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Essential oils from *Conamomum rubidum*: The phytochemical, microbiological, and molecular docking study

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This study explored the interaction of major components in essential oils from aerial parts and rhizomes of *Conamomum rubidum* (Lamxay & N.S.Lý) Škorničk. & A.D. Poulsen (*Zingiberaceae*) collected in Hongiao National Park (Lamdong province, Vietnam) to the relevant targets of microtubes. Δ-3-Carene (33.14% and 20.15%), β-phellandrene (14.63% and 25.61%), and 1,8-cineole (17.14 and 3.26%) were three major components in essential oils from aerial parts and rhizome of *C. rubidum*, respectively. The aerial parts oil possessed fungicidal activity against *Candida albicans* (MIC = MFC = 0.4%) and rhizome oil bactericidal activity against *Staphylococcus aureus* (MIC = MBC = 0.8%). A molecular docking study was performed with antimicrobial targets of *S. aureus* (2W9S, 1HSK, 2ZCQ) and *C. albicans* (3PVK, 5V5Z, 1IYL). Among the three components, β-phellandrene has the best affinity to the relevant targets of *S. aureus*, while Δ-3-care and 1,8-cineole exhibited good binding energy to the targets of *C. albicans* compared to β-phellandrene. Along with the *in vitro* activity test, the docking study demonstrated that β-phellandrene kept an important role in the ability to inhibit *S. aureus*. At the same time, Δ-3-carene and 1,8-cineole played a significant part in the capacity to inhibit *C. albicans*. By the ADMET calculations, Δ-3-carene, β-phellandrene, and 1,8-cineole can potentially be used as antimicrobial agents.

Keywords: Antimicrobial activity, *Candida albicans*, *Conamomum rubidum*, Essential oil, Molecular docking, *Staphylococcus aureus*

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Introduction

The Zingiberaceae family comprises 1,865 accepted species of aromatic perennial herbs belonging to about 63 genera. Many species in the family contain essential oils (EOs) in their tissues, which are widely employed as spices and medicine[1,2]. The genus *Conamomum* Ridl., including 12 species, is a small genus that belongs to the Ginger family. In Vietnam, the genus *Conamomum* consists of four species *Conamomum rubidum*, *C. odorum*, *C. pierreanum*, and *C. vietnamense*[2,3].

Among the species of the genus *Conamomum*, *C. rubidium* (Lamxay&N.S.Lý) Škorničk. & A.D. Poulsen (syn. *Amomum rubidum*) has been studied for its chemical composition as well as antimicrobial and larvicidal activities. Previous studies showed that the essential oil from *C. rubidum* rhizomes exhibited inhibitory activity against *Escherichia coli* (ATCC 25922) and *Fusarium oxysporum* (ATCC 48112) with minimum inhibitory concentration (MIC) values of 50 μg/mL[4]. In addition, the rhizome oil displayed larvicidal activity against *A. aegypti* with minimum lethal concentration, LC_{50} values of 22.85 and 22.62 μg/mL after 24 and 48 h, respectively[5]. Essential oils from leaves of *C. rubidium* also exhibited antimicrobial activity against *Pseudomonas aeruginosa* and *F. oxysporum*, while the stem oil possessed antimicrobial activity against *P. aeruginosa*, *F. oxysporum*, and *Candida albicans*[6]. *C. vietnamense* leaf and rhizome essential oils strongly inhibited the growth of Gram-positive bacteria *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6501[7]. Recently, four novel

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compounds, conarubins A-D were isolated from the whole plants of *C. rubidum* collected in Vietnam[8]. A new compound, conamonin A and eight known dihydrochalcones were also isolated from *C. rubidum*[9].

In this paper, we investigated the chemical composition of essential oils from aerial parts (leaves and stems) and rhizomes of *C. rubidium* growing in Hongiao National Park (Lamdong province, Vietnam) to identify the main components. Besides, the *in vitro* antimicrobial activity against *Staphylococcus aureus* and *C. albicans* of two aforesaid essential oils was evaluated. Finally, the docking study was conducted to calculate the binding energies of the ascendant compounds to the relevant targets of microtubes. To the best of our knowledge, we first demonstrated that *C. rubidum* essential oil has significant antimicrobial activity against *S. aureus* and *C. albicans*, which has been confirmed by molecular docking studies and ADMET (absorption, distribution, metabolism, excretion, and toxicity) calculations.

Materials and Methods

Plant material

The fresh whole plants of *C. rubidium* were collected at Hongiao National Park, Lam Dong province (12.188247ºN; 108.677594ºE; 1480m) on 29 December 2021. The scientific name of the sample was identified by one of the co-authors, Dr. Nguyen Quoc Binh, a botanist at the VNMN with the voucher specimens (SH 1190) deposited at VNMN.

