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RESEARCH ARTICLE

In-vitro Bioevaluation, Pharmacokinetics and Molecular Docking Study of unexplored Bisabolol-rich *Curcuma inodora* Blatt. essential oil from Konkan region: A biodiversity hotspot

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Abstract

Curcuma inodora Blatt., belonging to the Zingiberaceae family is an endemic species from peninsular India. The extraction and some of the biological applications of leaves essential oil of this species had been reported from South India. Although the Konkan region is a biodiversity hotspot, no report on the medicinal applications of essential oils in this species is available to date. Herein, the α–Bisabolol-rich rhizome essential oil from wild *C. inodora* was isolated by the hydrodistillation method and characterized by Gas Chromatography-Flame ionization detector (GC/ GC-FID) and Gas Chromatography High-resolution mass spectrometry (GC/HRMS) techniques for pharmacokinetics and molecular docking applications. The Hydrodistillation extract (HDREO) constituted 11 components with a major constituent, α-Bisabolol (66.13%). *In-vitro* anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory potential of rhizome oils were carried out. The pharmacokinetics of the major component, α-Bisabolol, like high GI absorption, zero Lipinski violation with good bioavailability score etc. support its candidacy as a drug. Further, docking was performed with the principal component α- Bisabolol. The least binding energy conformation of α–Bisabolol with anti-bacterial (3WGN.pdb), anti-fungal (1IYL.pdb), anti-oxidant (3MNG.pdb) and anti-inflammatory (1CX2.pdb) proteins were reported to be -7.01 kcal/mol, -8.15 kcal/mol, -7.82 kcal/mol and –7.71 kcal/mol respectively. This shows, the significant binding affinity of α–Bisabolol with proteins. So, the rhizome oil of this unexplored species can be utilized in developing novel phytopharmaceuticals and medicines after further study. To the best, this is a foremost report on the rhizome essential oil isolation by hydrodistillation method, its characterization and its bio-evaluation with pharmacokinetics and docking applications of this species.

Keywords

α-Bisabolol(α-Bis), *Curcuma inodora*, Hydro-distilled Rhizome Essential Oil (HDREO), Molecular docking, Pharmacokinetics

Introduction

Antimicrobial resistance, the serious threat to human being leads to about 7 lac deaths every year and estimates to be around 10 million deaths by 2050 (1). The present Covid-19 global pandemic also demands to search for novel antimicrobial, antioxidant and anti-inflammatory agents effective against newly emerging bacteria and viruses that are prone to present drugs.

Plants can be the source of formulating novel drugs, as they possess many useful bioactive molecules. WHO has declared a list of 21000 medici-

nal plants which are utilized for medicinal purposes across the world. In India alone, 2500 plants have been investigated and around 150 species are utilized by biopharmaceutical houses as medicines. Being the largest medicinal plant producer country, India is "the botanical garden of the world" (2).

The family Zingiberaceae, containing the genus *Curcuma, Hedychium, Zingiber, Amomum,* and *Alpinia,* among the kingdom Plantae is an extensively studied useful family which constitutes rhizomatous aromatic and medicinal plants having essential oils, which play a central role in warding off many infections. The essential oils containing a single or cluster of compounds with potent medicinal value have been part of many pharmaceutical formulations since earlier ages (3, 4).

The genus *Curcuma* is a widely investigated genus for its useful therapeutic value. To date, around 120 species under this genus are known worldwide, and around 40 species have been reported in India. While, in Maharashtra, 11 species are known and recognized to be utilized as food preservatives and spices and exhibit important medicinal properties. The *Curcuma* species like *C. longa, C. amada, C. aromatica, C. caesia, C. zeodaria, C. manga, C. aeruginosa* and *C. xanthorhizza* etc. have been stated to have medicinal worth (5).

Curcuma inodora is endemic to peninsular India and reported from the Western Ghat region. The term *inodora* represents the non-aromatic nature of its rhizomes. This could be the reason behind its underutilization for further study. The beautiful and attractive inflorescence has made it an important ornamental plant (6). In this view, this species has been exploited only for its ornamental potential and the medicinal importance of its essential oil remained an unexplored part, except for a few reports (7, 8). The geographical and environmental factors affect the chemical structure of essential oils (9). So, this study intended to explore the phytomedicinal properties of this plant from the Konkan region, a part of the Western Ghat, declared as a world heritage center by UNESCO.

