Potential Inhibitors of CYP51 Enzyme in Dermatophytes by Red Sea Soft Coral Nephthea sp.: In Silico and Molecular Networking Studies

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ABSTRACT: In this study, the n-hexane fraction of soft coral Nephthea sp. gathered from the Red Sea was evaluated for its antidermatophyte activity. The antidermatophyte activity was performed versus different fungi, including Microsporum canis, Trichophyton gypseum, and Microsporum mentagrophytes, using a broth microdilution method. The n-hexane fraction showed minimum inhibitory concentrations (MICs) against the tested dermatophytes of 104.2 ± 20.8, 125 ± 0.0, and 83.33 ± 20.83 μg/mL respectively. The chemical constitution of the lipoidal matter (n-hexane fraction) was characterized by gas chromatography coupled with a mass spectrometer (GC-MS). The unsaponifiable fraction (USAP) of Nephthea sp. showed relative percentages of hydrocarbons and vitamins of 69.61% and 3.26%, respectively. Moreover, the percentages of saturated and unsaturated fatty acids were 53.67% and 42.05%, respectively. In addition, a molecular networking study (MN) of the GC-MS analysis performed using the Global Natural Products Social Molecular Networking (GNPS) platform was described. The molecular docking study illustrated that the highest binding energy score for spathulenol toward the CYP51 enzyme was −8.3674 kcal/mol, which predicted the mode of action of the antifungal activity, and then the results were confirmed by the inhibitory effect of Nephthea sp. against CYP51 with an IC50 value of 12.23 μg/mL. Our results highlighted the antifungal potential of Nephthea sp. metabolites.

INTRODUCTION

Dermatophytes are keratin-loving fungi that commonly cause cutaneous infections in animals and humans such as ringworm and tinea.1−3 Usually, dermatophytes do not violate the living tissues but colonize the external layer of the skin.4 In addition, different symptoms typically appear within 2 weeks after direct contact between the human part and fungi.5 The most identified colonies belong to the three main genera Trichophytion, Microsporum, and Epidermophytion.6

The possible track of dermatophyte login to the host body is injured skin, scars, and burns.6 The fungal pathogens induce both immediate hypersensitivities as well as cell-mediated or delayed-type hypersensitivity.7 Microsporum canis (M. canis) causes tinea capitis and has a higher incidence in the winter season,8 while Trichophyton mentagrophytes (T. mentagrophytes) causes tinea pedis and and its incidence is increased in the hot season.9−10 Also, the geophilic dermatophyte of Microsporum gypseum (M. gypseum) appears during the rainy season and usually occurs from August to November when people come into direct contact with the soil.10,11

Despite the development of dermatophytosis treatment science and technology, it is still treated with commercially available topical and oral antifungal agents “from Whitfield’s ointment to azoles” with many side effects.6

Nature is considered an untapped source of biologically active metabolites. Recently, marine habitats have provided the drug market with unique skeletons with various diverse pharmacological activities.12,13 In addition, the treatment of cutaneous infections using natural plants and/or marine sources has displayed antidermatophyte potency.6,14−17 Marine Nephthea sp. is a known genus of the family Nephtheidae (20 genera) that comprises 12 species, and the members of this family are known as carnation corals, tree corals, or celt corals. They are distributed in the Indo-Pacific region.18

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Members of the genus *Nephthea* exhibit diverse bioactivities such as cytotoxic, antiviral, antihypertriglyceridemia, and antiphlogistic activities due to the presence of various chemical entities such as sesquiterpenes, diterpenes, and steroids. To the best of our knowledge, the antidermatophyte activities of the investigated *Nephthea* sp. fraction have not been previously evaluated. Therefore, the target of this study is the investigation of the antidermatophyte activity of the lipoidal matter of *Nephthea* sp. gathered from the Red Sea region. Additionally, the inhibitory action on the fungal CYP51 enzyme was determined to confirm the fungicidal activity. Moreover, an *in silico* study of the major identified components against the CYP51 enzyme was constructed. Finally, the chemical constituents of the investigated fraction and molecular networking based on the GC-MS data were reported (Scheme 1).