Isolation of essential oil

The fresh whole plants of *C. rubidium* were divided into two parts: aerial parts (leaves and stems) (5 kg) and rhizomes (4 kg). Each fresh part of the plant material was chopped into small pieces (5-10 mm). After that, they were immersed in water (20 L), and the essential oils were obtained by steam distillation for 4 hours using a Clavenger-type apparatus according to the USP. Then, the oils were dried using anhydrous sodium sulfate and kept in the refrigerator before the analysis.

Gas chromatography-mass spectrometry (GC-MS)

Sample preparation: the essential oils were diluted with chloroform to a concentration of $1x10^{-3}$ (v/v). Samples were analysed using the GC 7890A gas chromatograph system (Agilent, USA), using an Agilent 19091S-433 with HP-5MS column (30 m x 250 μm x 0,25 μm). Helium was used as carrier gas (1.0 mL/min). The sample injection mode was splitless; the injection volume was 1 μL. The inlet temperature was set at 250ºC. Temperature program was set up: initial temperature 60ºC, then increase 10ºC /min to 150ºC and hold for 5 min, then increase 3ºC/min to 250ºC; total analysis time for one sample is 43 minutes. Detector MSD 5975C (Agilent, USA) was installed to scan ionic fragments in the range 45-450 amu with an ionisation energy of 70 eV. Each component's spectral data was compared with the NIST and Wiley spectral libraries using NIST MSsearch 2.0 software. The retention indices (RI) of essential oil components were calculated using the *n*-alkane homologous sequence (C_8-C_{20}) and compared to RIs in the NIST Chemistry WebBook and the Adams database[10] to identify EO components. The relative percentage of each compound was obtained from the peak area.

Antimicrobial activity

Three standardised ATCC strains from library stock cultures were *Staphyloccocus aureus* ATCC 33591 (Gram-positive) and *Candida albicans* ATCC 10231 (yeast). Minimal inhibitory concentrations (MICs) were measured by microdilution on a 96-well plate from SPL in cation-adjusted Muller Hinton broth (CA-MHB), from Sigma-Aldrich for bacteria and Sabouraud dextrose broth (SDB) from Merck for yeast, following Clinical and Laboratory Standards Institute recommendations, with *Staphyloccocus aureus* ATCC 25923 (Gram-positive) (MSSA), and *Candida albicans* ATCC 10231 (yeast)[11,12].

The essential oils are dissolved in water, with 4% Tween 80. Their concentrations were calculated according to the percentage of EOs in the water. The reference standard is meropenem from Sigma-Aldrich for bacteria and itraconazole from Sigma-Aldrich for yeast. MIC is evaluated through 3 independent experiments.

Minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) was determined from MIC tests by subculturing wells with concentrations higher than MIC on TSA for bacteria and on SDA for yeasts, incubated for 24 h, and counting the colonies. In this study, the MBC is the lowest concentration required to kill >99.9% of microorganisms, compared with the initial inoculum (106 for bacteria and 105 for yeasts). The ratio MBC/MIC is a method used to evaluate bactericidal/fungicidal activities.

Molecular docking analysis

Molecular docking was performed to simulate the interaction between the main components in the *C. rubidium* EOs and some targets on *S. aureus* and *C. albicans*. Six selected targets included dihydrofolate reductase (DHFR) (PDB ID: 2W9S)[13], UDP-N-acetylenolpyruvoylglucosamine reductase (MurB) (PDB ID: 1HSK)[14], 4,4'-diapophytoene synthase (DAP synthase) (PDB ID: 2ZCQ)[15] from *S. aureus* and secreted asparic proteinase 2 (SAP2) (PDB ID: 3PVK)[16], lanosterol 14-alpha demethylase (LDM) (PDB ID: 5V5Z)[17], N-myristoyltransferase (NMT) (PDB ID: 1IYL)[18] from *C. albicans*. All these protein constructs downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org/) are good quality and suitable for simulation. Then, these proteins were imported into the ICM-Pro(x64) software for preparation in the following steps: adding hydrogen, removing unnecessary components, setting the MMFF force field, and determining the active centre to perform the docking process. The structures of the investigated compounds were prepared by downloading them from the PubChem Database, entering the ICM-Pro(x64) software and starting docking. The obtained results are the configurations with the lowest energy in 30 runs, analysing the interactions using Discovery Studio Client 2021 software.

