Bisabolol, a major constituent recognized for its pharmacological importance. Therefore, the α-Bisabolol enriched rhizome oil of *C. inodora* holds considerable therapeutic promise, justifying further pharmacological and clinical investigations for potential drug development.

Keywords: α-Bisabolol; *Curcuma inodora*; essential oil; sesquiterpenoids; Ultra-Sound Assisted Rhizome Essential Oil (USAREO)

Introduction

The Konkan region of India, located within the Western Ghats, is recognized as a global biodiversity hotspot. It is home to a high degree of floral and faunal endemism, which has garnered significant interest from researchers around the world (1). The recent COVID-19 pandemic exposed the limitations of existing drugs against various diseases, emphasizing the need for novel therapeutic alternatives. Plants and plant-based products offer promising substitutes for synthetic formulations, as they contain valuable bioactive compounds. Being the world's largest producers of medicinal plants, India has immense potential to develop these alternative drugs from plant resources (2).

The Zingiberaceae family ranks among the most prominent medicinal plant families, owing to its substantial therapeutic value. Among its members, the *Curcuma* and *Zingiber* genera have been extensively researched on a global scale (3). In contrast, this condition does not prevail in the Konkan region. Despite the rich availability of Zingiberaceae species, substantial research on these plants remains limited in this area. Our previous work has explored essential oils of some species like *Zingiber neesanum, Hedychium coronarium* and *Curcuma inodora* from this region (4-6). As a part of exploring the potency of the Zingiberaceae species from this region, we have also reported the synthesis and bioapplications of copper nanoparticles using *H. coronarium* leaves, highlighting their potential for water detoxification (7).

Curcuma inodora is an ornate plant owing to its gorgeous inflorescence. Its changing flower and bract color imparts additional value to its beauty and is useful as a cut flower crop. It is abundant in peninsular India. The label *inodora* is specifically used to highlight its non-aromatic nature, unlike other *Curcuma* species (8). This has led to limited studies on the therapeutic applications of this species from India and abroad. It is abundantly existing in the Konkan province. The life period of this species is between June to October i.e. the normal rainy season in India. The inflorescence of this species is used by locals in the Ganesh festival for decoration purposes. Further, the fresh rhizome paste is applied to wounds, cuts and insect bites. The decoction of the rhizomes is used topically to get relief from muscle pain and

arthritis, while the fresh rhizome paste is applied to eradicate skin infections, rashes and boils. These uses highlight the antimicrobial, anti-inflammatory and antiseptic properties of this species. Further, the ethanolic and methanolic leaf extracts of this species have been shown to exhibit antidiabetic properties. Moreover, the rhizome of this species has also been used against constipation and psychosomatic disorders. The smoke produced from burning dried rhizomes is traditionally used by the Korku tribals from Melghat, Amravati, Maharashtra, to induce a hypnotic or sedative effect. Fumes from the rhizome are inhaled to treat severe coughs and colds by Konda groups from Godavari district Andhra Pradesh, India. Anthocyanins, responsible for antioxidant and anti-inflammatory properties, have been reported in this species from the Konkan region. The major phytochemicals identified in this species include monoterpenes, sesquiterpenes, triterpenes, phenolic compounds, esters, fatty acids and related constituents (9-15).

Our previous work had focused on the bioassessment, pharmacokinetics and docking studies of α-Bisabolol, a major component resulting from this species's rhizome hydrodistillate extract (HDREO). However, there remained a critical gap in validating these findings through *in-vivo* pharmacokinetic and toxicological studies. The current work was designed to bridge this gap by integrating *in-vitro*, *in-silico* and *in-vivo* assessments of the ultrasound-assisted extracted essential oil, which differs in composition and potency from the HDREO. Furthermore, this study provides the first comprehensive toxicological evaluation (OECD 423 protocol) and histopathological support for the safety of α-Bisabolol rich oil. Thus, it contributes a crucial layer of experimental validation necessary before considering therapeutic or formulation prospects. As the properties of isolated oils are greatly influenced by isolation methods, geographical and environmental factors, this study can guide a detailed investigation of this species (16, 17).

Materials and Methods

Plant material

Fresh rhizomes (130 g) of *C. inodora* were collected in September 2021 from a forested area near the village of Nhave, Lonere-Raigad (Lat. 18.170895°, Long. 73.321895°). The species was taxonomically authenticated by the Botanical Survey of India, Western Regional Centre, Koregaon Park, Pune 411 001.

Isolation of oil

Solvent extraction

This was performed using 99.99 % ethanol as a solvent. The rhizomes were soaked in a beaker comprising a solvent and were enclosed with Al foil. For half an hour, the same was exposed to ultrasonic irradiation (5.5 L capacity, 230 V, 50 Hz and maximum temperature 70 °C). Then, after the centrifuge process, the solvent was removed by a rotary evaporator. After dehydrating the essential oil with anhydrous sodium sulfate was kept in the freezer and the percentage yield was calculated after three replications (4). The chemicals required were bought from Molychem, Mumbai.