**MATERIALS AND METHODS**

**Soft Coral Collection, Identification, and Extraction.** The investigated soft coral was gathered from the shores of the Red Sea (Hurghada, Egypt) by snorkeling in January 2020. The specimen was authenticated by Dr. El-Sayd Abed El-Aziz (Department of Invertebrates Laboratory, National Institute of Oceanography and Fisheries, Red Sea Branch, Egypt). *Nephthea* sp. (500 g) was stored at 4 °C, cut into small slices, and then extracted several times with a mixture of methanol and methylene chloride (1/1) and concentrated under vacuum to yield 6 g. The obtained extract was fractionated using *n*-hexane, concentrated to dryness to yield 2 g, and kept at 4 °C for further investigation.

**Antifungal Activity.** *Microsporum canis, Trichophyton mentagrophytes,* and *Microsporum gypseum* were collected from different habitats of dogs, humans, and soil, respectively. They were authenticated by Dr. Mona M. H. Soliman (Microbiology and Immunology Department, National Research Centre, Giza, Egypt).

**Isolation of Fungi and Characterization.** The skin scraping and hair samples, collected from infected humans and cats, were taken from the lesions using a blunt scalpel blade and cleaned with 70% alcohol. They appeared clinically as ringworm lesions. Hairs were pulled out from the lesion using sterile forceps and kept in a sterile envelope for further mycological examination. Soil samples were collected in sterile plastic bags. The hair bait technique was used to isolate dermatophytes from soil according to Em and Cu. The isolation and identification of dermatophytes were implemented according to Scott and Miller, while the isolates were identified microscopically according to Monika and Chinna.

**Broth Microdilution Method (MIC).** A microdilution assay was used to evaluate the antifungal susceptibility testing, according to Clinical and Laboratory Standards Institute (CLSI) guidelines in the M38-A mold document. The dermatophyte strains were subcultured on potato dextrose agar (PDA) (Merck Co., Darmstadt, Germany) and incubated at 30 °C for 5–7 days. Conidia were kept for 15 min in sterile saline, prior to being counted by a hemocytometer. The suspension was adjusted to $1 \times 10^4$ CFU mL$^{-1}$ in RPMI 1640 medium (Roswell Park Memorial Institute Medium) (with l-glutamine, without, according to Scott and Miller, sodium bicarbonate; Gibco-BRL, Grand Island, New York) buffered with MOPS (3-(N-morpholino)propanesulfonic acid; Serva, Feinbochemica GmbH, Germany). As well, a series of 2-fold serial dilutions were carried out for the *Nephthea* sp. *n*-hexane fraction from 1000 to 1.9 μg/mL. The inoculum and different concentrations of the fraction, as well as positive and negative controls, were incorporated in 96-well microtiter plates and incubated at 32 °C for 5 days. The minimum inhibitory concentration (MIC) was determined and compared with the positive control. Both positive and negative control wells were included in all of the tested plates. The experiments were performed in triplicate for each fungus sample.

**Sample Preparation for GC-MS analysis.** Preparation of Unsaponifiable Matter (USM) and Fatty Acid Methyl Ester
(FAME). An n-hexane fraction (1.42 g) was saponified under reflux using 10% alcoholic potassium hydroxide. The unsaponifiable matter was extracted with diethyl ether and evaporated to dryness after removal of excess alkali and kept for GC-MS analysis. The USM obtained (0.44 g) represented 30.99% of the total lipoidal matter of the n-hexane fraction. The free fatty acids were liberated after acidification, extracted with diethyl ether, dried in vacuo, and weighed. The fatty acids obtained (0.88 g) represented 61.97% of the total lipoidal matter of the n-hexane extract. The methylation of the obtained fatty acids was carried out with anhydrous methanol and diazomethane, and the samples were then kept for GC/MS analysis.

Gas Chromatography–Mass Spectrometry Conditions. The GC-MS analysis was performed using a Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS with a TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). An electron ionization (EI) system with a power of 70 eV was used. The mobile phase was helium gas at a constant flow rate of 1.0 ml min\(^{-1}\). The injector and MS transfer line temperatures were set at 280 °C. The oven temperature was programmed at an initial temperature of 50 °C (2 min), 50–150 °C at a rate of 7 °C min\(^{-1}\), 150–270 °C at a rate of 5 °C min\(^{-1}\) (2 min), and 270–310 °C at the final temperature at a rate of 3.5 °C min\(^{-1}\) (10 min). Quantification of all identified components was carried out using the relative peak area. The identification of the constituents was carried out by a comparison of their relative retention times and mass spectra with those of the NIST and Wiley library data of the GC-MS system.