Physicochemical properties and ADMET profile

In this study, ADMETlab 3.0 (https://admetlab3.scbdd.com/) - a web-based tool for predicting and analysing physicochemical and pharmacokinetic properties - was used for the three compounds with the highest concentration in the essential oil. The canonical SMILES codes of these compounds were directly obtained from the PubChem Database and input into the ADMETlab 3.0 website. Physicochemical parameters were then calculated to assess drug-likeness. The ADMET profile, including absorption, distribution, metabolism, excretion, and toxicity, was also predicted to evaluate their potential for use.

Results and Discussion

Analysis of essential oils

The yields of essential oils from aerial parts and rhizomes of *C. rubidium* were 0,1% and 0.25%, respectively. The chemical composition of EO samples was analysed using gas chromatography coupled with mass spectrometry (Table 1). The concentration of each component was calculated based on the percentage of peak area in the total ion chromatogram.

There were 27 components identified in EO from aerial parts of *C. rubidum* (accounting for 99.51%), with some dominant constituents being Δ-3-carene(33.14 %), 1,8-cineole(17.14%), β-phellandrene(14.63%), α-terpinol(6.35%), and terpinene-4-ol(4.91%). On the other hand, 21 components were detected in the rhizome oil (accounting for 96.52%). Some dominant constituents were β-phellandrene(25.61%), Δ-3-carene (20.15%), α-terpinol(8.62%), L-phellandrene (7.46%), andα-terpinolene(4.59%). The results showed that the essential oils from aerial parts and rhizomes of *C. rubidum* possessed many similar components such as Δ-3-carene (33.14 and 20.15%, respectively), β-phellandrene (14.63 and 25.61%, respectively), α-terpinol(6.32 and 8.62%, respectively). In addition, there are some other similar components with lower concentrations, such as: *α*-pinene,β-myrcene, L-phellandrene, γ-terpinene, andα-terpinolene.1,8-cineole was dominant in essential oil from aerial parts of *C. rubidum* (17.14%) but in lower content in the rhizome oil (3.26%).

The chemical compositions of essential oil from aerial parts of *C. rubidum* studied by us were quite like those in the previous study in which the dominant compounds in leaf oil and stem oil of *C. rubidum* were Δ-3-Carene (19.5 and 21.9%, respectively) and 1,8-cineole (37.7 and 1.9%, respectively)[4]. Besides, both our study and previous study showed that L-phellandrene, Δ-3-Carene, β-phellandrene were commanding in rhizome oil of *C. rubidum*. However, there were still some small differences between our study and previous studies. The essential oils of *C. rubidium* studied by us were characterised by a quite high amount of α-terpineol (6.35 and 8.62%) while these compounds were detected in very low content in all EOs from leaves, stems and rhizomes of *C. rubidum* in prior studies (0.2–2.5%). On the other hand, limonene was dominant in all EOs in previous studies (14.4–17.8%)[4], but was absent in both *C. rubidium* aerial parts oil and rhizome oil studied by us.

3-Carene is well known for its antimicrobial[19], antiinflamantory[20], and antifungal effects[21]. The 3-carene treatment suppressed the cytokine gene expression, such as interleukin-4 (IL-4), IL-5, and IL-13, reducing the lung epithelial cell thickness in the asthmatic model. These results suggest that

Table 1 — Chemical composition of essential oils from aerial parts and rhizomes of *C. rubidum*