Essential oil analysis

Gas Chromatography-High Resolution Mass spectrometry (GC/HRMS) analysis

GC/HRMS analysis was performed by using Agilent technologies 7890 gas chromatograph equipped with JEOL the Accu ToF GCV JMS-T100 GCV MS detector. Helium was the carrier gas used in GC with a flow rate of 1 mL/min. with HP5 column (30 m length x 0.25 mm diameter x 0.25 µm thickness) and fortified in GC during analysis. The samples to be analyzed- were diluted in 1:100 v/v ratios and acetone was the solvent used. The samples were inserted through an auto-injector (1:10 for a min) with a constant temperature of 250 °C. Initially, the temperature of GC was programmed at 60 °C for 2 min which was then programmed to 250 °C and increased up to 280 °C at 30 °C /min for 10 min. The GC/HRMS was scanned between 45-650 amu. The mass spectra were recorded at 70 ev (EI) and the compositions of essential oil were recognized based on R I, Library MS search (NIST) and by comparing MS with reported literature (18).

In-vitro antibacterial study

The broth dilution method was employed to appraise the MIC of extracted rhizome oil. The serial dilutions in the primary (1000, 500 and 250 µg/mL) and secondary (200, 100, 50, 25, 12.5, 6.25 µg/mL) screening were prepared. The highest dilution showing at least 99 % inhibition zone was considered as MIC. Two gram +ve bacteria - *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus, (*MTCC 442) and two gram -ve bacteria- *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 1688) were tested versus USAREO. Ampicillin was used as a reference drug. The results displayed are the product of triplicate analysis (19).

In-vitro antifungal study

Agar dilution protocol was employed to test USAREO against selected fungal pathogens viz. *Aspergillus clavatus, Candida albicans* and *Aspergillus niger*. The stock solutions of oil were prepared in DMSO, then incorporated in a specified amount of sterile molten dextrose agar for screening antifungal activities. To prepare inoculums, the stock of 100 mL of nutrient broth in 250 mL sterilized and clean conical flasks were heated at 27 °C for 24 hrs. before the experiment. The plates were kept in aseptic conditions and were allowed to diffuse through a potato agar dextrose medium. Further, the plates were incubated at 25 °C for 2 days. The dilution which showed 99 % inhibition was considered MIC concerning Griseofulvin as reference drug and the results displayed are the product of triplicate analysis (20).

The bacterial and fungal strains were cultured and their antimicrobial activities were evaluated at Microcare Laboratory, Gujarat.

In-vitro antioxidant activities

The antioxidant efficacy of USAREO was assessed by two assays viz. DPPH and NO following standard protocols (21, 22).

DPPH assay

The scavenging activity was measured using the stable radical DPPH by 96 well plate method. The methanolic DPPH (0.1 mM) solution was made ready and 1 mL of this solution was transferred to 3 mL of oil with varying concentrations (25, 50, 75 and 100 µg/mL). The absorbance was recorded at 517 nm after 30 min. The IC50 values were calculated as follows:

$$\text{Percentage scavenging} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

NO assay

This was determined using stable NO• radical cation following standard protocols. The sample concentrations employed and the percentage scavenging activity were decided by the same formula applied for the DPPH assay.

Ascorbic acid was the standard in both assays. All the tests were carried out in triplicate and the results are expressed as mean ± SD.

***In-vitro* anti-inflammatory activities**

The USAREO was examined for anti-inflammatory activities against human RBCs. The three different methods viz. Heat-induced hemolysis, inhibition of albumin denaturation and proteinase inhibitory action (23-25) were employed with five different concentrations of test samples - 25, 50, 75, 100, 125 and 150 µg/mL by following standard protocols. The standard drug used was diclofenac sodium. The solutions of both were made ready in isosaline (0.9 % NaCl).

Assay of heat-induced hemolysis

The reaction mixture (1 mL of oil and 1 mL RBCs (10 %)) was heated for 30 min at 56 °C. After incubation, the tubes were cooled under tap water. Then the mixture was centrifuged for 5 min at 2500 rpm and the absorbance was noted at 560 nm. A triplicate analysis was done. The tube containing only saline was used as a blank.

The percentage inhibition was decided as follows:

$$\text{Percentage inhibition} = 100 \times (A2/A1-1)$$

Where, A1 = Abs of the buffered saline solution

A2 = Abs of the test sample

Proteinase inhibitory action

The reaction mixture [0.06 mg trypsin + 0.25 mL 20 mM Tris-HCl buffer (pH 7.4) + 0.25 mL oil (different conc.)] was heated at 37 °C for 5 min and 0.25 mL of 0.8 % (w/v) casein was added. Then an additional 20 min incubation was carried and 0.5 mL of perchloric acid (70 %) was mixed to terminate the reaction. The cloudy suspension was centrifuged and the absorbance was noted at 210 nm against the buffer as blank. The experiment was performed in triplicate. The percentage inhibition was decided as follows:

$$\text{Percentage inhibition} = 100 \times (1 - A_2/A_1)$$

Where, A_1 = absorbance of the control and A_2 = absorbance of the sample

Inhibition of albumin denaturation

The reaction mixture [oil with different concentration + 1 % aqueous solution of bovine albumin fraction] was taken and the pH was maintained by a small amount of HCl (1N). The mixtures were heated for the first 20 min at 37 °C and then at 57 °C for the next 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. The percent inhibition was decided as follows:

$$\text{Percentage inhibition} = (\text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}} - 1) \times 100$$

The antioxidant and anti-inflammatory activities were evaluated at Biocyte Research Institute, Sangli, Maharashtra.