GC-MS Molecular Networking. Molecular networking (MN) is a simple computational process that may visualize and interpret mass data analysis. In addition, it can suggest the identical structures for all mass spectra within the data set and correlate the annotation between the unknown molecules and related molecules through the identical mass fragments. A molecular network (MN) for the GC-MS analysis data of the studied SAP and UNSAP Nephthea sp. lipoidal matters was constructed as follows. Thermo raw data files were transformed into the open format (mzML) using MS conversion that was supported by GNPS. The spectra in the network were then searched against GNPS GC-MS spectral libraries. The created MN was investigated and predicted using Cytoscape (vers. 3.9.0) which is open-source software for the analysis and exploration of MNs.

Molecular Docking Study. All of the molecular modeling studies were carried out using Molecular Operating Environment (MOE, 2019.0102) software. All minimizations were performed with MOE until an RMSD gradient of 0.1 kcal mol\(^{-1}\) Å\(^{-1}\) with the MMFF94x force field and the partial charges were automatically calculated. The X-ray crystallographic structure of human lanosterol 14α-demethylase (CYP51) complexed with ketoconazole (KKK) (PDB ID: 3LD6) was downloaded from the Protein Data Bank (https://www.rcsb.org/structure/3LD6). Water molecules and ligands were removed for each cocrystallized enzyme not involved in the binding. The protein was equipped for a docking study using the Protonate 3D protocol in MOE with default options. Docking binding sites were determined through a cocrystallized ligand (KKK). Moreover, the Triangle Matcher placement method and London dG scoring function were used for docking.

Inhibitory Activity of Lanosterol 14α-Demethylase (CYP51). Plate Reader Assay. The n-hexane fraction of Nephthea was screened for its inhibitory activity against lanosterol 14α-demethylase (CYP51) in comparison with the drug fluconazole as a reference at the Confirmatory Diagnostic Unit VACSERA, Cairo, Egypt. 7-Ethoxyresorufin (7-ER) is a fluorescent substrate and competitive suppressor of cytochrome P450 (CYP) isofrom CYP1A1 (IC\(_{50}\) = 0.1 μM). Upon enzymatic cleavage by CYP1A1 resorufin was released and its fluorescence was used to quantify CYP1A1 activity. Resorufin displays excitation/emission maxima of λ\(_{\text{max}}\) 572/580 nm, respectively. Resorufin and 7-ethoxyresorufin obtained from Sigma-Aldrich (St. Louis, MO, USA) are light-sensitive; therefore, this procedure should be carried out under yellow light to protect the integrity of the stock solutions. Incubations were prepared in a black 96-well plate, consisting of a substrate (7ER) and CaCYP51 bactosomes in 1pH 7.4 00 mM potassium phosphate buffer containing 5 mM magnesium chloride. Reactions were initiated by adding 40 μl of a 5x NADPH generating system (this can be omitted from wells containing blanks and standards). The formation of resorufin was measured fluorometrically every 30 s through the use of detection wavelengths (excitation/emission at 572/604 nm) chosen to minimize interference from NADPH and 7ER. The substrate 7-ethoxyresorufin and its metabolite resorufin are both available from Cypex.4

Statistical Analysis. All results are stated as mean ± SE (n = 3), and the statistical analysis of the inhibition activity (IC\(_{50}\)) against sterol 14α-demethylase (CYP51) was analyzed by a t test utilizing SPSS statistics 18.0 (Chicago, USA). The statistical significance was considered to be p < 0.05. GraphPad Prism 8.0 (GraphPad Prism Software Inc., San Diego, CA, USA) was used to visualize the results.

RESULTS AND DISCUSSION

The potential activity of the n-hexane fraction of Nephthea sp. was evaluated against the healthiest and purified dermatophyto-tis isolates M. canis, T. mentagrophytes, and M. gypseum.