No	RI^{cal}	RI^{lit}	Compounds	% Area		Classification
				Aerial parts oil	Rhizome oil	
1	931	931	α-Pinene	1.72	2.25	Monoterpene
2	943	951	Fenchene	0.33	-	Monoterpene
3	945	945	Camphene	0.28	-	Monoterpene
4	973	973	β-Pinene	0.37	3.08	Monoterpene
5	991	991	β-Myrcene	2.24	1.54	Monoterpene
6	1002	1002	L-Phellandrene	2.87	7.46	Monoterpene
7	1008	1008	Δ-3-Carene	**33.14**	**20.15**	Monoterpene
8	1015	1015	α-Terpinene	0.98	1.49	Monoterpene
9	1021	1022	m-Cymene	0.26	-	Monoterpene
10	1023	1023	p-Cymene	2.25	-	Monoterpene
11	1023	1023	o-Cymene	-	1.99	Monoterpene
12	1027	1031	β-Phellandrene	**14.63**	**25.61**	Monoterpene
13	1030	1030	1,8-Cineole	**17.14**	3.26	Monoterpenoid
14	1049	1049	β-(E)-Ocimene	0.2	-	Monoterpene
15	1058	1058	γ-Terpinene	1.84	2.62	Monoterpene
16	1087	1087	α-Terpinolene	3.32	4.59	Monoterpene
17	1093	1091	2-Nonanone	0.26	-	Other
18	1100	1095	Linalool	1.93	0.54	Monoterpenoid
19	1102	1101	2-Nonanol	0.39	-	Other
20	1112	1112	D-Fenchyl alcohol	0.42	1.04	Monoterpenoid
21	1121	1117	Dehydrosabinaketone	0.28	0.59	Other
22	1164	1164	Borneol	1.16	-	Monoterpenoid
23	1165	1165	Endo-Borneol	-	2.12	Monoterpenoid
24	1177	1177	Terpinene-4-ol	4.91	4.01	Monoterpenoid
25	1190	1190	α-Terpinol	6.35	8.62	Monoterpenoid
26	1220	1223	Fenchyl acetate	-	0.88	Monoterpenoid
27	1253	1253	Geraniol	0.75	-	Monoterpenoid
28	1256	1257	Linalyl acetate	0.49	-	Monoterpenoid
29	1286	1285	Bornyl acetate	-	2.40	Monoterpenoid
30	1405	1402	Methyl eugenol	0.69	-	Other
31	1522	1520	(E)-Calamenene	-	1.16	Sesquiterpene
32	1523	1523	(Z)-Calamenene	0.31	-	Sesquiterpene
33	1562	1564	Nerolidol	-	1.12	Sesquiterpenoid
Monoterpenes				64.43	70.78	
Monoterpenoids				33.15	22.87	
Sesquiterpenes				0.31	1.16	
Sesquiterpenoids				-	1.12	
Others				1.62	0.59	
Identified				99.51	96.52	

essential oil 3-carene has an anti-asthmatic effect[22]. Numerous *in vitro* studies showed that some compounds found in our essential oils have promising cholinesterase inhibitory activity, such as δ-3-carene, 1,8-cineole, etc.[23,24,25,26] Compounds that act as cholinesterase inhibitors still represent the only pharmacological treatment of Alzheimer´s disease[27].

1,8-cineole exhibits an array of biological properties, including anti-inflammatory, antioxidant, antimicrobial, bronchodilatory, analgesic, and pro-apoptotic effects[28]. Recent evidence has also indicated its potential role in managing conditions such as Alzheimer's disease, neuropathic pain, and cancer[28].

The main biological activities of essential oils rich in phellandrene are antimicrobial, antitumoral and repellent. Such essential oils can also be used as biopesticides and activity food preservative[29].

Antimicrobial activity of the essential oils

Interestingly, the rhizome oil possessed bactericidal activity against *S. aureus* (MIC = MBC = 0.8%) while the essential oil from aerial parts was ineffective (MIC > 1.6%) (Table 2). On the opposite

Table 2 — Antimicrobial and antifungal activity of EOs from aerial parts and rhizomes of *C. rubidum*

Samples	Antimicrobial activity			
	S. aureus		*C. albicans*	
	MIC (%)	MBC (%)	MIC (%)	MFC (%)
Conamomum rubidum aerial parts oil	>1.6	>1.6	0.4	0.4
Conamomum rubidium rhizome oil	0.8	0.8	>1.6	>1.6
Meropenem	0.032*	0.032*		
Itraconazol			0.016*	0.016*

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimum fungicidal concentration.
*The unit is mg/L, result coincide with the usual value, in all 3 independent experiments.

side, the essential oil from aerial parts of *C. rubidium* exhibited fungicidal activity against *C. albicans* (MIC = MFC = 0.4%), while the rhizome oil was ineffective (MIC > 1.6%). Meropenem was used as a positive control for *S. aureus,* and itraconazol was used as a positive control for *C. albicans*.

According to the WHO, the infectious diseases caused by *S. aureus* are the most commonly met in medical institutions. They are challenging for global health care, as they develop high resistance to already known medicines. The American Medical Association reported that the death rate from *S. aureus* exceeds the death rate from AIDS[26]. The number of people hospitalised with resistant staphylococcus strains in the United States more than doubled to more than a quarter of a million[30,31]. Today, a promising approach in the battle against antibiotic-resistant strains is the use of medicines from plant materials, especially those that contain essential oils[32,33]. Such plant-origin medicines are characterised by a diverse chemical composition, which significantly complicates the development of resistance in pathogenic strains of *S. aureus*.

C. albicans is the most common and important type of the genus. Infections caused by *C. albicans* occur when the body's immune system is compromised, resulting in conditions such as oral candidiasis, vaginal candidiasis, skin and nail candidiasis, and severe systemic fungal infection. Traditional chemical drug treatments for *C. albicans* infection have limitations, including the potential for the development of drug resistance[34]. Therefore, essential oils have gained significant attention due to their antibacterial activity and intestinal regulatory effects. It makes them an ideal focus for eco-friendly antifungal research. Thus, *C. rubidum* essential oil is a promising agent for treating infections caused by *S. aureus* and *C. albicans*.