In-silico pharmacokinetics study

An in-silico ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis of the principal phytochemical constituents identified in the rhizome oil was conducted to evaluate their drug likeness and pharmacokinetic profiles. The study utilized the SwissADME online server (http://www.swissadme.ch/), a widely accepted computational tool for predicting molecular properties relevant to oral bioavailability and drug development. Parameters such as lipophilicity (Log P), solubility, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, cytochrome P 450 (CYP 450) inhibition and Lipinski's rule of five were assessed for each compound. These predictive models provide insights into the potential therapeutic viability, safety and systemic behavior of the active components, aiding in the rational selection of lead molecules for further experimental validation (26).

In-vivo pharmacokinetics, oral toxicity

Ethical consideration

The animals under study were used only after approval of the Ethical Committee (IAEC Approval No. SCI/IAEC/2022-23/05 dated 29th May 2022) of SCITESLA laboratories, Navi Mumbai, Maharashtra.

Acute oral toxicity

This was determined following OECD guidelines 423 (27), with 12 female rats of the age between 6-8 weeks with bodyweight 210-232 g brought from the National Institute of Bioscience, Pune. Different doses of USAREO up to 2000 mg/kg were administered and observed for 72 hrs concerning changes in behavior, toxic allergies and mortality.

Housing conditions: Temp 22 ± 2 °C, Light 12:12 hrs. light-dark cycle, HR:30-45 %.

ADME study

This was performed on 06 Wistar rats of either sex, aged between 8-12 weeks old with bodyweight 190-225 g by using a validated method (28).

The bioavailability and metabolic studies were carried out using male Sprague-Dawley rats with α-Bisabolol as standard. Overnight fasted rats were divided into various groups and administered with a test sample at the dose of 100 mg/kg at a volume of 10 mL/kg orally.

To study enzymatic metabolism blood samples from each rat were withdrawn in an Eppendorf tube at various intervals viz. 0, 0.02, 0.5, 1, 2, 4, 8, 10, 12, 18 and 24 hrs. Then the blood samples were centrifuged at 5000 rpm for 10 min for plasma separation. Subsequently, 200 µL of plasma was mixed with 200 µL of CH_3CN to precipitate proteins, which were then separated by centrifugation. The clear supernatant was used for the determination of concentration using a microplate reader (EPOCH, BioTek-Agilent, USA). The volume of distribution (Vd) was estimated based on the distribution of oil in body tissue rather than plasma while the elimination rate constant (K_{el}) was determined from the excretion rate and plasma half-life ($t_{1/2}$) represented half time to reduce the concentration of test sample.

Histopathology studies

This was done following the literature method (29). The rats were sacrificed after toxicity studies by cervical dislocations and vital organs like heart, brain, lungs, kidney and liver were removed. Then, immediately washed with cold normal saline and used for histoarchitecture studies. The organs were immersed in buffered (pH 7.2) formalin overnight. The next day, 2 mm thick slices were taken and placed in the cassette with the base side towards the bottom. After paraffin embedding, a 7 µm section was sliced and stained with hematoxylin-eosin. The stained sections were examined under the microscope and photographs were taken.

Statistical analysis

The data are presented as mean ± SEM.

Results and Discussion

Composition of isolated oil

The average oil yield obtained by ultra sound assisted solvent extraction was 0.69 % (0.9 mL from 130 g rhizomes). The compositions of USAREO were detected using GC/GC-FID and GC/HRMS analysis (Table 1).

Ultrasound assisted solvent extraction produced a pale yellow essential oil comprising 16 constituents, accounting for 99.87 % of the total oil content. The major components identified were α-Bisabolol (45.64 %), β-Thujene (17.25 %), β-Bisabolene (14.24 %) and Pregn-14-en-2-ol (8.28 %). The oil composition included 45.64 % oxygenated sesquiterpenoids, 14.24 % sesquiterpene hydrocarbons, 18.15 % monoterpene hydrocarbons, 0.36 % oxygenated monoterpenes and 21.48 % other compounds.

Compared to our previous report on the hydrodistilled rhizome extract of this species, the present study shows a higher oil yield and a greater number of total components (6). Several constituents are common to both studies, including α-Bisabolol (66.13 %), β-Thujene (4.23 %) and 4-Terpineol (2.99 %). The earlier extract exhibited a higher percentage of oxygenated sesquiterpenes (77.65 %) and a lower proportion of sesquiterpene hydrocarbons (3.09 %). In contrast, the current extract contains 45.64 % oxygenated sesquiterpenes and 14.24 % sesquiterpene hydrocarbons.

