

The Anti-inflammatory Effect and Molecular Docking of Essential Oils Extracted from Rhizomes of *Distichochlamys benenica* and *Distichochlamys citrea*

Gia-Buu Tran¹, Ty Viet Pham², Musa Turkmen³, Nguyen Hoai Nguyen⁴, and Bich Hang Do^{1*}

¹ Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, VIETNAM

² Faculty of Chemistry, University of Education, Hue University, 34 Le Loi, Hue City, VIETNAM

³ Faculty of Agriculture, Department of Crop Fields, Hatay Mustafa Kemal University, 31034, Hatay, TURKEY

⁴ Faculty of Biotechnology, Ho Chi Minh City Open University, 97 Vo Van Tan Street, District 3, Ho Chi Minh City, VIETNAM

Abstract: *Distichochlamys* genus, belonging to the ginger family, are native to Vietnam and there has not been many research mentioned about biological properties of this genus. In this study, essential oils were extracted from the rhizomes of *Distichochlamys benenica* (DBEO) and *Distichochlamys citrea* (DCEO), and the components were identified using gas chromatography-mass spectrometry. Further, for the first time, the anti-inflammatory activity of these essential oils was evaluated *in vitro* using nitric oxide inhibitory assay and *in silico* via binding with the inflammatory cyclooxygenase proteins (COX-1 and COX-2). The main constituents of DBEO and DCEO, according to the results, were 1,8-cineole, (*E*)-citral and (*Z*)-citral. The nitric oxide release was inhibited by DBEO and DCEO, with IC₅₀ values of 27.13±2.43 and 37.44±3.02 µg/mL, respectively. Moreover, the COX-1 and COX-2 proteins formed strong bonds with 1,8-cineole, (*E*)-citral, and (*Z*)-citral, whose free energies roughly ranged from -54.78 to -84.08 kcal/mol. These results suggested that by inhibiting multiple inflammatory proteins, DBEO and DCEO may have a promising anti-inflammatory effect.

Key words: *Distichochlamys citrea*, *Distichochlamys benenica*, essential oil, docking study, inhibition of NO release

1 Introduction

Zingiberaceae is a large flowering family with above 13,000 species, which are adapted in tropical and subtropical climates, and widely grow in Southeast Asian countries such as Vietnam, Malaysia, Indonesia, etc.¹⁾ *Zingiberaceae* family consists of many commonly used medicinal plants and spices such as ginger, turmeric, galangal, cardamom, white ginger lily, etc. Recently, several new members of the *Zingiberaceae* family have been found and recorded in the flora of Vietnam. Among them, some species of *Distichochlamys* genus, such as *Distichochlamys orlowii*, *Distichochlamys rubrostriata*, *Distichochlamys citrea* and *Distichochlamys benenica* are endemic species of Vietnam. In 1995, Newman firstly collected *D. citrea*, from Bach Ma National Park, Thua Thien Hue, Vietnam, and also established the new genus for this species, the genus *Distichochlamys*²⁾. Furthermore, *D. benenica* is the last

member belonging to the genus and has been first recorded in Ben En National Park, Thanh Hoa, Vietnam by Nguyen and Leong-Škorničková (2012)³⁾.

Some researchers have reported on the bioactivities of two species of *Distichochlamys* genus, *D. benenica* and *D. citrea*. For example, Huong *et al.* investigated the chemical profile of essential oil of *D. benenica* (DBEO) and demonstrated its mosquito larvicidal and anti-microbial activities of the oil⁴⁾. In addition, Hoang *et al.* (2020) also reported the acetylcholinesterase inhibitory activity of DBEO⁵⁾. Although the biochemical composition of essential oils from *D. citrea* (DCEO) has been published⁵⁾, there have been no papers mentioning the bioactivity of DCEO. The bioactivity of extracts and compounds isolated from *D. benenica* and *D. citrea* was discovered. Particularly, Pham *et al.* suggested that *trans*-o-coumaric acid, a natural product isolated from the rhizomes of *D. benenica*, exhib-

*Correspondence to: Bich Hang Do, Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, VIETNAM

E-mail: dobichhang@tdtu.edu.vn

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ited a strong anti-inflammatory and anti-microbial effects⁶⁾. The antibacterial activities of *D. citrea* have also been reported in several studies. For example, the *n*-hexane extract of *D. citrea* could effectively eradicated *Streptococcus pyogenes*, the pathogen responsible for streptococcal pharyngitis⁷⁾. Moreover, the extracts of *D. citrea* also exhibited antioxidant and α -glucosidase inhibitory effects⁸⁾. These findings suggest that the two species, *D. benenica* and *D. citrea*, as promising medicinal plants for application in the pharmaceutical industry.