The present investigation intended the essential oil isolation from the rhizomes of *C. inodora*, its characterization, biological efficacies along with pharmacokinetics and docking studies with the key constituent, α- Bisabolol (α-Bis).

Materials and Methods

Plant material

The 225 g rhizomes of *C. inodora* species were gathered from the forest area situated near the village Kachale, located on Lonere-Shriwardhan road, (SH-99), (Lat. N 18°9 ' 16.2936 "Long. E73° 17 58.9704 ') in Sept. 2019. The species was validated by Dr. Priyanka Ingle, Botanical Survey of India (BSI), Pune.

Isolation of oil

Hydrodistillation

The fresh rhizomes of plants were washed, dried and transferred to Clevenger's apparatus. Around one and a half-liter of distilled water was added and continued assembly for around 8 hrs. at the 80^0 to 90 ^{0}C temperature. The oil was collected in vials and dehydrated by using anhy Na_2SO_4.

Essential oil analysis

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

GC/HRMS analysis was accomplished by Agilent technologies 7890 Gas chromatograph equipped with JEOL The Accu ToF GCV JMS-T100 GCV MS detector. 'He' was the carrier gas used in GC with 1.0 mL/min flow rate, and the HP5 column also called EB5column (30 m length x 0.25 mm diameter x 0.25 µm thickness) was fortified in GC during analysis. The samples to be analyzed- were diluted in a 1: 100 v/v ratio and acetone was used as a solvent. The samples were inserted through an auto-injector (1:10 for a minute) with a constant temperature of 250 ^{0}C. Initially, the temperature of GC was programmed at 60 ^{0}C for 2 min, then to 250 ^{0}C and increased up to 280 ^{0}C at 30 ^{0}C/min for 10 min. The GC/HRMS was scanned between 45-650 amu. The Mass Spectra (MS) was recorded at 70 ev (EI) and the compositions of oil were recognized based on R. I., Library MS search (NIST), and by comparing MS with reported literature (10).

Gas Chromatography (GC) Analysis

Shimadzu QP-2010 Ultra Gas Chromatograph with HP5MS column (length 30 m, film thickness:0.25 µm with max. temp. 250 ^{0}C) was used for gas chromatographic analysis**.** 'He' was the carrier gas used with a 1ml/min flow rate. An oven temperature initially was 50 ^{0}C which was further programmed to 250 ^{0}C. The flame ionization detector was a detector used in the analysis. The injector and detector temperatures were 260 ^{0}C and 280 ^{0}C respectively. The percentage composition was calculated on the base of the Area normalization method and was compared using standards.

In-vitro Antibacterial Study

The MIC of oil was done by the Broth dilution method with DMSO as a diluent (11). 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 – *S. aureus* (MTCC 96), *S. pyogenus (*MTCC 442), and two-gram -ve bacteria- *E. coli* (MTCC 443) and *P. aeruginosa* (MTCC 1688) were tested versus HDREO. Ampicillin and Ciprofloxacin were used as reference drugs. The results displayed are the product of triplicate analysis.

In-vitro Antifungal Study

Agar dilution protocol was applied to assess HDREO versus some selected fungal strains – *C. albicans* (MTCC227), *A. niger* (MTCC 282) & *A. clavatus* (MTCC 1323) (11).

The stock solutions of HDREO 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 ^{0}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 ^{0}C for 2 days. The dilution which showed 99% inhibition was considered MIC concerning Griseofulvin and Nystatin as reference drugs and the results displayed are the product of triplicate analysis.

In-vitro Antioxidant activity

Two assays viz. DPPH and NO were employed to check the anti-oxidant efficacy of HDREO following standard protocols (12, 13).

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 1.0 mL of this solution was transferred to 3.0 mL of HDREO with varying concentrations (25, 50, 75, and 100) µg/mL). The absorbance was recorded at 517 nm after 30 min (12). The IC50 values were calculated as follows:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

NO assay

This was determined using stable NO• radical cation following standard protocols (13). The sample concentrations employed and the % scavenging activity was decided by the same formula applied for the DPPH assay.

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

In-vitro Anti-inflammatory Activities

The HDREO was examined for anti-inflammatory activities against human RBCs. The 3 different methods viz. Heat-induced hemolysis**,** Inhibition of albumin denaturation and Proteinase inhibitory action 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 HDREO and 1 mL RBCs (10%)) was heated for 30 min. at 56 ^{0}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 (14). The tube containing only saline was used as a blank.