The presence of Caryophyllene (31.33 %), Benzofuran (21.29 %), Cyclohexane (11.50 %), Phytol (3.19 %), α-Bisabolol (2.32 %), along with other compounds in the hydrodistilled

Table 1. Chemical composition of *C. inodora* essential oil

Sr. No.	Components	RI[1]	RI[2]	By USASE (%)	Identification
1.	α–Bisabolol	1683	1683	45.64	MS, RI
2.	β-Thujene	968	971	17.25	MS, RI
3.	4-Terpinol	1161	1177	0.36	MS, RI
4.	β- Bisabolene	1500	1509	14.24	MS, RI
5.	m-Cymene	1013	1015	2.04	MS, RI
6.	Eucalyptol	1023	1023	0.90	MS, RI
7.	Acetic acid, 17-[4-chloro -5-methoxy-1,5-dimethylhexyl]-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl	3268	3270	0.40	MS, RI
8.	17-[1,5-Dimethyl -3-phenylthiohex-4-enyl]-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopent(a) phenanthren-3-ol	3742	3745	1.30	MS, RI
9.	5H-Cyclopropa [3,4] benz [1,2-e]azulen-5-one,9,9 a bis[acetyloxy]-3-[(acetyloxy)methyl-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a,-dodecahydro,3,4a,7b-trihydroxy-1-ol	3533	3533	0.32	MS, RI
10.	Mibemycin b,13-chloro-5-demethoxy-28-deoxy-6,28, epoxy-5-[hydroxyimino]-25-(1-Methylethyl) -(6R,13R,25R)	4391	4389	3.22	MS, RI
11.	Pregn-14-en-2-ol,(5α ,20S)	2102	2100	8.28	MS, RI
12.	Cyclopropanecarbohydrazide,2,2,3,3-tetramethyl-N2-(4-nitrophenyl)	2357	2350	1.69	MS, RI
13.	9-(4-Fluoro-phenyl)-12-imino-10,11,-dioxa-tricyclo[6.2.2.01,6]dodecane-7,7,8-tricarbonitrile	2746	2740	2.66	MS, RI
14.	Ursodeoxycholic acid	2896	2890	0.47	MS, RI
15.	Pregna-20-ol-3-on-19-oic acid lactone	2428	2430	0.58	MS, RI
16.	Docosahexaenoic acid,1,2,3-propanetriyl ester	7462	7450	0.52	MS, RI
	Total chemical components (percentage oil detected)			16 (99.8 %)	
	Monoterpene (Hydrocarbons)	-	-	18.15 %	
	Monoterpene (Oxygenated)	-	-	0.36 %	
	Sesquiterpene (Hydrocarbons)	-	-	14.24 %	
	Sesquiterpene (Oxygenated)	-	-	45.64 %	
	Others			21.48 %	

- Compounds not detected

NA- Not available

RI1- Retention index values detected on the HP5 column

RI2- Retention index values from literature (Adams 2007)

MS- Mass spectrum from NIST and Wiley libraries

RI-Retention indices (reported)

USASE-Ultra Sound Assisted Solvent Extraction

extract of dried leaves of this species reported from South India (9).

Alloaromadendrene oxide-I (53.3 %), retinal (11.3 %), β-Bisabolene (7.7 %) and andrographolide (5.7 %) as the major constituents in the methanolic rhizome extract of this species were reported (11). Similarly previous works reported curzerenone (20.8 %), germacrone (11.1 %) and eucalyptol (5.3 %) as the predominant components in the rhizome extract obtained via hydrodistillation in Malaysia (12).

α-Bisabolol, a prominent oxygenated sesquiterpene, is commonly derived from the essential oils of various ornamental and edible plants. It exhibits a broad spectrum of biological activities, including anticancer, cardioprotective, neuroprotective, anti-inflammatory, antimicrobial and antinociceptive properties, making it one of the most widely utilized herbal constituents (30). In our previous study and the present investigation, α-Bisabolol was identified as a major component, indicating that the rhizomes of this species serve as a significant natural source of this bioactive compound (6).

β-Thujene, a hydrocarbon monoterpene, is a key constituent in the essential oils of various plant species and is known for its diverse biological activities, particularly its well-documented antioxidant potential (31). β-Bisabolene, a sesquiterpene, is also widely reported in essential oils across numerous species and demonstrates cytotoxic activity against mammary carcinoma cells, along with synergistic antibacterial effects against selected pathogens (32).

In-vitro antibacterial activities

The antibacterial activities of USAREO are tabulated in Table 2. The USAREO showed outstanding antibacterial activities against *S. pyogenus, P. aeruginosa* and *E. coli* with Ampicillin as a reference drug. The percentage efficiency values of USAREO tabulated in Table 2 indicate that USAREO has more phenomenal antibacterial potential than the standard used. This might be due to the synergistic action of α–Bisabolol and β-Bisabolene in the oil (33).

When compared with previous studies the HDREO exhibited admirable activities versus *S. aureus* and *P. aeruginsoa.* So, both the mixtures exhibited outstanding antibacterial activities owing to the key components (6).

In-vitro antifungal activities

The *in-vitro* efficacy of USAREO versus certain strains is depicted in Table 3. Out of these three fungal strains, the oil exhibited excellent antifungal potential versus *C. albicans (MTCC-227)* concerning the reference drug Griseofulvin. The percentage efficiency of USAREO, tabulated in Table 3, suggests its better efficacy against *C. albicans* than Griseofulvin. This can be attributed to the excellent antifungal agents like α-Bisabolol in synergy with β- Bisabolene reported recently (34). Upon comparison, the HDREO showed excellent antifungal potential versus *C. albicans* only, but was not efficient against the other two strains, like USAREO (6).