Antifungal Results. Figure 1 shows that the n-hexane fraction of Nephthea sp. has antifungal activity against isolated dermatophytes. The highest activity against M. gypseum was observed with MIC = 83.33 ± 20.83 μg/mL, followed by M. canis with MIC = 104.2 ± 20.8 μg/mL and T. mentagrophytes with MIC = 125 ± 0.0 μg/mL.

Chemical Profile of USAP Fraction. The unsaponifiable fraction (USAP) was subjected to a GC-MS analysis, and the relative percentages of the total hydrocarbons and total

Figure 1. Antifungal activity (MIC) of Nephthea sp. against dermatophyte isolates. All results are given as means ± SE.
oxygenated compounds were 37.34% and 33.68%, respectively. The sesquiterpene widdrene was the major identified component of nonoxygenated hydrocarbons with a content of 20.24% (Table 1 and Figure 2), followed by methyl 3,5-tetradecadiynoate (13.16%). Spathulenol with a content of 7.14% is a tricyclic sesquiterpene alcohol that has a basic skeleton similar to that of the azulenes. Additionally, an acyclic diterpene alcohol, phytol, was identified with a content of 1.39% and can be used as a precursor for the manufacturing of the synthetic forms of vitamin E and vitamin K1. A polyene chromophore structure (retinal), which is considered as the active skeleton of vitamin A, was detected with a content of 1.87%.

Molecular networking based on GC-MS data was used to visualize the identical compounds having similar fragments (Figure 3). Eighteen clusters were connected to visualize 152 nodes together through 289 edges. The MN of the USAP fraction of *Nephthea* sp. revealed that widdrene (thujopsene), methyl 3,5-tetradecadiynoate, and spathulenol were the most predominant skeletons in the tested sample. The compounds were recognized on the basis of GNPS libraries and the highest matching factor (SI ≥ 700).

Table 1. Chemical Compositions of USM of *Nephthea* sp. Identified by GC-MS Analysis