The essential oil studied in article[6] possessed low antimicrobial activity and is ineffective on gram-negative and gram-positive bacteria (including *S. aureus*), and *C. albicans*. Meanwhile, the essential oil in our study is effective on both microorganisms, with the aerial parts oil being effective against *C. albicans* and the rhizome oil being effective against *S. aureus*. Thus, we showed for the first time that the essential oil of different organs of *C. rubidium* is effective against the microorganisms *S. aureus* and *C. albigans.* Also, we calculated the binding affinity of the main compounds in essential oil to the microtubes using molecular docking and performed the ADMET calculations to confirm this. These studies help to show which components are important to the antimicrobial activity of essential oils.

Docking study of selected components

Among the identified compounds, Δ-3-carene, β-phellandrene and 1,8-cineole were three dominant compounds in the obtained essential oils (Table 1). It is expected to explore the interaction of three components with relevant antimicrobial targets. To do so, a molecular docking study was performed with two relevant antimicrobial targets.

The interactions between the molecules and the targets were simulated, and the results were described in Table 3, Fig. 1 and 2. The selected targets for *S. aureus* included DHFR (2W9S), MurB (1HSK), and DAP synthase (2ZCQ), all of which play critical roles in the bacterium's growth and toxicity, involving glycine synthesis, purine synthesis, DNA precursor synthesis; peptidoglycan biosynthesis; and the production of the yellow-orange carotenoid[13-15]. The three main components in the EOs exhibited strong binding capabilities to *S. aureus* targets with negative binding energies (ranging from -6.80 kcal/mol to -18.94 kcal/mol) (Table 3). Furthermore, these compounds formed bonds with residues in the active centres of the target enzymes primarily through

Table 3 — Binding energy (kcal/mol) calculated via docking study between key components and relevant targets

	Targets	Components		
		Δ-3-Carene	β-Phellandrene	1,8-Cineole
S. aureus	2W9S	-9.12	-13.34	-7.87
	1HSK	-7.90	-16.11	-6.80
	2ZCQ	-9.95	-18.94	-7.32
C. albicans	3PVK	-10.20	-8.53	-9.61
	5V5Z	-14.97	-7.22	-9.85
	1IYL	-11.23	-6.27	-10.07