The % inhibition was decided as follows:

$$\% \text{ Inhibition} = 100 \text{ x } (A2/A1\text{-}1)$$

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 HDREO (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 (14). The experiment was performed in triplicate. The % inhibition was decided as follows:

$$\% \text{ Inhibition} = 100 \times (1 - A_2/A_1)$$

Where, A_1 = abs. of the control, and A_2 = abs. of the sample.

Inhibition of albumin denaturation

The reaction mixture [HDREO (with different conc.+ 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 (14). Percent inhibition was decided as follows:

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

Pharmacokinetics study

An *in-silico* chemical absorption, distribution, metabolism, excretion and toxicity (ADMET) study of α-Bis was performed using the Swiss-ADME server to predict its possible use in the drug/s development (15).

Molecular docking study

For the binding mode of α-Bisabolol (α-Bis) with the anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory target protein, we employed molecular docking calculations using AutoDock4.2 software (16). α-Bis showed excellent anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory potentials. The crystal structure of the anti-bacterial, cytoskeleton ftsz (3WGN.pdb), anti-fungal, N-myristoyl transferase (1IYL.pdb), anti-oxidant, Peroxiredoxin (3MNG.pdb) and anti-inflammatory, cyclooxygenase-2 (1CX2.pdb) were taken from the protein database. Moreover, the ftsz is a cytoskeleton filamentous protein essential for bacterial cell division and is a potential anti-bacterial target (17), the *N*-myristoyl transferase (PDB ID 1IYL) from *Candida albicans* was selected as the anti-fungal target. N-myristoyl transferase (NMT) catalyzes the transfer of the fatty acid myristate from myristoyl-CoA to the N-terminal glycine of substrate proteins. Next, peroxiredoxin is an antioxidant enzyme, that maintains the balance of ROS levels against augmentation of ROS production during the pathogenesis of CVD *(18)* and cyclooxygenase-2 is COX-2, an enzyme accountable for inflammation and pain (19).

The atomic coordinates of the α-Bis were generated using the Discovery Studio Visualizer (20). For the docking study, grid boxes of 126×126×126 were built around the protein receptors. Next, we employed a blind docking protocol (16) to explore a binding mode of α-Bis. Here, we kept all the receptor target proteins rigid and α-Bis as a flexible molecule. The docked conformations were produced by applying the Lamarckian Genetic Algorithm. These conformations were grouped using an all-atom RMSD with a cut-off of 4 A. These clusters were then scrutinized based on

van der Waals, binding, electrostatic energy etc. The least binding energy conformation of α-Bis was analyzed for the interactions employing the Discovery Studio visualizer (20).

Results and Discussion

Chemical composition

An average yield of Hydrodistillate extract was 0.57 % (1.3 mL from 225 g). The compositions of HDREO were detected using GC/GC-FID and GC/HRMS analysis.

Constituents in HDREO

The hydrodistillation resulted in yellow-colored oil. It constituted 11 components, comprising 99.92% essential oil (Table 1). The key constituents were α- Bis (66.13%) and Bisabolol oxide-II (7.63%).

germacrone (11.1% & 7.5%) and eucalyptol (5.3% & 5.8%) as key components in the rhizome and leaves extract respectively from Malaysia by hydrodistillation technique (8). While another study reported some common components like Retinal which exist in the HDREO in a lower % (7) and β–Farnesene in a higher amount when compared (8). This highlights the influence of geographical and terrestrial factors on the chemical structure of bioactive components.

The existence of α–Bis, the **"**most-used herbal component" responsible for excellent bioactivities with high skin healing properties, is a major part of HDREO (21). Also, Bisabolol oxide-II, an important bioactive compound (21), β–thujene, the useful antioxidizing agent (22) and 4-terpinol, an excellent antiviral compound (23) exists in substantial amounts.

Table 1. Chemical constituents of *C. inodora* rhizome oil and comparison with the literature.