| no. | Rt (min) | rRt | Kovats index | molecular formula | identified compound | molecular weight | base peak (m/z) | rel area (%) | library |
|-----|----------|-----|--------------|-------------------|---------------------|------------------|----------------|-------------|---------|
| 1   | 11.31    | 0.53| 1085         | C₇H₁₆O            | neoheptanol         | 116              | 43             | 0.05        | GNPS    |
| 2   | 13.85    | 0.65| 1162         | C₉H₂₀            | 2,2,3,4-tetramethylpentane | 128          | 57             | 0.18        | GNPS    |
| 3   | 16.28    | 0.77| 1229         | C₁₀H₂₂          | 2,4,4-trimethyl-1-hexene | 126          | 71             | 0.13        | GNPS    |
| 4   | 17.85    | 0.84| 1270         | C₁₀H₂₀O         | α-ionone            | 192              | 121            | 0.62        | GNPS    |
| 5   | 18.18    | 0.86| 1278         | C₁₀H₂₄          | 1,4-cadinadiene     | 204              | 161            | 0.19        | GNPS    |
| 6   | 18.54    | 0.88| 1287         | C₁₀H₂₄O₄        | deoxyerysicealactone | 276              | 43             | 0.31        | GNPS    |
| 7   | 19.22    | 0.91| 1305         | C₁₀H₂₄          | α-cubebene          | 204              | 161            | 0.84        | Wiley9  |
| 8   | 19.95    | 0.94| 1332         | C₁₀H₂₄          | aristolene          | 204              | 105            | 1.23        | Mainlib |
| 9   | 20.32    | 0.96| 1346         | C₁₀H₂₄          | α-muromelic         | 204              | 105            | 2.14        | Wiley9  |
| 10  | 21.04    | 1   | 1371         | C₁₀H₂₄          | widdrene (thujopsene) | 204          | 119            | 20.24       | Wiley9  |
| 11  | 21.82    | 1.03| 1398         | C₁₀H₂₄          | α-selinene          | 204              | 93             | 3.11        | Wiley9  |
| 12  | 22.36    | 1.06| 1419         | C₁₀H₂₄          | α-gurjunene         | 204              | 81             | 3.38        | Wiley9  |
| 13  | 22.87    | 1.08| 1439         | C₁₀H₂₄          | cadinene            | 204              | 161            | 3.19        | Wiley9  |
| 14  | 23.86    | 1.13| 1476         | C₁₀H₂₄O₂        | methyl 3,5-tetradecadiynoate | 234          | 91             | 13.16       | Mainlib |
| 15  | 24.03    | 1.14| 1482         | C₁₀H₂₂          | α-vitirenene        | 202              | 159            | 1.93        | Mainlib |
| 16  | 24.58    | 1.16| 1502         | C₁₀H₂₄O        | lanceol, cis        | 220              | 93             | 0.58        | Mainlib |
| 17  | 25.02    | 1.19| 1516         | C₁₀H₂₄O        | spathulenol         | 220              | 43             | 7.14        | Mainlib |
| 18  | 25.53    | 1.21| 1532         | C₁₀H₂₄O        | retinal             | 284              | 91             | 1.87        | Wiley9  |
| 19  | 30.96    | 1.47| 1700         | C₁₀H₂₄O₃       | verticellol         | 290              | 121            | 1.48        | Wiley9  |
| 20  | 31.75    | 1.50| 1728         | C₁₀H₂₄          | α-elemene           | 204              | 81             | 1.28        | Mainlib |
| 21  | 32.40    | 1.54| 1750         | C₁₀H₂₄O        | nonadecanone        | 282              | 58             | 1.37        | Wiley9  |
| 22  | 33.90    | 1.61| 1800         | C₁₀H₂₄O        | heptadecanol        | 256              | 55             | 2.74        | Mainlib |
| 23  | 34.43    | 1.63| 1819         | C₁₀H₂₄O        | phytol              | 296              | 71             | 1.39        | Mainlib |
| 24  | 35.10    | 1.66| 1843         | C₁₀H₂₄          | elemol              | 222              | 59             | 3.95        | GNPS    |
| 25  | 35.19    | 1.67| 1846         | C₁₀H₂₄          | β-elemene           | 204              | 81             | 0.04        | GNPS    |
| 26  | 35.38    | 1.68| 1853         | C₁₀H₂₄          | terpinolene         | 136              | 93             | 0.05        | GNPS    |
| 27  | 35.48    | 1.68| 1856         | C₁₀H₂₄O        | lanosterol          | 426              | 95             | 0.04        | GNPS    |
| 28  | 37.48    | 1.78| 1954         | C₁₀H₂₄O        | dotriacontyl isobutyl ether | 523          | 57             | 0.04        | GNPS    |
| 29  | 37.75    | 1.79| 1974         | C₁₀H₂₄O₄       | lobohedleolide      | 330              | 53             | 0.03        | GNPS    |
| 30  | 40.61    | 1.93| 2174         | C₁₀H₂₆O₂       | squalane            | 422              | 57             | 0.08        | GNPS    |

rel % of total identified compounds 72.87
rel % of identified hydrocarbons 69.61
rel % of identified vitamins 3.26

*aRt*: retention time relative to that of widdrene (Rt = 21.04 min)

**Figure 2.** Total ion chromatogram (TIC) for GC-MS of the unsaponifiable matter of *Nephthea* sp.
Figure 5 illustrates the molecular network constructed from the GC-MS data and visualizes the identical fragments inside the same cluster. Fifty-five nodes were connected through 148 edges and visualized in 10 clusters. The designed MN reveals that cyclohexyl propanoic acid, undecenoic acid, and arachidonic acid skeletons are the most prevalent in the tested sample (Figure 5).

Molecular Docking Analysis. Fungal CYP51 has been demonstrated to be the biochemical target for commercial fungicides; through an examination of the binding interactions of KKK (ketoconazole) to the active site of the CYP51 enzyme, it shows strong hydrogen-bond interactions with Phe77, Phe234, Gly307, Ile379, Cys449 and Met487 (Figure 6).