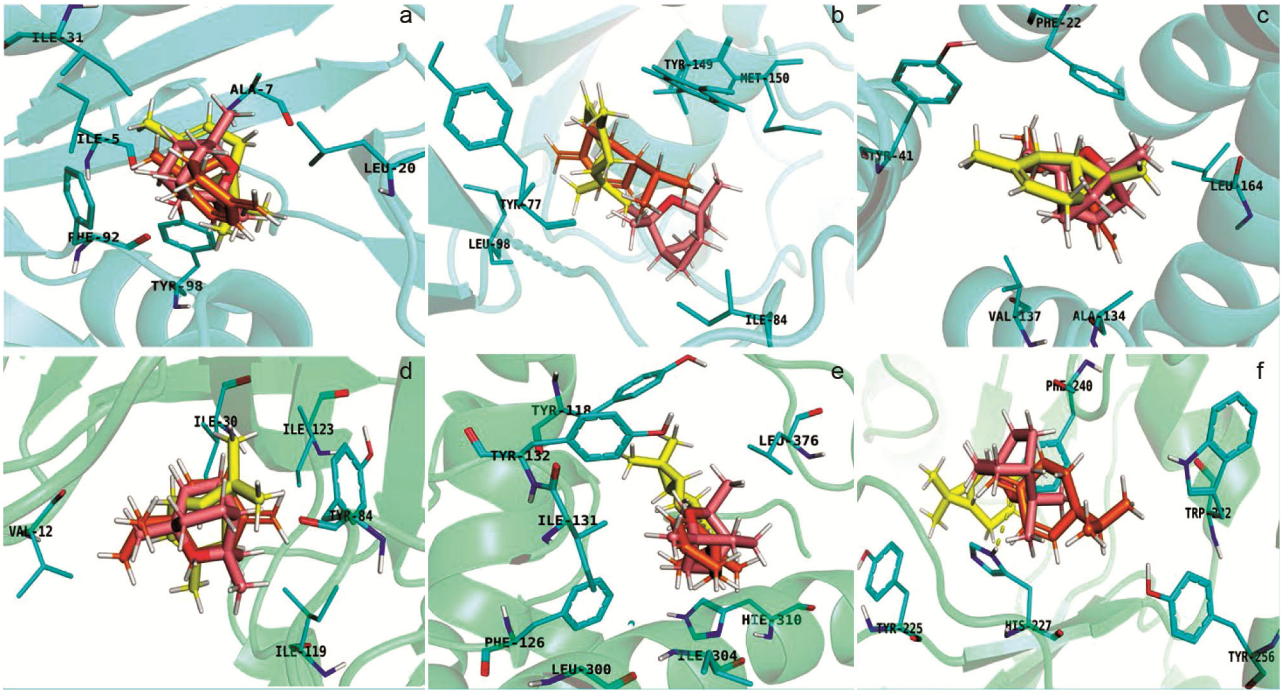

Fig. 1 — 3D interactions of Δ-3-carene (yellow), β-phellandrene (orange), and 1,8-cineole (pink) at the active sites of the proteins, a) 2W9S; b) 1HSK; c) 2ZCQ; d) 3PVK; e) 5V5Z; and f) 1IYL.

hydrophobic interactions (alkyl, pi-alkyl). The docking results revealed that all three major components in the essential oils can inhibit *S. aureus*. Among them, β-phellandrene (14.63% in aerial parts oil, 25.61% in rhizome oil) displayed significantly better binding affinities with the three *S. aureus* targets compared to Δ-3-carene (33.14% in aerial parts oil, 20.15% in rhizome oil) and 1,8-cineole (17.14% in aerial parts oil, 3.26% in rhizome oil). Along with the *in vitro* activity test results that demonstrated rhizome oil's superior inhibitory effect on *S. aureus* compared to aerial parts oil, it can be concluded that β-phellandrene is the component that decisively contributed to the inhibitory capability of the essential oils from the rhizomes of *C. rubidum* against *S. aureus*.

For *C. albicans*, the selected molecular docking targets included SAP2 (3PVK), LDM (5V5Z), and NMT (1IYL). These molecular targets have all been proven to play essential roles in the yeast's survival and invasion in the host, making them potential targets for antifungal drug design. Specifically, SAP2 could cleave proteins, potentially cleaving environmental proteins to provide amino acids for growth while also cleaving host proteins, leading to invasion and inflammation[35]. LDM plays a vital role in the ergosterol synthesis pathway, a primary sterol component in the yeast cell membrane with various functions[11]. NMT attaches myristate to yeast cell proteins, contributing to cell membrane binding, signalling, and other crucial cellular functions[12]. The molecular docking results showed that the major