Sr. No.	Components	RI[1]	RI[2]	By HD (%)	Identification	By Santoshkumar R & Yusuf A (2019) From rhizomes	By Malek S.N. *et al.* (2006)	
							Rhizome extract	Leaves extract
1.	β - thujene	968	971	4.23	MS, RI	-	-	-
2.	4-terpinol	1161	1177	2.99	MS, RI	-	-	-
3.	Oxiranemethanol,3-methyl-3-(4-methyl-3-pentyl)	1269	1269	3.37	MS, RI	-	-	-
4.	Cyclopropanemethanol,α,2-dimethyl-2-[(4-methyl-3-pentenyl)-[1α(R*)2α]	1299	1299	7.45	MS, RI	-	-	-
5.	β - farnesene	1452	1454	3.09	MS, RI	-	0.6	-
6.	D-nerolidol	1522	1527	1.15	MS, RI	-	-	-
7.	Trans-Z-α-Bisabolene epoxide	1531	1531	1.02	MS, RI	-	-	-
8.	6,11-Dimethyl -2,6,10-dodecatrien-1-ol	1634	1634	1.72	MS, RI	-	-	-
9.	Bisabolol oxide-II	1646	1646	7.63	MS, RI	-	-	-
10.	α - Bisabolol	1683	1683	66.13	MS, RI	-	-	-
11.	Retinal	2466	2466	1.14	MS, RI	11.3	-	-
	Total Chemical Components (% oil detected)			11 (99.92%)	-	NA	55 (89.3%)	32 (66.9%)
	Monoterpene (Hydrocarbons) (1)	-	-	4.23%	-	NA	NA	NA
	Monoterpene (Oxygenated) (2,3)	-	-	6.36%	-	NA	NA	NA
	Sesquiterpene (Hydrocarbons) (5)	-	-	3.09%	-	NA	NA	NA
	Sesquiterpene (Oxygenated) (6,7,8,9,10)	-	-	77.65%	-	NA	NA	NA
	Others (4,11)	-	-	8.59 %	-	NA	NA	NA

- Compounds not detected, NA- Not Available, RI[1]- Retention index values detected on the HP5 column, RI[2]- Retention index values from literature (Adams 2007), MS- Mass Spectrum from NIST and Wiley libraries, RI-Retention indices(reported).

The HDREO constituted 77.65% oxygenated sesquiterpenoids, 3.09% sesquiterpene hydrocarbons, 4.23 % monoterpene hydrocarbons, 6.95% oxygenated monoterpenes and 8.59 % other compounds.

Reports are from south India on - Retinal (11.3%) and β- Bisabolene (7.7%) as the chief constituents in the methanolic rhizome extract of this species (7).

Reports are on curzerenone (20.8 % &16.9%),

In-vitro antibacterial activities

The antibacterial activities of HDREO are tabulated in Table 2. The hydrodistillation extract has exhibited excellent antibacterial activities against *S. aureus* and *P. aeruginosa* with Ampicillin and Ciprofloxacin as reference drugs. This can be accredited to the key component, α–Bis, as proved by a recent report (24).

Table 2. MICs of HDREO against bacterial pathogens

Test Pathogens	MICs of HDREO (µg/ml)	MICs (µg/ml) of Ampicillin (Standard)	MICs (µg/ml) of Ciprofloxacin (Standard
E. coli (MTCC-443)	250	100	25
P. aeruginosa (MTCC-1688)	125	100	25
S. aureus (MTCC-96)	100	250	50
S. pyogenus (MTCC-442)	250	100	50

In-vitro antifungal activities

An *In-vitro* efficacy of HDREO tested against selected strains are depicted in Table 3. The HDREO exhibited excellent antifungal action against *C. albicans* (MTCC-227) concerning reference drugs Griseofulvin and Nystatin, credited to excellent antifungal agents like α–Bis in combination with Bisabolol oxide-II, reported recently (25).

Table 3. MICs of HDREO against fungal pathogens

Test pathogens	MICs of HDREO (µg/ml)	MICs (µg/ml) of Griseofulvin (Standard)	MICs (µg/ml) of Nystatin (Standard)
C. albicans (MTCC-227)	500	500	100
A. niger (MTCC-282)	1000	100	100
Clavatus (MTCC-1323)	1000	100	100

In-vitro antioxidant activities

The antioxidant efficacy of HDREO was appraised by DPPH and NO assays. From Table 4, it is clear that HDREO has exhibited excellent *In-vitro* antioxidant activities in both assays concerning ascorbic acid as standard. The IC_{50} values of HDREO are closer to the reference drug. These excellent activities of HDREO are accredited to excellent antioxidants i.e., α–Bis alone or synergistic action of α- Bis and Bisabolol oxide-II (26).

