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Phytochemical screening and antioxidant and cytotoxic activities of ethyl acetate subfractions of soft coral *Nepthea* sp. growing in Southeast Sulawesi

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ABSTRACT

Nepthea sp. is a soft coral that grows abundantly in the seas of Southeast Sulawesi, Indonesia. However, there is no information available regarding its pharmacological or chemical characteristics. As a result, the goal of this research was to uncover the chemical profile of the *Nepthea* sp. ethyl acetate subfractions, as well as their antioxidant and anticancer potential. The sample was extracted with ethyl acetate and then fractionated using vacuum liquid chromatography with Si-gel as an adsorbent and a chosen solvent as an eluent. Phytochemical tests, Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy (LC-MS/MS), and total phenolic content were used to determine the chemical content. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals were used to test the antioxidant potency, whereas MCF-7 cell lines were used in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide experiment to evaluate cytotoxicity. The fractionation of the ethyl acetate extract (160 g) produced six subfractions: Fractions A (35.2 g), B (4.3 g), C (5.9 g), D (10.7 g), E (26.5 g), and F (15.4 g). According to the DPPH and ABTS results, fraction E has the highest antioxidant potency (IC_{50} = 67.39 ± 1.56 and 54.12 ± 0.95 mgl^{-1}, respectively), and fraction C has the highest anticancer activity (IC_{50} = 72.82 ± 1.30 mgl^{-1}). Fraction C components include 3-acetyl-3,4-dihydro-5,6-dimethoxy-2(1)H-benzopyrone, oxyphyllenone B, and unidentified chemicals, according to LC-MS/MS data ($C_{15}H_{21}NO$, $C_{21}H_{33}NO_2$, $C_{15}H_{23}NO_3$, $C_{15}H_{21}NO_2$, $C_{15}H_{21}NO_3$, and $C_{45}H_{84}O_{14}$). Rengyolester, piperolactam-C9:1(8E), valine, and unidentified chemicals ($C_{52}H_{79}N_3O$, $C_{32}H_{51}NO_7$) make up fraction E. As a result, the ethyl acetate extract and its subfractions from *Nepthea* sp., especially fractions C and E, can be used as a source of raw materials for anticancer agents and antioxidants, respectively.

INTRODUCTION

Soft coral, specifically *Nepthea* sp. from Southeast Sulawesi, Indonesia, was chosen as the sample to continue our research on the chemical and pharmacological aspects of marine natural resources. We had previously worked with sponges, and through the process of isolation and structural determination, we were able to get a new compound from *Clathria* sp., called clathruohate (Sahidin *et al*., 2018), as well as the chemical screening of various sponges (Sahidin *et al*., 2020). Antihyperlipidemic properties (Wahyuni *et al*., 2019), anti-inflammatory properties (Fristiohady *et al*., 2019), antioxidant properties, and acute toxicity (Fristiohady *et al*., 2020) are some of the biological activities of the sponges growing in Southeast Sulawesi.

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The Indo-Pacific region (Seah *et al*., 2015), the Mauritius and Rodrigues Island (Jahajeeah *et al*., 2021), and the Brazilian Coast (Almeida *et al*., 2014) are all thought to be home to *Nepthea* sp. (Nephtheidae). According to the study, a derivate of tetraprenyl-benzoquinone generated by *Nepthea* sp. and a guaiane-based sesquiterpene discovered from *Nepthea chabroli* (Almeida *et al*., 2014), a molecule from *Nepthea* sp., called nephtoacetal, is cytotoxic to the HeLa cell lines (Zhang *et al*., 2013). Furthermore, erectasteroids A–H from *Nepthea erecta* are active against the HT-29 and P-388 cell lines (Cheng *et al*., 2007), and nebrosteroids A–H from *N. chabroli* have anti-inflammation activity (Huang *et al*., 2008).

However, no research on the chemical and pharmacological features of *Nepthea* growing in Southeast Sulawesi has been published yet. Soft corals whose studies have been reported from around the Sulawesi island include the following: soft coral from the South China Sea, *Sarcophyton solidum*, which produces diterpenoids (Zhu *et al*., 2015), *Sinularia depressa* from the South China Sea which generates sinulasterols A–C that play a role in cancer prevention through anti-inflammatory actions (Yang *et al*., 2020), and *Lobophytum* sp. from Selayar, South Sulawesi, the ethyl acetate extracts of which are active as antibacterials and antioxidants (Putra *et al*., 2016). Meanwhile, the mapping phase of a soft coral project in Southeast Sulawesi is still ongoing (Pedoja *et al*., 2018; Wanda *et al*., 2018).