Table 2. Chemical compositions of FAME of Nephthea sp. identified by GC-MS Analysis

| no. | R_t (min) | R_t/R_c | Kovats index | molecular formula | identified compound | molecular weight | base peak (m/z) | rel area (%) | library |
|-----|-----------|---------|--------------|-------------------|--------------------|------------------|----------------|-------------|---------|
| 1   | 4.24      | 0.32    | 769          | C_6H_7NO_4        | xanthurenic acid   | 205              | 187           | 0.01        | GNPS    |
| 2   | 4.77      | 0.36    | 807          | C_13H_26O_2       | lauric acid        | 214              | 87            | 0.04        | GNPS    |
| 3   | 8.58      | 0.65    | 992          | C_14H_28O_2       | myristic acid      | 228              | 74            | 0.70        | Wiley9  |
| 4   | 10.76     | 0.82    | 1069         | C_11H_20O_2       | cyclopropane pentanoic acid, 2-undecyl | 310 | 43 | 0.05 | Wiley9 |
| 5   | 12.55     | 0.96    | 1123         | C_16H_30O_2       | palmitoleic acid   | 254              | 237           | 1.92        | Wiley9  |
| 6   | 13.04     | 1       | 1138         | C_12H_26O_2       | cyclohexylpropanoic acid | 156 | 74 | 43.99 | Wiley9 |
| 7   | 14.47     | 1.10    | 1179         | C_16H_32O_2       | palmitic acid      | 256              | 74            | 0.15        | Mainlib |
| 8   | 15.20     | 1.16    | 1199         | C_17H_34O_2       | margaric acid      | 270              | 73            | 0.29        | Wiley9  |
| 9   | 16.52     | 1.26    | 1236         | C_18H_38O_2       | α-eleostearic acid | 278 | 67 | 2.19 | Mainlib |
| 10  | 16.83     | 1.29    | 1244         | C_19H_36O_2       | undecenoic acid    | 198              | 55            | 29.87       | Wiley9  |
| 11  | 17.41     | 1.33    | 1259         | C_20H_40O_2       | stearic acid       | 284              | 74            | 8.15        | Wiley9  |
| 12  | 18.21     | 1.39    | 1279         | C_18H_36O_2       | oleic acid         | 282              | 73            | 0.06        | GNPS    |
| 13  | 18.71     | 1.43    | 1291         | C_18H_36O_2       | linoleic acid      | 280              | 67            | 0.13        | Mainlib |
| 14  | 20.17     | 1.54    | 1340         | C_20H_40O_2       | arachidonic acid   | 304              | 79            | 6.37        | Wiley9  |
| 15  | 20.31     | 1.55    | 1354         | C_22H_44O_2       | docosahexanoic acid | 328 | 79 | 1.05 | Mainlib |
| 16  | 21.50     | 1.64    | 1387         | C_20H_40O_2       | arachidic acid     | 312              | 74            | 0.30        | Wiley9  |
| 17  | 27.51     | 2.10    | 1591         | C_22H_44O_2       | adrenic acid       | 332              | 79            | 0.46        | Mainlib |

Rel % of saturated fatty acids 53.67
Rel % of unsaturated fatty acids 42.05
Rel % of total identified compounds 95.73
Others 0.01

"rR_c: retention time relative to cyclohexylpropanoic acid (R_t = 13.04 min)."
The docking setup was first validated by self-docking of the cocry stallized ligand (KKK) in the proximity of the binding site of the enzyme; the docking score ($S$) was $-12.6869$ kcal/mol, and the root mean square deviation (RMSD) was $2.3919$ Å (Figure 7.).

Fungal CYP51 was docked with the major detected components of GC-MS, revealing that spathulenol showed the highest binding energy score ($-8.3674$ kcal/mol) among the tested compounds, indicating a higher fitting ability, followed by cyclohexylpropanoic acid ($-8.0406$ kcal/mol) and then undecanoic acid and widdrene. However, the cyclohexylpropanoic acid derivative showed a higher number of interactions with the amino acids in the active site of the tested enzyme. The results are summarized in Table 3 and Figures 8–11.