Table 4. IC_{50} values of HDREO in antioxidant assays

Sr. No.	Test samples	Mean IC_{50} values (µg/ml)	
		DPPH assay	NO assay
1.	HDREO	38.24±0.69	64.61 ± 1.31
2.	Standard (Ascorbic acid)	61.5 ± 1.72	70.17± 1.52

In-vitro anti-inflammatory activities

The anti-inflammatory efficacy of HDREO were checked by 3 methods. The result is tabulated in the following table.

From Table 5, it seems that HDREO has exhibited excellent anti-inflammatory activities. In Proteinase inhibition, the anti-inflammatory activity values of HDREO are very closer to the sodium diclofenac.

Table 5. Anti-inflammatory activities of HDREO

Sr. No.	Test samples	Methods used		
		Albumin denaturation (µg/ml)	Membrane stabilization (µg/ml)	Proteinase inhibition (µg/ml)
1.	HDREO	63.42 ± 2.01	51.54 ± 1.73	62.99 ± 1.09
2.	Standard (Diclofenac sodium)	87.06 ± 0.61	72.12 ± 3.61	69.06 ± 1.06

The HDREO showed exceptionally well anti-inflammatory activities in all three methods. These exceptionally well activities of HDREO can be credited to an outstanding anti-inflammatory agent i.e., α–Bis in the sample (26).

Pharmacokinetics (ADMET) study of α-Bisabolol

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

Table 6. Pharmacokinetic properties of α-Bisabolol

Properties	Values
Physicochemical Properties	
Molecular Formula	$C_{15}H_{26}O$
Molecular weight	222.37g/mol
Number of heavy atoms	16
Number of hydrogen bond donor and acceptor	1
TPSA	20.03 $^0A^2$
Lipophilicity	
Log Po/w (iLogP)	3.46
Pharmacokinetics	
Water Solubility	1.01e-01 mg/ml; 4.55e-04 mol/l
Class	Soluble
P-gp substrate	No
GI absorption	High
BBB permeability	Yes
Log Kp (skin permeation)	-4.97 cm/s
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Druglikeness	
Lipinski	Yes, zero violation
Ghose, Veber, Egan	Yes
Bioavailability score	0.55

Table 6 depicts that the α-Bis shows a topological surface area (TPSA) > 30 $^0A^2$ suggesting very good brain penetration and high lipophilicity value, Log Po/w (iLogP) as 3.46. Further, the α-Bis shows good pharmacokinetics like high GI absorption, no P-glycoprotein(p-gp) indicating better intestinal absorption. The brain-blood-barrier (BBB) permeant is too yes, justifying its suitability. Also, the interaction with the Cytochrome P family indicates its effectiveness and non-toxic nature. Moreover, the α-Bis 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, the α-Bis shows good ADMET properties; hence, it seems an ideal candidate for the drug design.