In-vitro antioxidant activities

The antioxidant efficacy of USAREO was checked by DPPH and NO assays. The results are tabulated in Table 4.

From Table 4, it is clear that USAREO has exhibited excellent *in-vitro* antioxidant activities in both the assays particularly in the NO assay, considering ascorbic acid as the standard. The IC_{50} values of USAREO are closer to the standard. The excellent antioxidant activities of USAREO are supposed to be due to excellent antioxidants i.e. β–Thujene, α–Bisabolol alone or synergistic action of α-Bisabolol and Bisabolene (31,34). When compared with earlier works, HDREO also exhibited outstanding activities in NO assay (6).

In-vitro anti-inflammatory activities

The anti-inflammatory efficacy of USAREO is tabulated in Table 5.

As presented in Table 5, USAREO demonstrated remarkable anti-inflammatory potential. In particular, its activity in the proteinase inhibition assay was closely aligned with that of the reference drug, Diclofenac sodium. A similar trend was previously reported for HDREO, which also exhibited strong proteinase inhibitory activity (6), consistent with the current observations. The potent anti-inflammatory response in both essential oils is likely attributable to the presence of α-Bisabolol, a compound known for its significant anti-inflammatory properties (34). The comparable inhibition levels to the standard may be explained by a higher proportion of α-Bisabolol in the samples, although this was not specifically quantified in the present study. This further supports the ethnomedicinal literature reported for this plant concerning anti-inflammation.

Table 2. MICs of USAREO against bacterial pathogens

Test pathogens	MICs of USAREO (µg/mL)	MICs (µg/mL) of Ampicillin (Standard)	Percentage efficiency of USAREO
E. coli (MTCC-443)	62.5	100	160
P. aeruginosa (MTCC-1688)	50	100	200
S. aureus (MTCC-96)	100	250	250
S. pyogenus (MTCC-442)	25	100	400

Table 3. MICs of USAREO against fungal pathogens

Test pathogens	MICs of USAREO (µg/mL)	MICs (µg/mL) of Griseofulvin (Standard)	Percentage efficiency of USAREO
C. albicans (MTCC-227)	250	500	200
A. niger (MTCC-282)	500	100	20
clavatus (MTCC-1323)	1000	100	10

Table 4. IC50 values of USAREO in antioxidant assays

Sr. No.	Test samples	Mean IC_{50} values (µg/mL)	
		DPPH assay	NO assay
1.	USAREO	33.22 ± 0.77	58.47± 1.11
2.	Standard (Ascorbic acid)	61.5 ± 1.72	70.17± 1.52

Table 5. Anti-inflammatory activities of USAREO

Sr. No.	Test samples	Methods used		
		Albumin denaturation (µg/mL)	Membrane stabilization (µg/mL)	Proteinase inhibition (µg/mL)
1.	USAREO	45.29 ± 1.68	41.79 ± 1.44	58.28 ± 2.08
2.	Standard (Diclofenac sodium)	87.06 ± 0.61	72.12 ± 3.61	69.06 ± 1.06

In-silico pharmacokinetics (ADMET) study of principal components

The physicochemical and pharmacokinetics properties along with bioavailability radar and boiled egg graph of key components are tabulated in Table 6 and Fig. 1 respectively, retrieved from the Swiss ADME server.

From Table 6, the topological surface area (TPSA) > 30° A^2 suggests very good brain penetration with a high lipophilicity value, Log Po/w (iLogP) in the range 2.62-3.49. Exceptionally, the α-Bisabolol shows good pharmacokinetics such as high GI absorption, no P-glycoprotein(p-gp) indicating better intestinal absorption. The brain-blood-barrier (BBB) permeant is too yes for α-Bisabolol and β–Thujene, justifying their suitability. Also, the interaction of these phytomolecules with the Cytochrome P family indicates their effectiveness and non-toxic nature. Furthermore, the α-Bisabolol shows drug-likeness properties such as zero Lipinski violation, Ghose, Veber and Egan parameters are too yes with a very good bioavailability score of 0.55. Overall, all the major components exhibit good ADMET properties.

Fig. 1(A) indicates that all the molecules are inside a pink area showing their drug-likeness properties with a bioavailability contour. As depicted in Fig. 1(B), the presence of these molecules in and near the yolk with a red point confirms their high brain penetration probability with no P-gp (35).

In-vivo pharmacokinetics, oral toxicity study

Acute oral toxicity

The administration of different test sample concentrations to rats resulted in no moribund or death, even no significant behavioral changes. So, the findings suggested the test sample does neither induce any toxic reaction nor any mortality; thus, LD50 value is > 2000 mg/kg (36).