When a pathogen invades the body, the immune system responds by releasing mediators such as histamine, prostaglandins, leukotrienes, and cytokines leading to inflammation. In addition, it enhances vascular permeability, thereby attracting leukocytes to the inflamed site, facilitating pathogen clearance. Nevertheless, dysregulated inflammation can result in various illnesses, including cancer, diabetes mellitus, autoimmune, neurodegenerative, and cardiovascular diseases⁹⁾. Currently employed in inflammation treatment, nonsteroidal anti-inflammatory drugs, present several potential side effects, including the exacerbation of pre-existing heart failure, hypertension, gastrointestinal ulcers, severe cardiovascular events, and acute renal failure¹⁰⁾. Hence, it is imperative to explore alternative therapeutic sources with anti-inflammatory properties to mitigate the side effects associated with medication.

Essential oils (EOs) are aromatic, volatile liquids extracted from whole plants or their parts using various distillation techniques. Due to their diverse chemical constituents, mainly terpenes (monoterpenes, sesquiterpenes) and non-terpenic compounds such as eugenol, cinnamaldehyde, and saffrole, EOs exhibit a wide range of biological characteristics¹¹⁾. Many studies have indicated the potential anti-inflammatory activity of essential oils (EOs)^{12–14)}. Therefore, in this study, the EOs were extracted from the rhizomes of two *Distichochlamys* species, namely *D. benenica* and *D. citrea*. The anti-inflammatory activity was assessed *in vitro* via nitric oxide (NO) inhibition assay and the binding of compounds in *Distichochlamys* species to inflammatory proteins was evaluated using docking model.

2 Materials and Methods

2.1 Materials

2.1.1 Chemicals and cell line

The lipopolysaccharides (LPS) derived from *Escherichia coli*, sodium nitrite, sulfanilamide, N-1-naphthylethylenediamine dihydrochloride, dimethyl sulfoxide (DMSO), and dexamethasone were provided by Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were supplied by Life Technologies, Inc. (Gaithersburg, MD, USA). The Griess Reagent System was provided by Promega Corporation (WI,

USA).

2.1.2 Plant sample

Fresh *D. benenica* (located at 16° 12' 21.3" N 107° 17' 17.0" E) and *D. citrea* (located at 16° 12' 13.8" N 107° 17' 49.3" E) were gathered in October 2023 at A Luoi, Thua Thien Hue, Vietnam. The morphological structures of the plants were identified by Dr. Do Xuan Cam, Hue University of Agriculture and Forestry. Specimens of those species was deposited in the Faculty of Chemistry, University of Education, Hue University (Fig. 1).

2.2 Methods

2.2.1 Isolation of the essential oils

Rhizomes weighing 15.0 kg each underwent a series of extraction steps to EOs. The hydrodistillation procedure was carried out in accordance with a previously published study¹⁵⁾, including: the rhizomes were meticulously washed and subsequently cut into smaller pieces to enhance the extraction efficiency. The chopped rhizomes were subjected to hydro-distillation using a custom stainless steel apparatus. The extraction process was facilitated using an electromagnetic stove, ensuring controlled and efficient distillation. This procedure was continued for a duration of 4 h, resulting in EOs, characterized by their white color, were collected from the distillation process. The yields were determined to be 0.35% (v/w) for *D. citrea* and 0.37% (v/w) for *D. benenica*. The collected essential oils were dried over Na₂SO₄ to remove any residual water content. Subsequently, the oils were carefully stored at a temperature of 6°C to maintain their integrity for subsequent analyses.

2.2.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was performed using a Shimadzu Technologies GCMS-QP2010 Plus chromatograph equipped with a fused silica Equity-5 capillary column (30 m length, 0.25 mm diameter, 0.25 μ m film thickness; Supelco, USA). The analytical parameters were configured as follows: carrier



Fig. 1 Specimens of *D. benenica* (left) and *D. citrea* (right).

helium flow: 1.5 mL/min, injector and interface temperatures: 280°C, temperature ramp: 60°C (2 min hold) to 240°C (20 min hold) at 3°C/min, then to 280°C at 5°C/min (30 min hold), split ratio: 10:1, inlet pressure: 93.2 kPa, injection volume: 1.5 µL, ionization voltage: 70 eV, detector voltage: 0.82 kV, MS scan mass range: 40–450 amu at a sampling rate of 0.5 scan/s. Retention indices were calculated by co-injecting constituents with a homologous series of *n*-alkanes (C₇–C₄₀). Chemical identification relied on comparing RI values with literature data. MS fragmentations were cross-referenced with NIST 11 and WILEY 7 Libraries, using known compositions of EOs.