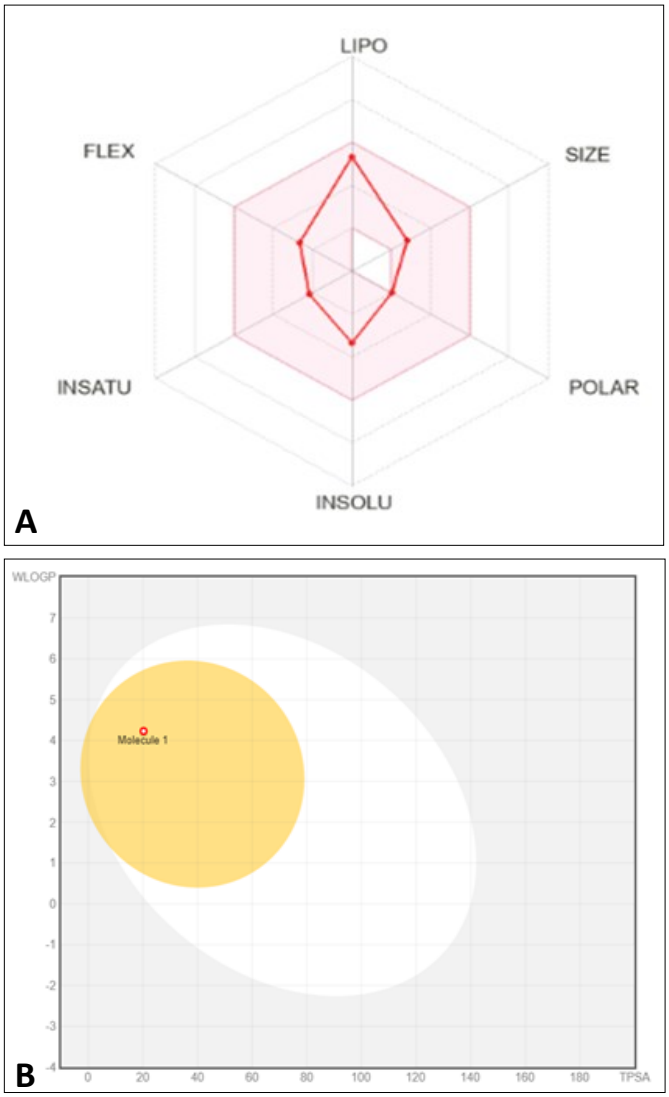

Fig. 1. Bioavailability radar (**A**) and Boiled-egg graph of α-Bisabolol(**B**)., (Molecule 1 represents α-Bisabolol).

Fig. 1(A) indicates the molecule is inside a pink area showing its drug-likeness with bioavailability contour while as shown in Fig. 1(B) the presence of α-Bis in yolk with a red point confirms its high brain penetration probability with no P-gp (27).

Hence, we performed the docking study with the different receptors, to explore the binding mode and affinity.

Molecular docking of α-Bisabolol with target receptors

To study the binding mode of α-Bis with target anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory receptors, molecular docking techniques were employed using AutoDock4.2 software.

The least binding energy conformation of α-Bis with anti-inflammatory (1CX2.pdb), anti-oxidant (3MNG.pdb), anti-bacterial (3WGN.pdb), anti-fungal (1IYL.pdb) was reported to be –7.71 kcal/mol, -7.82 kcal/mol, -7.01 kcal/mol, -8.15 kcal/mol respectively (Fig. 2, 3 and Table 7). To identify the bonding-non-bonding interactions of anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory receptors with α-Bis, we further analyzed the docked complex as depicted in Fig. 2 and 3.