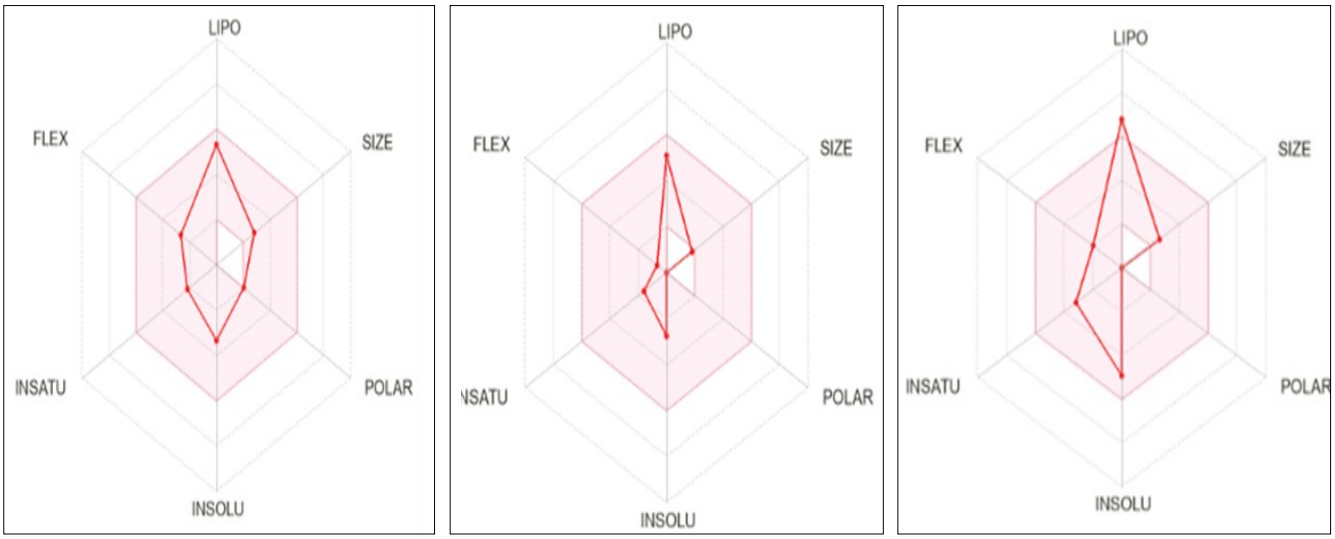
ADME studies

The ADME studies results are tabulated in Table 7.

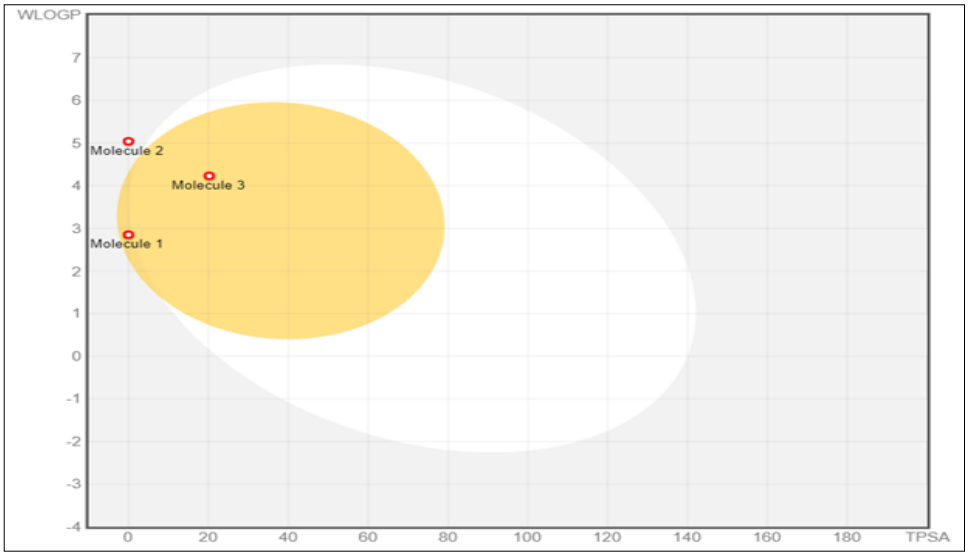
From the above table, the K_{el} value for the test sample and standard suggests a normal elimination profile of first order. The greater V_d value of the test sample indicates larger tissue distribution and low protein binding. The drug clearance value of the test sample is higher than the standard, indicating lesser AUC and, thus, lower residence time in the systematic circulation and early reduction in plasma concentration. The lower AUC values, from Fig. 2, of the test sample (356) with standard

Table 6. Pharmacokinetic properties of principal components

Properties	α-Bisabolol	β–Thujene	β-Bisabolene
Physicochemical properties			
Molecular formula	$C_{15}H_{26}O$	$C_{10}H_{16}$	$C_{15}H_{24}$
Molecular weight	222.37 g/mol	136.23 g/mol	204.35 g/mol
Number of heavy atoms	16	10	15
Number of hydrogen bond donor and acceptor	1	0	0
TPSA	20.03^0A^2	0.0^0A^2	0.0^0A^2
Lipophilicity			
Log Po/w (iLogP)	3.46	2.62	3.49
Pharmacokinetics			
Water Solubility	1.01e-01 mg/mL; 4.55e-04 mol/L	2.33e-01 mg/mL; 1.71e-03 mol/L	2.48e-03 mg/mL; 1.21e-05 mol/L
Class	Soluble	Soluble	Moderately soluble
P-gp substrate	No	No	No
GI absorption	High	Low	Low
BBB permeability	Yes	Yes	No
Log Kp (skin permeation)	-4.97 cm/s	-4.71 cm/s	-3.03 cm/s
CYP1A2 inhibitor	No	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	Yes	No	Yes
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Drug likeness			
Lipinski	Yes, zero violation	Yes, 1 violation MLOGP>4.15	Yes, 1 violation MLOGP>4.15
Ghose, Veber, Egan	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55