2.2.3 Inhibition of NO release

The macrophage RAW264.7 cell line was grown in DMEM culture media supplemented with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 10% FBS, 100 U/mL penicillin G, and 0.4 mg/mL streptomycin sulfate. The cell was cultured with 5% CO₂ at 37°C in an incubator. Upon reaching approximately 80% confluency after 3–5 days of growth, the cells were subcultured at a ratio of 1:3. The experiments utilized healthy cells that had undergone multiple subcultures.

The NO inhibition assay was performed following a previous protocol with slightly modification¹⁶. A 96-well plate was seeded with 2×10^5 cells per well, and the cells were cultured for 24 h at 37°C and 5% CO₂ atmosphere. Prior to the experimental testing, the culture medium was replaced with FBS-free DMEM for 3 h. The cells were treated with different concentrations of DBEO and DCEO (100, 20, 4, and 0.8 µg/mL) for 2 h, followed by a 24-h treatment with 10 µg/mL LPS to induce NO production. The wells without DBEO and DCEO were served as the negative control, while dexamethasone at concentrations of 100, 20, 4, and 0.8 µM were utilized as the positive control. 100 µL of cell culture medium was added into a fresh well of a 96-well plate, following by adding 50 µL of 0.1% (w/v) N-1-naphthylethylenediamine dihydrochloride (diluted in water) and 50 µL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid (Griess Reagent System). A spectrophotometer set to 540 nm was used to measure the amount of nitrite produced by the mixtures after they had been incubated for 10 min at room temperature. FBS-free DMEM was utilized as a blank.

The formula used to determine the inhibition of NO production:

$$\% \text{ inhibition} = 100\% - [\text{NO}_{\text{sample}}/\text{NO}_{\text{LPS}}] * 100$$

The Table Curve 2Dv4 software was utilized to determine the IC₅₀ of inhibition.

2.2.4 Docking study

The RCSB Protein Data Bank's crystal structure data was used in the *in silico* investigation, which was carried out with Maestro software's Schrödinger 2021-3 (New York, USA). 1,8-cineole, (*Z*)-citral, and (*E*)-citral were chosen to test the binding ability with inflammatory proteins, such as

cyclooxygenase-1 (COX-1) (Uniprot ID: P22437) and cyclooxygenase-2 (COX-2) (PDB ID: 1CVU). The Protein Preparation Workflow tool was used to prepare the proteins, which included generating het states, optimizing hydrogen bonds, supplementing missing side chains, adding missing hydrogen atoms, and eliminating unnecessary water molecules. To guide the binding process between ligands and proteins, receptor grids were generated at the positions of native ligands in each structure after preparing the proteins. Subsequently, the Glide Docking method was used to dock the chemical structures of 1,8-cineole, (*Z*)-citral, and (*E*)-citral with the receptor grids. The MM-GBSA method will be employed to estimate the free energy of the complexes formed between the ligands and receptors.

2.2.5 Statistical analysis

Every experiment was conducted in triplicate, and the mean ± standard error was used to describe the data. GraphPad Prism 9.0.0.121 (GraphPad Software, Inc., San Diego, CA) was used to analyze the data, and *p*-values of less than 0.05 were regarded as statistically significant.

3 Results

3.1 GC-MS

Through GC-MS analysis, a total of 29 compounds, constituting 100.0%, were identified in DBEO. The major components were oxygenated monoterpenes (85.1%), followed by monoterpene hydrocarbons (7.6%), non-terpenic compounds (5.2%), sesquiterpene hydrocarbons (1.7%), and oxygenated sesquiterpenes (0.4%). The primary compounds in DBEO were 1,8-cineole (20.6%), (*E*)-citral (19.8%), and (*Z*)-citral (14.7%). Several compounds, including geraniol, bornyl acetate, borneol, α-terpineol, terpinen-4-ol, and others, were present in percentages greater than 1.0% (Table 1, Fig. 2a).