This article describes the cytotoxicity and antioxidant properties of the ethyl acetate extract and its subfractions of soft coral *Nepthea* sp., as well as a chemical study of those samples using phytochemical screening, LC-MS/MS analysis, and total phenolic content (TPC) to learn more about soft corals from Southeast Sulawesi.

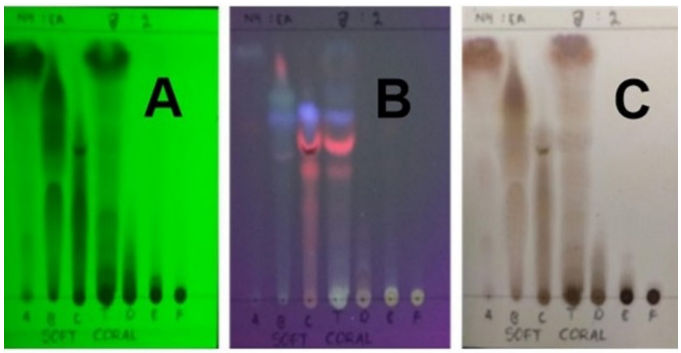

Figure 1. TLC chromatogram of ethylacetate extract (T) and the fraction A–F. (A) λ 254 nm (short); (B) λ 366 nm (long); and (C) $CeSO_4$ + heat.

MATERIALS AND METHODS

General procedures

Methanol, ethyl acetate, n-hexane, Aquades, and acetone were all analytical grade compounds. The other materials used were Kieselgel 60 F_{254} 0.25 mm (Merck), Si-gel 60 GF_{254} p.a (Merck®), silica 60 G (Merck®), cerium sulfate ($CeSO_4$) (Merck®), ascorbic acid (Merck®), gallic acid (Merck®), quercetin (Merck®), doxorubicin (Merck®), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The LC-MS/MS study used Waters ACQUITY UPLC I–Class together with the Xevo G2-X2 Quadrupole Time-of-Flight Mass Spectrometer.

Collection of *Nepthea* sp.

Nepthea sp. was taken on the reef slopes of the Saponda Islands in Indonesia's Province of Southeast Sulawesi. Scuba diving at a depth of 4–10 m was used to collect the sample. The material was collected and put in separate ice containers before being returned to the laboratory for further analysis. Soft coral identification was made by the specialist staff of Universitas Halu Oleo's Faculty of Fisheries and Marine Science (Baru Sadarun, Ph.D.), and the specimen was placed at the Faculty of Pharmacy.

Fractionation and extraction

Fresh samples of *Nepthea* sp. (3 kg) were extracted with ethyl acetate at room temperature (3 × 10 l, 24 hours each time), and a dark brown extract was produced after being compressed under lower pressure. The extract was fractionated using vacuum liquid chromatography with Si-gel as an adsorbent and a combination of n-hexane: ethyl acetate (polarity rising) and 100% of methanol as eluent, yielding six fractions (A–F).

Screening of phytochemical contents

Screening of secondary metabolites in all the samples was done using the Harborne methods (Sadarun *et al*., 2022).

Total phenolic content

Total phenolic compounds were determined using the Folin–Ciocalteu reagent with slight adjustments to Singleton and Rossi's description (Chandra *et al*., 2014).

Table 1. Weight and chemical profiles the samples.

	Ethyl acetate extract	Fraction					
		A	B	C	D	E	F
Weight (g)	160.0	35.2	4.3	5.9	10.7	26.5	15.4
Flavonoids	-	-	-	-	-	-	-
Saponins	+	-	-	+	+	+	+
Tannins/phenolics	+	-	-	+	+	+	+
Alkaloids	+	-	-	+	+	+	+
Terpenoids	+	+	+	+	+	+	-
TPC (mgGAE/g Ex) $y = 0.0063x + 0.1284$ $R^2 = 0.9885$	23.9 ± 0.87	0	0	2.69 ± 0.42	36.2 ± 1.25	73.11 ± 1.86	68.77 ± 2.03

LC-MS/MS analysis

The LC-MS/MS standard operating procedure was prepared for the chemical identification of the soft coral *Nepthea* sp. ethyl acetate extract and its subfractions (A–F). The UNIFI software was used to identify the mass-to-charge ratio (m/z) values of all peaks obtained from the LC-MS/MS analysis using the MSE identification method. The chemicals found were thoroughly examined using the Dictionary of Marine Natural Products (Blunt and Munro, 2008).