Molecule 1 (β-Thujene) **Molecule 3 (α-Bisabolol)** **Molecule2 (β-Bisabolene) 1A**



1B

Fig. 1. Bioavailability radar (1A) and Boiled-egg graph (1B) of major components.

(834.08) with maximum conc. (Cmax) 672 and 1157.15, suggest a lower bioavailability value for the pharmacodynamic response as shown in Table 7 and Fig. 2 (37).

Histopathological studies

This study revealed no significant alteration in the major organs, shown as in photomicrographs. This suggests, the non-toxic nature of USAREO.

Kidney

Fig. 3A shows normal tubular, glomerular on and glomerular histoarchitecture with a few vacuolization.

Brain

Fig. 3B shows normal histoarchitecture of the brain and did not display any pathological alteration with the sample treatment.

Pancreas

Fig. 3C shows no significant change in histoarchitecture and did not elicit pancreatic necrosis or pathological changes.

Heart

Fig. 3D shows the histoarchitecture of normal cellular and cardiac cells with no signs of pathological alterations.

Liver

Fig. 3E shows normal histoarchitecture with a small number of resident lymphocytes and no portal/septal degree of inflammation was observed.

Overall, no significant alteration in the organ structure was observed indicating the non-toxic nature of USAREO (38).

Table 7. In-vivo pharmacokinetic properties of USAREO and standard

Sr. No.	Parameters	Test sample	α-Bisabolol (standard)
1.	Elimination rate constant (K_{el})	0.194	0.114
2.	Half-life($t_{1/2}$)	3.55	6.03
3.	Volume distribution (V_d)	4.95	3.73
4.	Clearance (L/hrs.)	0.96	0.42
5.	AUC_{1-t} (ng/hrs/mL)	342.73	509.76
6.	$AUC_{0-\infty}$ (ng/hrs/ mL)	13.59	323.71
7.	AUC (ng/hrs/ mL)	356	834.08
8.	C_{max} (ng/ mL)	672	1157.15
9.	T_{max} (hrs)	2	2

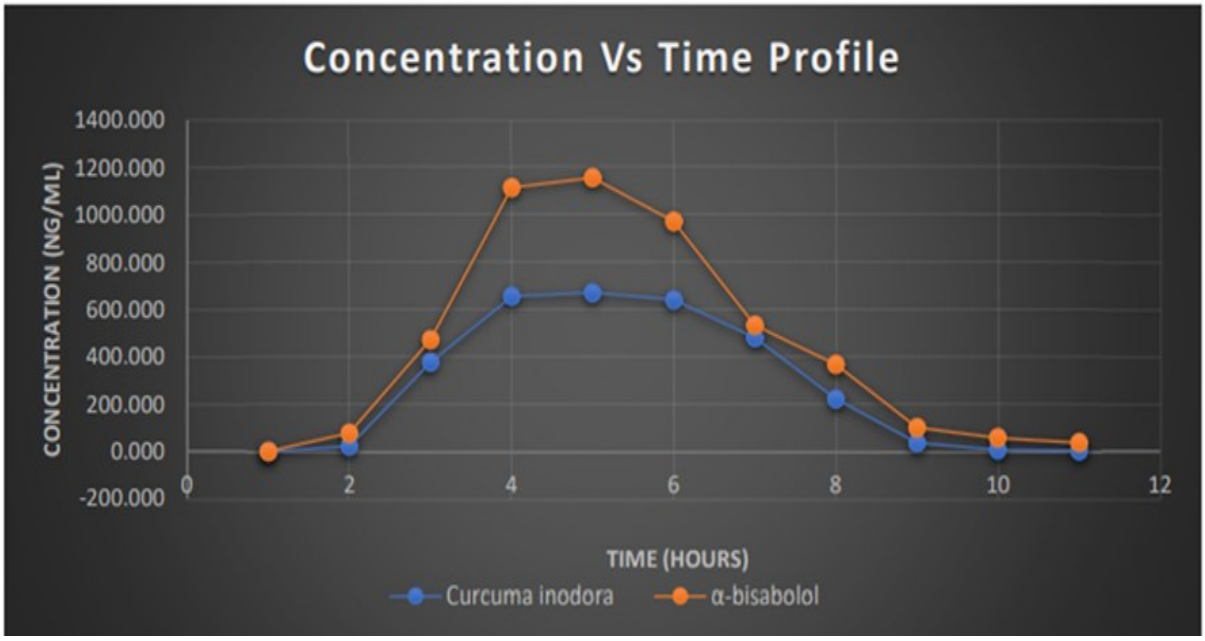


Fig. 2. Plasma concentration Vs. Time profile for C. inodora E O and Bisabolol (standard).