In DCEO, 19 compounds were identified, constituting 100.0%. This oil was predominantly composed of oxygenated monoterpenes (93.2%). Unlike DBEO, DCEO did not contain sesquiterpene hydrocarbons and oxygenated sesquiterpenes. It also included monoterpene hydrocarbons (3.4%) and non-terpenic compounds (3.4%). The principal compounds in *D. citrea* rhizome oil were 1,8-cineole (39.9%), (*E*)-citral (20.7%), and (*Z*)-citral (12.4%). Other notable compounds included benzyl geraniol, α-terpineol, and linalool (Table 1, Fig. 2b).

The comparison of the two oils revealed differences in the presence of certain compounds, such as camphene, sabinene, γ-terpinene, *endo*-fenchyl acetate, *exo*-fenchyl acetate, bornyl acetate, β-elemene, (*E*)-caryophyllene, γ-murolene, γ-himachalene, β-selinene, pogostol, and nootkatone, which were absent in DCEO.

Table 1 Chemical compositions of DBEO and DCEO.

No	Compounds	RI exp	RI lit	<i>D. benenica</i>	<i>D. citrea</i>
1	α -Pinene	933	932	1.3	0.4
2	Camphene	947	946	0.4	–
3	<i>n</i> -Heptanol	965	959	–	0.1
4	Sabinene	972	969	0.5	–
5	β -Pinene	976	974	3.1	0.8
6	6-methyl-5-hepten-2-one	986	981	1.8	0.9
7	β -Myrcene	991	988	1.6	1.3
8	<i>o</i> -Cymene	1024	1022	0.3	0.2
9	Limonene	1028	1024	–	0.7
10	1,8-Cineole	1033	1026	20.6	39.9
11	γ -Terpinene	1058	1054	0.5	–
12	Linalool	1101	1095	4.1	3.0
13	<i>exo</i> -Isocitral	1145	1140	1.8	1.0
14	(<i>Z</i>)-Isocitral	1164	1160	–	1.2
15	Borneol	1165	1165	3.9	1.3
16	Terpinen-4-ol	1178	1174	3.2	2.9
17	Ethyl-3(2-furyl)-propanoate	1183	1178	3.4	2.4
18	α -Terpineol	1192	1186	3.3	3.1
19	<i>endo</i> -Fenchyl acetate	1221	1218	1.3	–
20	<i>exo</i> -Fenchyl acetate	1230	1229	1.0	–
21	(<i>Z</i>)-citral	1245	1235	14.7	12.4
22	Geraniol	1258	1249	7.1	5.2
23	(<i>E</i>)-citral	1277	1264	19.8	20.7
24	Geranyl acetate	1288	1279	0.4	2.5
25	Bornyl acetate	1385	1284	4.0	–
26	β -Elemene	1393	1389	0.2	–
27	(<i>E</i>)-Caryophyllene	1420	1417	0.3	–
28	γ -Murolene	1485	1478	0.7	–
29	γ -Himachalene	1487	1481	0.3	–
30	β -Selinene	1494	1489	0.3	–
31	Pogostol	1656	1651	0.2	–
32	Nootkatone	1806	1806	0.2	–
Total				100	100
Monoterpene hydrocarbons				7.6	3.4
Oxygenated monoterpenes				85.1	93.2
Sesquiterpene hydrocarbons				1.7	0.0
Oxygenated sesquiterpenes				0.4	0.0
Non-terpenic compounds				5.2	3.4

RI exp: Retention indices relative to *n*-alkanes (C₇-C₄₀) on Equity-5 column; RI lit: Retention indices from Adams book and the NIST standard database; Bold: Compounds with greater than 5%; – : Not identified