**Table 3. Docking Results of Major Detected Components of GC-MS of Nephthea sp. on the Binding Sites of Fungal CYP51**

| $S$ (kcal/mol) | amino acids interacting group | type of interaction | length (Å) |
|----------------|------------------------------|--------------------|-----------|
| $-8.0406$ | His236 CH$_2$ (cyclohexyl) | H-bond (nonclassical) | 4.17 |
| Met378 O (C=O) | Met487 CH$_3$ | H-bond (nonclassical) | 3.60 |
| Met487 OH | Met487 CH | H-bond (nonclassical) | 4.38 |
| Met487 CH$_2$ | Met487 CH | H-bond (nonclassical) | 4.39 |
| Met487 CH$_3$ | Met487 CH | H-bond (nonclassical) | 4.17 |

Inhibition of Fungal CYP51 Enzyme. One-third of the agrochemical fungicides used are azole drugs that target inhibition of the CYP51 enzyme, which belongs to the cytochrome P450 monooxygenase (CYP) superfamily. CYP51 enzyme is considered a critical step in the synthesis of ergosterol, that is fungal-specific sterol. The therapeutic azole antifungal compounds emerged in orally administrated forms during the 1980s, first with ketoconazole and then later with fluconazole and itraconazole. These drugs are used extensively due to the widespread incidence of fungal infections associated with AIDS but also are associated with cancer chemotherapy and organ transplantation and are used in the intensive care unit. Thus, more detailed information on the activity of CYP51 inhibitors is important toxicologically so that further applications may emerge. The most popular antifungal agent that inhibits the biosynthesis of lanosterol 14α demethylase (CYP51) and ergosterol in the fungal cell membrane is the drug fluconazole, which was used in this study as a reference drug (ST) with $IC_{50} = 2.27 \pm 0.05 \mu$g/mL. In addition, the $n$-hexane fraction of *Nephthea* sp. showed inhibitory activity against CYP51 with $IC_{50} = 12.23 \pm 0.29 \mu$g/mL (Figure 12).
Moreover, this is the first report of the antifungal activity of the n-hexane fraction of soft coral Nephthea sp. using a broth microdilution method and a Candida albicans CYP51 plate reader assay. Undoubtedly, there is a relationship between the antifungal activity of the investigated fraction and its main components.

In the present study, the major chemical constituents of the unsaponifiable fraction are sesquiterpene, widdrene (thujopsene), and the tricyclic sesquiterpene alcohol spathulenol, which have been previously reported to have high antimicrobial activities.\(^9\)\(^,\)\(^10\) In addition, several fatty acids (methylated and hydroxylated fatty acids) and their derivatives were identified from the FAME fraction that previously exhibited antifungal activity targeting the cell membrane. They cause leakage of intracellular components and cell death through an increase in membrane fluidity.\(^31\)

In previous studies, widdrene (thujopsene) was a volatile component of the heartwood extract of the cedar Callitropsis nootkatensis with activity against Phytophthora ramorum\(^12\) and showed potent antifungal activity at low concentrations (0.1%, 1%, and 10%), against 16 fungal strains, particularly against Gonytrichum macrocladum (GMB), Eurotium herbariorum (EHA), and Penicillium decumbens (PDT) using a disk diffusion method.\(^43\) Likewise, it showed antibacterial activity against several strains of Cryptococcus neoformans.\(^14\) Additionally, Ashe juniper showed significant antifungal activity against four species of wood-rot fungi, due to its high content of thujopsene (over 30%).\(^57\) There are few data concerning the effect of thujopsene on dermatophytes. This may be the first study concerning the effect of this compound on dermatophytes. The previous findings suggested that the content of widdrene (thujopsene) might explain its vital role in antidermatophytosis.

In addition, essential oils showed antifungal activity against dermatophytes and Candida spp.\(^46\)\(^–\)\(^49\) For instance, the essential oil of Croton argyrophylloides showed antifungal activity against M. canis due to its contents of spathulenol and bicyclogermacrene through a synergistic effect.\(^50\) Furthermore, the extracts and fractions of Jatropha neopauciflora (Pax) were also shown to have antifungal activity, particularly against Trichophyton mentagrophytes, due to its major contents of β-sitosterol, spathulenol, coniferyl alcohol, and lupeol.\(^51\) These findings exhibited the roles of terpene, sterol, and phenylpropanoid activities.

Methyl 3,5-tetradecadiynoate is a methylated fatty acid detected in the USAP of Nephthea sp. as a major component. It was previously reported that 12-methyltetradecanoic (12-Me 14:0) acid inhibits the formation of appressorium in the rice pathogen Magnaporthe oryzae.\(^52\) However, its mechanism of action is still unknown and needs further investigation.