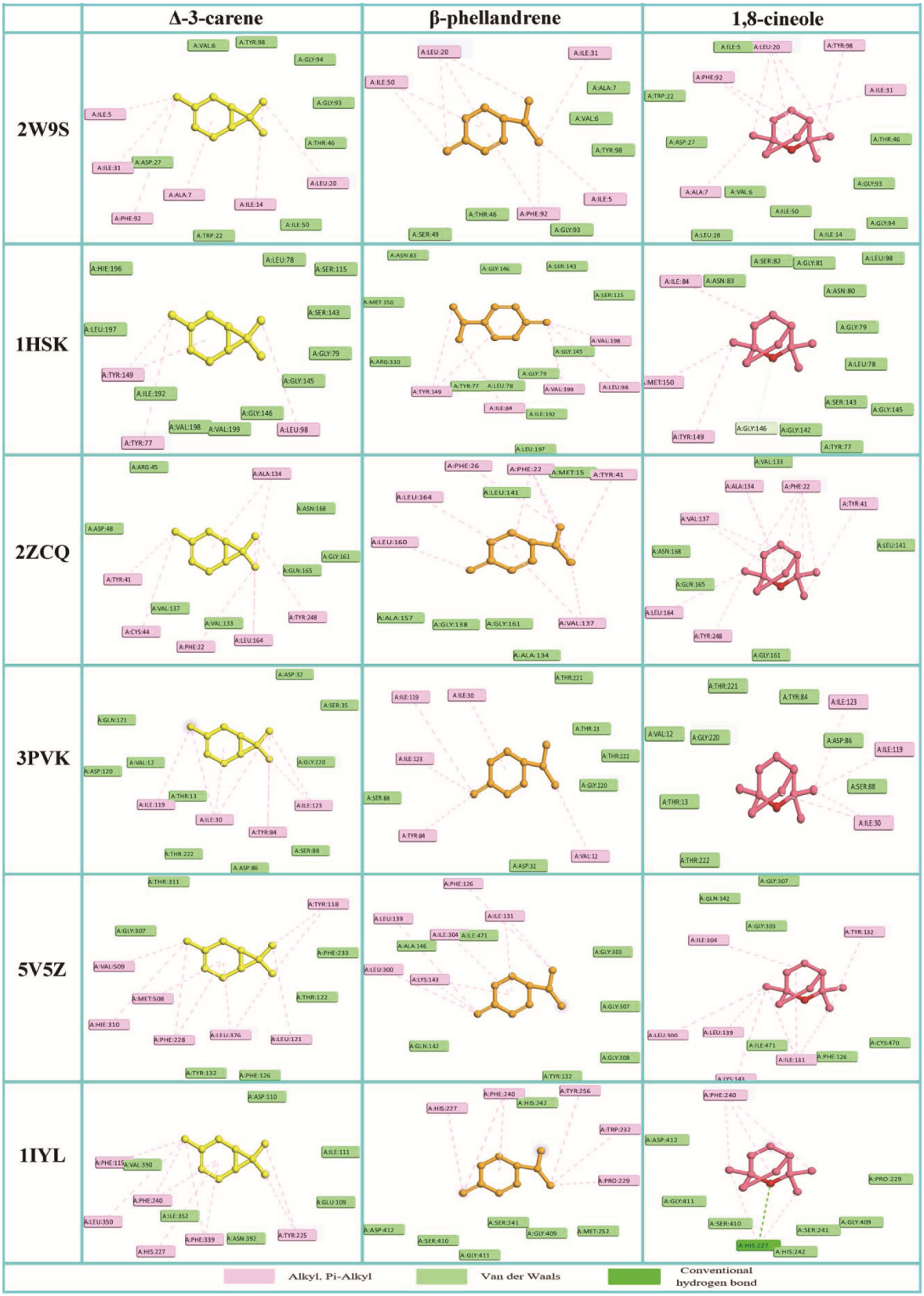

Fig. 2 — 2D interactions of Δ-3-carene, β-phellandrene, and 1,8-cineole towards the residues in the active sites of the targets.

Table 4 — Physicochemical properties

Parameters	Δ-3-carene	β-phellandrene	1,8-cineole	Optimal range
Molecular Weight	136.13	136.13	154.14	100 – 600
Van der Waals Volume	161.767	167.687	173.194	
Density	0.862	0.812	0.89	
Num. H-bond acceptors	0	0	1	0 – 12
Num. H-bond donors	0	0	0	0 – 7
Num. rotatable bonds	0	1	0	0 – 11
Num. rings	1	1	3	0 – 6
Num. atoms in the biggest ring	7	6	6	0 – 18
Num. heteroatoms	0	0	1	1 – 15
Formal charge	0	0	0	-4 – 4
Num. rigid bonds	8	7	9	0 – 30
Flexibility	0	0.143	0	
Stereo centers	2	1	0	≤ 2
TPSA	0	0	9.23	0 – 140
Fsp^3	0.8	0.6	1.0	≥ 0.42
LogP	4.623	4.169	2.168	
LogD	3.522	3.178	2.175	
LogS	-4.671	-3.932	-1.383	
Druglikeness				
Lipinski rule	Accepted	Accepted	Accepted	
Pfizer rule	Rejected	Rejected	Accepted	
GSK rule	Rejected	Rejected	Accepted	

components in *C. rubidium* essential oils have the potential to inhibit the activities of all three *C. albicans* targets with negative binding energies (ranging from -6.27 kcal/mol to -14.97 kcal/mol) (Table 3) and the ability to interact with residues in the active sites of these target enzymes. The interactions formed between the ligands and receptors primarily involve hydrophobic interactions (alkyl, pi-alkyl) and conventional hydrogen bonds (Fig. 1 and 2). Comparing the binding affinities of the three components in the essential oils to the three *C. albicans* targets, it can be observed that the two major components found in the highest proportion in aerial parts oil (Δ-3-carene and 1,8-cineole) exhibited stronger binding affinities compared to the major component in rhizome oil (β-phellandrene). Especially, Δ-3-carene displayed the highest binding affinity with all three *C. albicans* targets. Based on these results and *in vitro* inhibitory activity findings, it can be concluded that Δ-3-carene and 1,8-cineole played crucial roles in inhibiting *C. albicans* of the EOs from the aerial parts of *C. rubidum*.

Physicochemical properties and ADMET profile

ADMETlab 3.0 is a chem-informatic tool developed Li Fu *et al.*[36]. The physicochemical parameters were calculated using this web-based tool and are presented in Table 4. The results showed that the parameters of the three compounds are quite similar. Most parameters fell within the optimal range, and all three compounds complied with Lipinski's Rule. 1,8-cineole adhered to all three drug-likeness principles of Lipinski, Pfizer, and GSK. It could be observed that Δ-3-carene and β-phellandrene had relatively high logP and logD values, while logS was quite low, which may have affected the absorption ability of these compounds.