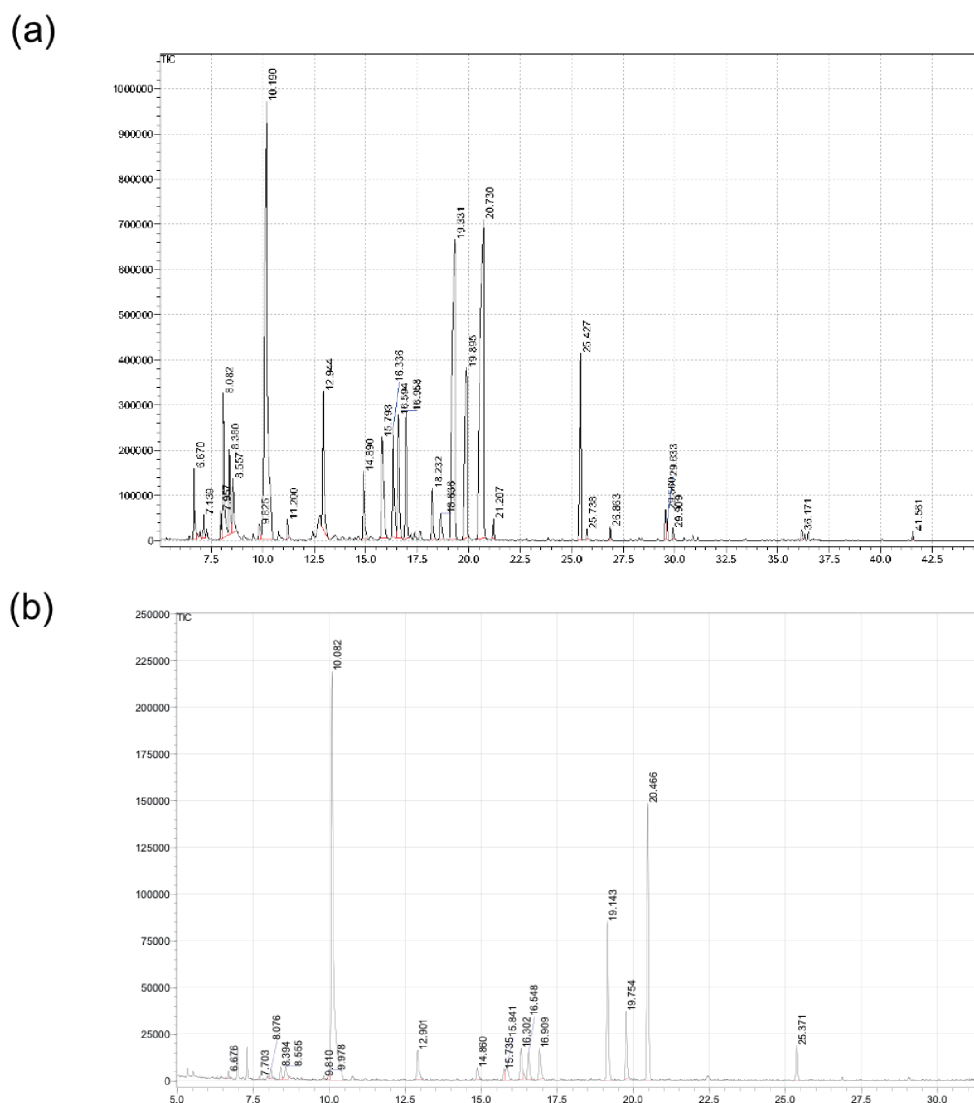


Fig. 2 GC-MS of DBEO (a) and DCEO (b).

3.2 Inhibition of NO release

The anti-inflammatory effect of DBEO and DCEO were evaluated *in vitro* using the NO inhibition assay with RAW264.7 cell lines. The cells were incubated with tested samples, followed by treatment with LPS to enhance the NO production. The NO release was measured using Griess Reagent System and measured at 540 nm. The results were expressed in Fig. 3. Both DBEO and DCEO exhibited effective inhibition of NO release with a maximum of 77.91-87.50% of inhibition at a concentration of 100 $\mu\text{g/mL}$. The inhibition is dose-dependent at the tested concentrations similar to dexamethasone. The IC_{50} of DBEO was 27.13 ± 2.43 and that of DCEO was 37.44 ± 3.02 $\mu\text{g/mL}$, compared to 5.77 ± 0.20 $\mu\text{g/mL}$ of dexamethasone. This result revealed that both DBEO and DCEO showed potential anti-inflammatory activity via inhibition of NO release of macrophage cells.

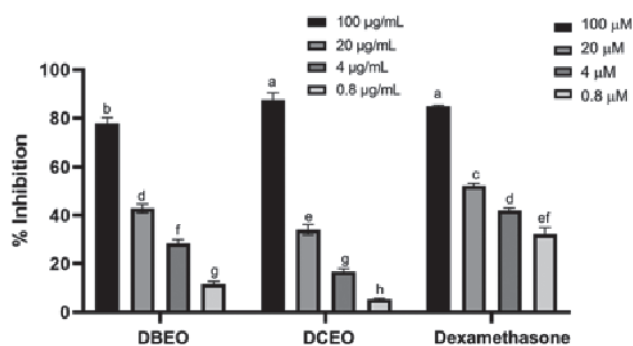


Fig. 3 The percentage of NO production that was inhibited following DBEO and DCEO treatment. The various superscript letters (a, b) indicate statistical differences between groups ($p < 0.05$); Turkey's test is performed after a one-way ANOVA.