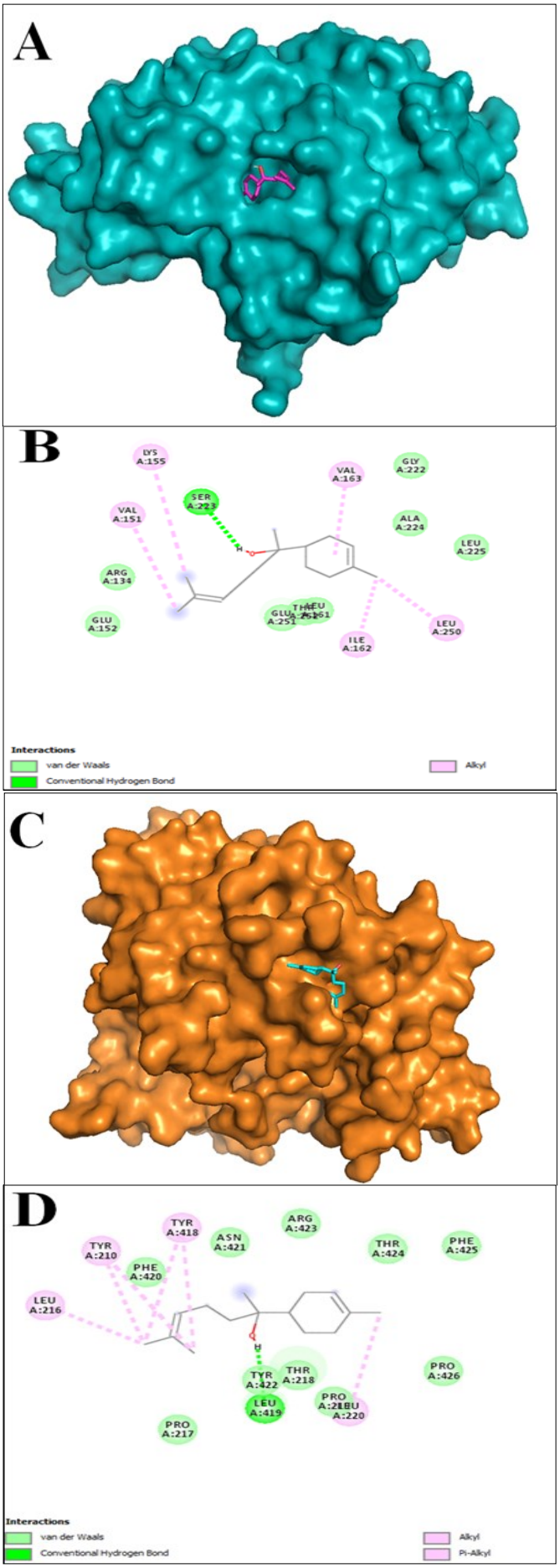

Fig. 2. Binding mode of α-Bisabolol with anti-bacterial (2WGN.pdb) and anti-fungal (1IYL.pdb) receptor after molecular docking. Here, anti-bacterial and anti-fungal receptors are shown in the space-fill model with cyan and orange color respectively. α-Bisabolol is shown in stick model magenta and cyan color in the anti-bacterial and anti-fungal receptors respectively.

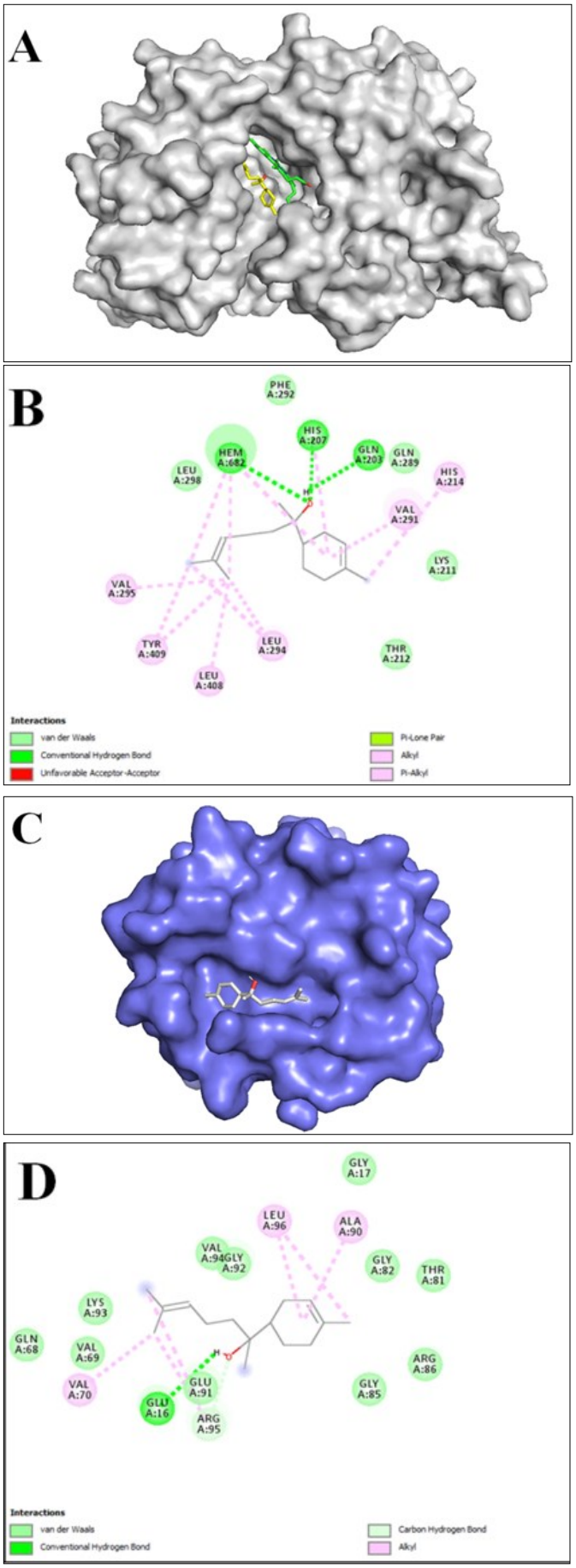

Fig. 3. Binding mode of α-Bisabolol with anti-inflammatory (1CX2.pdb) and anti-oxidant (3MNG.pdb) receptor after molecular docking. Here, anti-inflammatory and anti-oxidant receptors are shown in the space-fill model with grey and blue color respectively. Bisabolol is shown in stick model yellow and grey color in anti-inflammatory and anti-oxidant receptors respectively. The Heme group in the anti-inflammatory receptor is shown on a stick with green color.