The ADMET profiles of the three compounds are presented in Table 5. The results indicated these compounds exhibited good absorption potential, with human intestinal absorption ≥ 30% and high permeability in Caco-2 and MDCK assays. Additionally, the oral bioavailability of all compounds was predicted to be ≥ 30%, with 1,8-cineole predicted to be ≥ 50%. Most compounds were not affected by P-glycoprotein, although Δ-3-carene was predicted to inhibit P-glycoprotein. Regarding distribution, it was noteworthy that β-phellandrene and 1,8-cineole had high plasma protein binding ratios, and all three compounds were predicted to be capable of crossing the blood-brain barrier. In terms of metabolism, the compounds were predicted to be substrates and inhibitors of certain liver enzymes. The clearance of these compounds was all moderate, ranging from 5 to

Table 5 — ADMET properties

Properties	Δ-3-carene	β-phellandrene	1,8-cineole
Absorption			
Caco-2 permeability	High	High	High
MDCK permeability	High	High	High
Pgp-inhibitor	Yes	No	No
Pgp-substrate	No	No	No
Human intestinal absorption	≥ 30%	≥ 30%	≥ 30%
$F_{20\%}$	≥ 20%	≥ 20%	≥ 20%
$F_{30\%}$	≥ 30%	≥ 30%	≥ 30%
$F_{50\%}$	< 50%	< 50%	≥ 50%
Distribution			
Plasma protein binding	69.4%	98.5%	92.7%
Volume distribution (L/kg)	2.944	1.275	2.767
Blood-brain barrier penetration	Yes	Yes	Yes
The fraction unboud in plasma	30.8%	1.6%	8.8%
Metabolism			
CYP interaction	CYP2C19 inhibitor CYP2C19 substrate CYP2C9 inhibitor CYP2D6 substrate CYP2B6 substrate	CYP2C19 inhibitor CYP2C19 substrate CYP2C9 substrate CYP2B6 substrate CYP2C8 inhibitor	CYP2C19 inhibitor CYP2C19 substrate CYP2C9 substrate CYP2D6 substrate CYP2B6 inhibitor CYP2B6 substrate CYP2C8 inhibitor
Excretion			
Clearance (mL/min/kg)	12.729	9.886	9.895
$T_{1/2}$ (h)	1.801	0.939	1.14
Toxicity			
hERGblockers	No	No	No
Human hepatotoxicity	No	No	No
Drug-induced liver injury	No	No	No
Drug-induced nephrotoxicity	No	No	No
AMES toxicity	No	No	No
Hematotoxicity	No	No	No
Rat oral acute toxicity	No	No	No
FDAMDD	No	No	No
Eye irritation	Yes	Yes	Yes

15 mL/min/kg. According to predictions, nearly all compounds were non-toxic to the heart, liver, kidneys, and blood and did not induce genetic mutations or acute toxicity in rats. However, they all caused eye irritation.

Conclusion

This study focused on the chemical composition and antimicrobial activity of essential oils from aerial part oil and rhizome oil of *C. rubidum*. The aerial part essential oil exhibited stronger antifungal activity against *C. albicans* while rhizome oil possessed stronger antibacterial activity against *S. aureus* than the oil from aerial parts. 27 components were identified in aerial part oil of *C. rubidium*, accounting for 99.51% with Δ-3-carene (33.14 %),1,8-cineole (17.14%), β-phellandrene (14.63%), α-terpinol (6.35%), terpinene-4-ol (4.91%) were dominant compounds while 21 components (accounting for 96.52%) were detected in rhizome oil with β-phellandrene (25.61%), Δ-3-carene (20.15%), α-terpinol (8.62%), L-phellandrene (7.46%), and α-terpinolene(4.59%) were ascendant components. The aerial parts oil possessed fungicidal activity against *C. albicans* (MIC = MFC = 0.4%), and rhizome oil bactericidal activity against *S. aureus* (MIC = MBC = 0.8%). The docking study demonstrated that β-phellandrene kept an important role in the ability to inhibit DHFR of *S. aureus* while Δ-3-carene and 1,8-cineole played a significant part in the capacity to inhibit SAP2 of *C. albicans.* The ADMET calculations indicated that all three compounds, Δ-3-carene, β-phellandrene, and 1,8-

cineole, can potentially be used as antimicrobial agents. In the future, the biological activity of *C. Rubidum* essential oil and especially its main components mentioned above should be investigated in other microorganisms as well.

Conflict of interest

The authors declare that there are no conflicts of interest.

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