Table 2 XP-IDF docking scores and MM-GBSA estimation of 1,8-cineole, (*Z*)-citral, and (*E*)-citral with COX-1 and COX-2.

Compounds	XP-IDF Docking score	MM-GBSA estimation (kcal/mol)	No. of H-bonds	Residues
COX-1 (Uniprot ID: P22437)				
Diclofenac	− 9.659	− 63.81	1	Tyr 387
1,8-Cineole	− 6.526	− 54.78	0	–
(<i>Z</i>)-Citral	− 5.405	− 57.51	1	Tyr 387
(<i>E</i>)-Citral	− 5.114	− 54.78	1	Arg 122
Compounds	XP-IDF Docking score	MM-GBSA estimation (kcal/mol)	No. of H-bonds	Residues
COX-2 (PDB ID: 1CVU)				
Diclofenac	− 9.831	− 84.08	2	Arg 2120, Tyr 2355
1,8-Cineole	− 6.043	− 71.25	0	–
(<i>Z</i>)-Citral	− 5.862	− 66.52	1	Tyr 2385
(<i>E</i>)-Citral	− 6.210	− 70.92	0	–

3.3 Docking analysis

The binding of 1,8-cineole, (*Z*)-citral, and (*E*)-citral with COX-1 and COX-2, the main inflammatory proteins in the arachidonic acid pathway was performed using Maestro software's Schrödinger. According to the findings, all three of the compounds under investigation had favorable free energy levels with COX-1 and COX-2. As seen **Table 2** and **Fig. 4**, the values for COX-1 and COX-2, respectively, ranged from − 54.78 to − 63.81 kcal/mol and − 66.52 to − 84.08 kcal/mol. These values are statistically equivalent to the values for the positive control diclofenac, which are − 63.81 and − 84.08 kcal/mol. Furthermore, the effective binding of compounds in DBEO and DCEO was demonstrated through hydrogen bond formation. Particularly, (*Z*)-citral, and (*E*)-citral formed 1-2 H-bonds with COX-1 and COX-2. In light of these findings, it is clear that 1,8-cineole, (*Z*)-citral, and (*E*)-citral display possible binding interactions with the two proteins that cause inflammation. Moreover, these results are consistent with the *in vitro* data shown in **Fig. 3**, which showed that both DBEO and DCEO effectively inhibited inflammation, nearly equal to that of the positive control.

4 Discussion

The purpose of this study was to extract EOs from the rhizomes of *D. benenica* and *D. citrea*, and subsequently analyze their constituents using GC-MS. An *in vitro* assay was utilized to validate the inhibitory effect of DBEO and DCEO on inflammation, while a docking model was utilized to assess the binding of primary compounds with inflammatory proteins, such as COX-1 and COX-2.

This study focuses particularly on the rhizome samples

of *D. benenica* and *D. citrea* collected in 2023 from Thua Thien Hue, examining their chemical compositions in detail and comparing them with previous samples from other regions and years^{2,3}. DCEO from Quang Binh were rich in 1,8-cineole (43.67%), β -linalool (14.79%), and neryl acetate (10.81%)¹⁷. In Thua Thien Hue, DCEO contained 1,8-cineole (30.34%), (*E*)-citral (20.88%), (*Z*)-citral (11.05%), neryl acetate (9.90%), and *cis*-geraniol (7.61%)¹⁷. DCEO from Quang Tri showed high levels of (*E*)-citral (18.38%), (*Z*)-citral (13.98%), neryl acetate (11.11%), and *cis*-geraniol (8.89%) while in Quang Nam, the major components were (*E*)-citral (19.13%) and (*Z*)-citral (13.49%)¹⁷. DCEO from Nghe An contained notable compounds such as 1,8-cineole (23.0%), (*E*)-citral (18.9%), (*Z*)-citral (15.0%), *cis*-geraniol (9.3%), and α -cedrol (5.2%)¹⁸. These results indicate significant chemical diversity in DCEO based on the region of collection. Different methods of distillation might affect the concentration of compounds. Specifically, in this study, DCEO were extracted using hydro-distillation revealed 1,8-cineole (39.9%), (*E*)-citral (20.7%), (*Z*)-citral (12.4%), α -terpineol (3.1%), β -pinene (0.8%), and α -pinene (0.4%). DCEO obtained from steam distillation showed high levels of (*E*)-citral (22.56%), (*Z*)-citral (15.21%), α -terpineol (12.40%), β -pinene (7.06%), and 1,8-cineole (5.24%)¹⁹. DCEO from microwave-assisted hydrodistillation resulted in high concentrations of (*E*)-citral (26.12%), (*Z*)-citral (17.89%), α -terpineol (14.87%), 1,8-cineole (9.18%), α -pinene (5.35%), and β -pinene (5.17%)¹⁹. These differences in extraction methods underscore the impact of the extraction process on the essential oil composition. These findings highlight the importance of continuous study on the chemical profiles of *Distichochlamys* species to understand their potential applications and the underlying