Garg in 1993 showed that saturated fatty acids having short chains ranging from C7 to C11 are more toxic to skin fungi in comparison to the corresponding long chains of >12. As well as odd-numbered carbon, chain fatty acids are slightly more toxic than their corresponding even-numbered one carbon-less fatty acid. Polyunsaturated fatty acids were found to be more toxic than their corresponding even-numbered one carbon-less fatty acid.\(^3\)\(^,\)\(^4\) This conclusion is in agreement with our present study, which stated that cyclohexylpropanoic acid C3:0 and undecenoic acid (11:1) may be responsible for the antifungal activity against selected dermatophytes.

In addition, Garnier et al. in 2020 showed that propanoic (propionic) and acetic acids were the most abundant

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**DISCUSSION**

Most dermatophyte infections are not life threatening and respond well to currently available topical treatment with over the counter (OTC) fungal agents. However, some dermatophyte infections require complex treatment regimens and are more resistant to traditional antifungal therapies.\(^3\)\(^8\) In addition, the emergence of the resistance of microbes, including fungi and yeasts, toward the available antimicrobial agents requires a search for other antimicrobials.
fermentation products for Propionibacterium jensenii and were shown to exhibit promising antifungal activities in dairy products. However, propionic acid was only quantified at high levels in the P. jensenii fermentate 322 ($9.94 \pm 21.28$ mg/g). The antifungal activity of propanoic acid (propionic acid) has been previously reported in the literature.

Moreover, unsaturated fatty acids such as undecenoic acid (11:1), which contains a fixed bond, were identified; when undecenoic acid is inserted into the membrane, it increases motional freedom inside the membrane and increases oxidative stress, encouraging its fungicidal activity. 

Also, undecenoic acid is used for the production of the bioplastic nylon-11, which is used in the treatment of fungal infections of the skin. Undecenoic acid (C11:1) is a short-chain unsaturated fatty acid and is more toxic to dermatophytes than long-chain fatty acids (>C 12:0); it completely inhibited the growth of species such as T. mentagrophytes, T. mentagrophytes var. interdigitale, T. rubrum, M. canis and M. gypseum at <0.5 mM, suggesting the highest activity of this fatty acid in the range of C7–C13 series. Likewise, it has been previously used in treating tinea pedis produced by T. mentagrophytes and T. rubrum. In addition, it has been utilized in curing dermatomycosis caused by T. rubrum, Epidermophyton inguinale and M. audouinii (Carolina et al., 2011). Moreover, onychomycosis is caused by T. rubrum. Additionally, McDonough et al. in 2002 found that the medium-chain fatty acids (MCFAs) undecanoic acid (11:0), 10-undecenoic acid (11:1 Delta 10), and lauric acid (12:0) can affect the growth of Saccharomyces cerevisiae in a dose-dependent manner. This study is a companion to that of Ells and co-workers, who in 2009 demonstrated that the polyunsaturated fatty acid arachidonic acid (20:4) may affect the growth of fungal CYP51. The Global Natural Products Social Molecular Networking rostrum and a molecular docking study predicted spathulenol efficacy against the CYP51 enzyme that might be responsible for antifungal activity, which was then confirmed in vitro by the inhibitory effect of Nephthea sp. against CYP51. Further clinical studies will support these findings and explore the detailed mechanism of action.

### CONCLUSION

In this study, we investigated the unsaponifiable and saponifiable materials of Nephthea sp. by GC-MS that could be helpful in the authentication of marine soft coral. This is the first documentation of molecular networks toward the lipoidal matter by GC-MS analysis in addition to the promising antidermatophyte activity of Nephthea species against M. gypseum, M. canis, and T. mentagrophytes through inhibition of fungal CYP51. The Global Natural Products Social Molecular Networking rostrum and a molecular docking study predicted spathulenol efficacy against the CYP51 enzyme that might be responsible for antifungal activity, which was then confirmed in vitro by the inhibitory effect of Nephthea sp. against CYP51. Further clinical studies will support these findings and explore the detailed mechanism of action.

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**Notes**

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