Table 7. Hydrogen bonding interactions of α-Bis with anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory receptors

Protein	Binding energy (kcal/mol)	Atoms involved in the interactions	Distance (Å)	Angle (°)	Fig. Ref
Anti-bacterial	-7.01	: Bis:H -SER223: OG	2.0067	133.559	2B
Anti-fungal	-8.15	: Bis:H - LEU419:O	1.98146	161.725	2D
Anti-oxidant	-7.82	: Bis:H - GLU16:OE2	2.09748	138.842	3D
		ARG95:HD1 -: Bis:O	1.69538	166.679	
Anti-inflammatory	-7.71	HIS207:HE2 -:O- Bis:	2.0414	149.002	3B
		HEM682: ND -:O- Bis:	3.24903		
		: Bis:H - GLN203:OE1	2.69489	164.399	

In table-7, α – Bisabolol is denoted as Bis

Docking study with Anti-bacterial and Anti-fungal receptors

The analysis of the anti-bacterial (2WGN.pdb)-Bis complex (Fig. 2A) reveals that the α-Bis is stabilized by the conventional 'H'bonding interactions Ser223 (2.00 Å) as depicted in Table 7 and Fig. 2B. In addition, the α-Bis also forms alkyl type of interactions Val151, Lys155, Ile162, Val163, Leu250 while Arg134, Gly222, Ala224, Leu225, Glu152, Glu251, Thr252, Leu261 forms van der Waals interactions as depicted in Fig. 2B.

The analysis of anti-fungal (1IYL.pdb) with α-Bis complex (Fig. 2C) shows that the α-Bis is stabilized by the 'H' bonding interactions with Leu419 (1.98 Å) as revealed in Fig. 2D. Also, Leu216, Leu220, Tyr210, Tyr418 from the π-alkyl type interactions with α-Bis and Pro217, Pro216, Thr218, Tyr422, Phe425, Pro426, Thr424, Arg423, Asn421 and Phe420 forms van der Waals interactions as depicted in Fig. 2D.

Docking study with Anti-inflammatory and Anti-oxidant receptors

The analysis of the anti-inflammatory (1CX2.pdb)-α-Bis complex reveals that the α-Bis is stabilized by the conventional 'H' bonding interactions His207 (2.01 Å), Gln203 (2.69 Å), and HEM (3.24 Å) as shown in Table 7 and Fig. 3B. Also, His214, Val291, Leu294, Leu408, Tyr409, Val295 form π-alkyl type of interactions and Lys211, Thr212, Gln289, Phe292 and Leu298 forms van der Waals interactions with α-Bis, depicted in Fig. 3B.

The analysis of anti-oxidant (3MNG.pdb) with α-Bis complex (Fig. 3C) shows that the α-Bis is stabilized by the 'H' bonding interactions with Glu16 (2.09 Å) and Glu91 and Arg95 forms CH-O type of interactions (Fig. 3D). Also, Val70, Ala90 and Leu96 from the alkyl type of interactions with α-Bis and Gly17, Gly82, Gln68, Thr81, Arg86, Gly85, Gly92, Val94, Arg95 forms van der Waals interactions, depicted in Fig. 3D.

Conclusion

This study reveals the influence of geographical and environmental factors on the phytochemical composition of unexplored *C. inodora* rhizome oil and further state that the HDREO can be a rich source of the 'most-used herbal component' i.e., α–Bisabolol, reported for its distinct identity.

The various *in-vitro* biological applications of

HDREO, especially its antibacterial, antioxidant and anti-inflammatory potentials indicate that it will be useful in formulating the respective drugs.

Further, the pharmacokinetics of α –Bisabolol, such as high GI absorption, no P-gp, zero Lipinski violation with good bioavailability score etc. support its candidacy as an ideal drug. While the docking study displayed that, the α–Bisabolol has substantial binding affinity and interaction with the target anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory proteins affirming its drug-likeness.

Overall, the rhizome oil of this unexplored species can be utilized in developing novel phytopharmaceuticals and medicines after further study.

This study further recommends carrying out a detailed *in-vivo* investigation of rhizome oil of this species for the formulation of antibacterial, antifungal, antioxidant and anti-inflammatory drugs. Also, suggests studying the efficacy of rhizome oil in skin care ointments, apparently after further study.

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Authors contributions

PL designed the study. PN carried out experimental work and wrote the manuscript. PL, HM, and PN finalized the manuscript.

Compliance with ethical standards

Conflict of interest: Authors state no conflict of interest.

Ethical issues: None

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