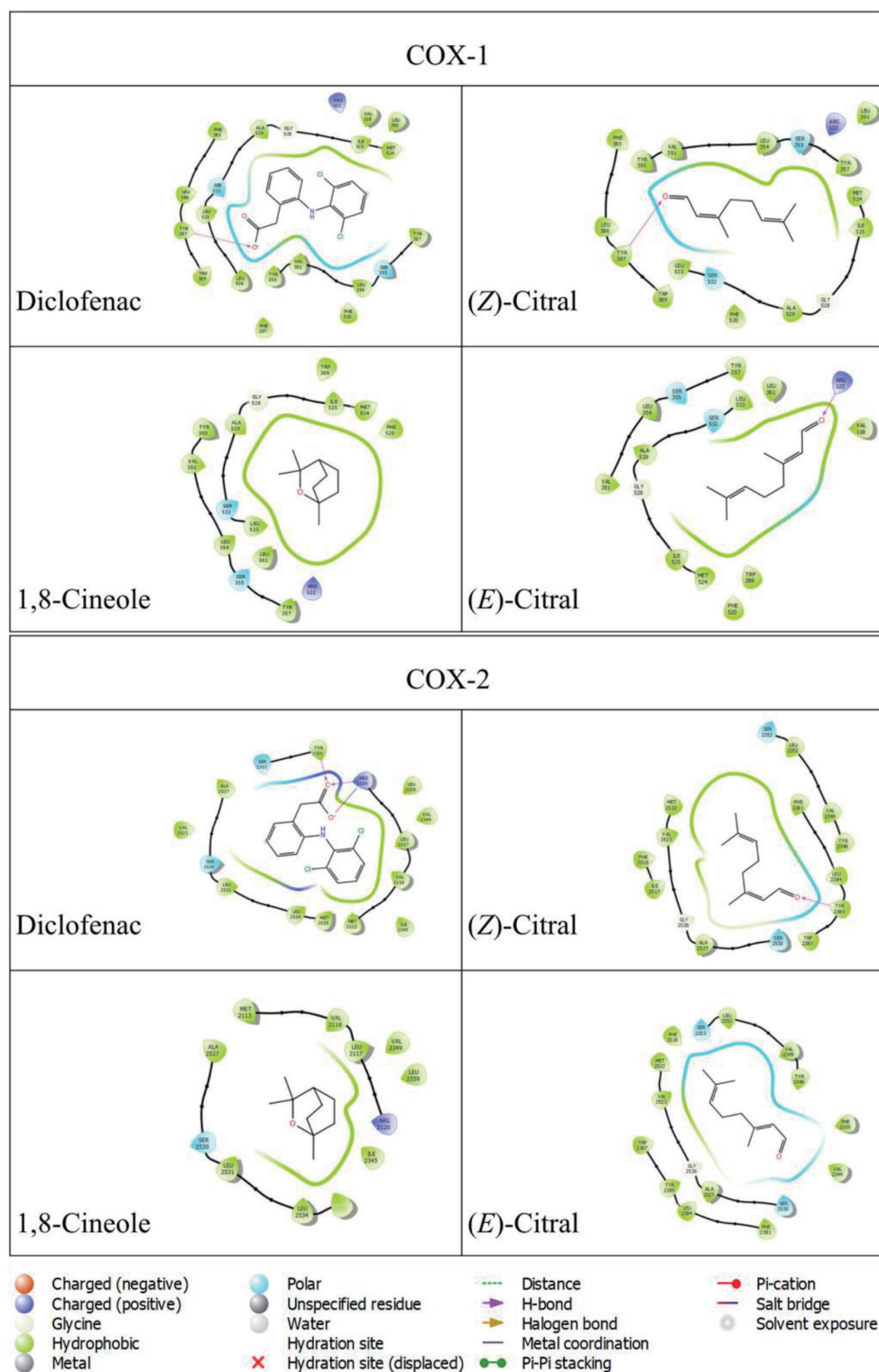


Fig. 4 The bindings of 1,8-cineole, (Z)-citral, and (E)-citral with COX-1 and COX-2.

factors driving their chemical diversity.