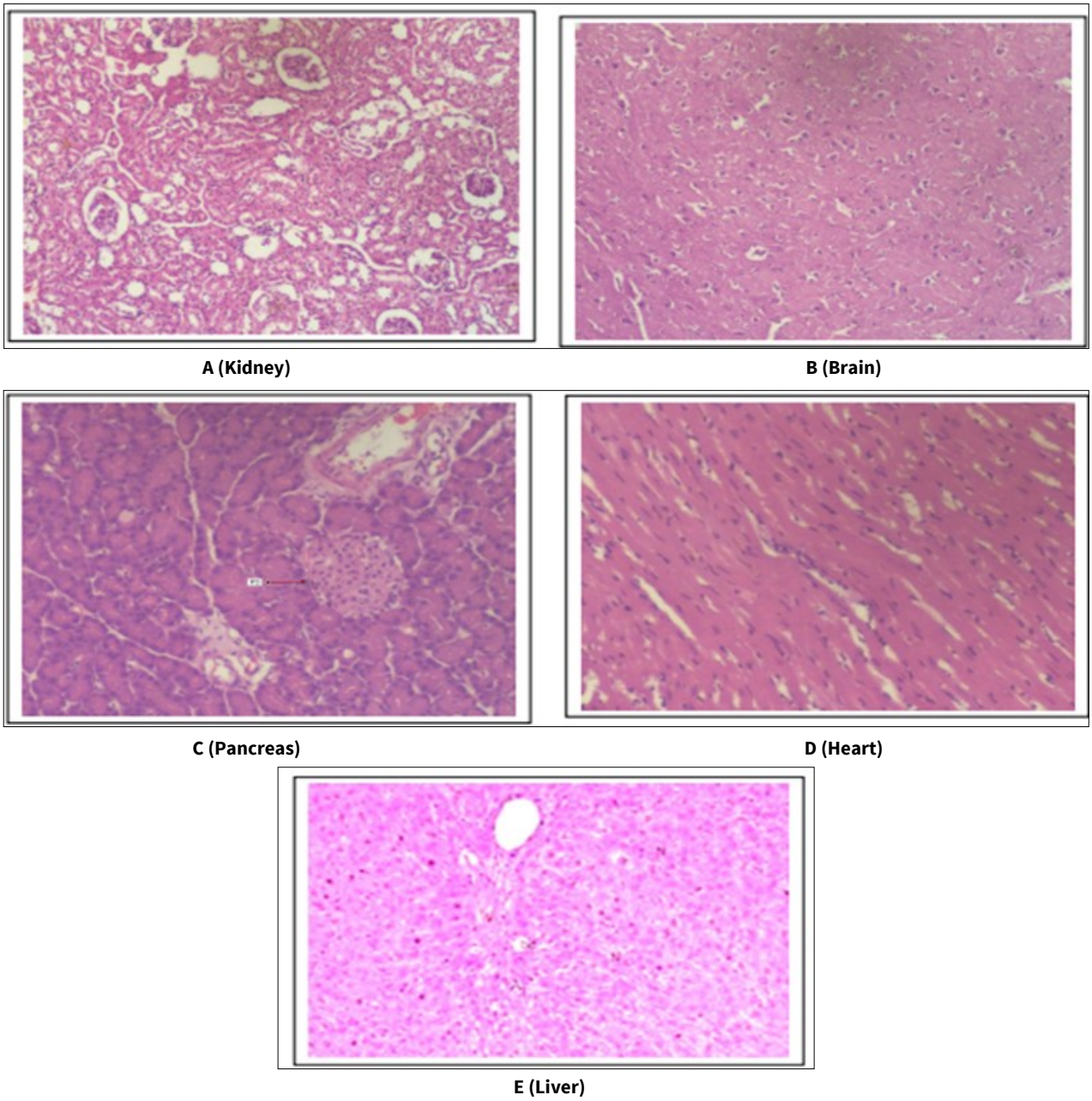


Fig. 3. Photomicrographs of vital organs.

Conclusion

This study reports, for the first time, the presence of α-Bisabolol, along with β-Thujene and β-Bisabolene, as key constituents in the rhizome oil of *Curcuma inodora* via solvent extraction. Among these, α-Bisabolol emerged as a major bioactive compound that is likely responsible for the significant *in-vitro* antimicrobial, antioxidant and anti-inflammatory activities exhibited by the USAREO. The oil was found to be non-toxic, as supported by *in-silico* ADMET profiling and *in-vivo* studies, further reinforcing its therapeutic potential. Given the well-established pharmacological and dermatological benefits of α-Bisabolol, *C. inodora* rhizomes represent a promising natural source for the development of novel pharmaceutical and cosmeceutical formulations. Future work should focus on the isolation, quantification and targeted application of α-Bisabolol from this species to harness its full therapeutic value.

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Authors' contributions

PL and PN designed the study. PN carried out experimental work and wrote the manuscript. PL, HM, AS, AG, KM and PN revised and finalized the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare no conflict of interest.

Ethical issues: None

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