Zingiberaceae is a well-known family for anti-inflammatory activity. Many studies have mentioned that EOs extracted from *Zingiberaceae* family effectively reduced in-

flammation *in vitro* and *in vivo*. Specifically, EOs from *Zingiber ottensii* Valetton significantly reduced the ear and paw edema in rats. The activity of EOs from *Z. ottensii* might be via inhibiting COX-2, PGE-2 and tumor necrosis

factors α (TNF- α) expression²⁰. EOs from the fruits of *Alpinia oxyphylla* Miq. reduced mouse edema by inhibiting the expression of TNF- α , COX-2, interleukin-6 (IL-6), and nuclear factor-kappa B (NF- κ B)²¹. EOs from *Alpinia oxyphylla* Miq., *Kaempferia galanga* L., *Amomum kravanh* Pierre ex Gagnep., and *Alpinia galanga* (Linn.) Willd exhibited significantly anti-inflammatory effects by markedly down-regulating the expression levels of COX-2, TNF- α , interleukin-6 (IL-6), and interleukin-1 in adjuvant arthritis rats²². EOs of *Z. zerumbet* exhibited significant anti-inflammatory activity in both acute and chronic animal models²³. EOs (128 μ g/mL) significantly inhibited the production of proinflammatory mediators nitric oxide (NO) ($92.73 \pm 1.50\%$) and cytokines TNF- α and IL-6 in LPS-induced RAW264.7 macrophages without any cytotoxic effect²⁴. In our study, both DBEO and DCEO effectively inhibited the NO production in RAW264.7 cells with IC₅₀ of 27.13 ± 2.43 and 37.44 ± 3.02 μ g/mL, respectively, similar to the positive control. At concentration of 100 μ g/mL, DBEO and DCEO reduced NO production up to $87.5 \pm 2.98\%$. The *in silico* results also confirmed the good binding of main compounds in DBEO and DCEO with COX-1 and COX-2 (Figure 3-4, Table 2). These results were in accordance with our finding about the anti-inflammation of DBEO and DCEO via reducing the NO production and targeting COX-2. There has been no study about anti-inflammation of EOs from *Distichochlamys* genus. The main compositions in DBEO and DCEO including 1,8-cineole, (Z)-citral, and (E)-citral might be explained for the high anti-inflammatory activity of these Eos^{25–31}. However, there are several issues with this study. First, DBEO and DCEO were extracted using hydrodistillation, which produced 0.35% to 0.37% of the yield and component amount as selected in Table 1. Second, the anti-inflammatory property of EOs were evaluated using a NO release inhibition model, and the binding between EOs' compounds and inflammatory proteins was predicted using a docking model. In order to compare the yields and bioactivities of the EOs, different extraction techniques should be employed in subsequent studies. Additionally, a number of *in vitro* studies should be carried out to clarify EOs' anti-inflammatory mechanism, such as western blot or real-time polymerase chain reaction tests of the protein expressions associated with inflammatory signaling pathways. To enhance the bioactivities of EOs, other activities like anti-cancer, anti-microbial, anti-diabetic, etc. activities should be investigated.

5 Conclusions

An essential oil, characterized by its oily and fragrant nature, can be extracted from a plant that has been shown to exhibit a wide range of bioactivities. The primary com-

ponents of the EOs extracted from the rhizomes of *D. citrea* and *D. benenica* in this study were 1,8-cineole, (E)-citral, and (Z)-citral. By preventing the release of NO and by tightly binding with the inflammatory proteins COX-1 and COX-2, DBEO and DCEO demonstrated encouraging anti-inflammatory activity both *in vitro* and *in silico*. These results suggest that DBEO and DCEO have promising anti-inflammatory properties and could be used in the treatment of diseases relating to inflammation.

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Author Contribution Statement

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of Interest

The authors state no conflict of interest.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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