Antioxidant and cytotoxic activities

The antioxidant activity of those samples was measured by the DPPH radical (Sahidin *et al*., 2020) and the ABTS method (Wahyuni *et al*., 2021). The cytotoxicity property was evaluated toward the MCF-7 cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay *in vitro* (Asasutjarit *et al*., 2021).

RESULTS AND DISCUSSION

The study of the chemical and pharmaceutical aspects of soft coral *Nepthea* sp. (3 kg) from the Southeast Sulawesi Sea, starting with sample extraction using ethyl acetate, yielded 160 g of the extract. The ethyl acetate extract of *Nepthea* sp. was fractionated into six fractions (A–F). The compounds profile of each fraction was analyzed using thin-layer chromatography (TLC), which is shown in Figure 1.

The chemical contents in the ethyl acetate extract and fractions A–F of *Nepthea* sp. are quite different, as shown by the TLC chromatogram in Figure 1. Qualitatively, terpenoids, alkaloids, and phenolics are compounds produced by *Nepthea* sp. based on phytochemical screening. Flavonoids are extremely rare and nearly undetectable in this sample. Fractions A and B do not contain phenolic compounds. Detailed data on the weight and chemical profile of samples are presented in Table 1.

Fraction C has the best cytotoxic potential against MCF-7 breast cancer cells lines compared to other samples with IC_{50} 72.82 ± 1.30 mgl^{-1}, and fraction E had the most antioxidant potential compared to other samples with IC_{50} 67.39 ± 1.56 mgl^{-1} (DPPH) and IC_{50} 54.12 ± 0.95 mgl^{-1} (ABTS) (Table 2 and Fig. 2). The difference in the biological activity of each fraction is strongly influenced by the content of its secondary metabolites.

Fractions C and E were studied in depth because they demonstrated the greatest promise for anticancer and antioxidant properties, respectively. Table 3 presents the chemicals identified and unidentified in the ethyl acetate extract, fractions C and F, of *Nepthea* sp. based on LC-MS/MS data. In addition, Figure 3 shows the identified structure of the identified compounds.

Figure 3 shows that the majority of the compounds found in *Nepthea* sp. are terpenoids, particularly sesquiterpenes and diterpenes, lactone, and nitrogenous compounds like **8** and **9**. The number of terpenoids detected in *Nepthea* sp. is comparable to those found in other soft corals, such as sarcophine (Saleh *et al*., 2020), terpenoids from *Lobophytum crassum*, and steroids from *Sarcophyton pauciplicatum* (Florean *et al*., 2020; Saleh *et al*., 2020). The majority of the unidentified molecules are nitrogen compounds, and alkaloids are assumed to represent a type of secondary metabolites that include nitrogen atoms.

Table 2. Biological properties of all samples.

Assays		Ethyl acetate extract	Fraction						Standard (positive control)	
			A	B	C	D	E	F	Ascorbic acid	Doxo rubicin
DPPH	Regression equation	$y = 0.0996x + 8.9634$	$y = 0.1118x + 3.1098$	$y = 0.1382x + 3.9431$	$y = 0.1972x + 9.2886$	$y = 0.2927x + 18.902$	$y = 0.561x + 12.195$	$y = 0.5325x - 3.8399$	$y = 0.7467x + 42.85$	
		$R^2 = 0.9889$	$R^2 = 0.9256$	$R^2 = 0.9383$	$R^2 = 0.9023$	$R^2 = 0.9985$	$R^2 = 0.9972$	$R^2 = 0.9593$	$R^2 = 0.9857$	
	IC_{50} in $mg.l^{-1}$	412.01 ± 5.78	419.41 ± 4.22	333.26 ± 2.41	206.45 ± 3.12	106.25 ± 2.13	67.39 ± 1.56	101.1 ± 1.83	9.58 ± 0.57	
ABTS	Regression equation	$y = 0.0813x + 3.1465$	$y = 0.1226x + 2.338$	$y = 0.1307x + 22.3115$	$y = 0.3438x - 2.673$	$y = 0.6747x - 2.1664$	$y = 0.7921x + 7.1358$	$y = 0.5888x - 2.8482$	$y = 1.1854x + 39.518$	
		$R^2 = 0.9848$	$R^2 = 0.958$	$R^2 = 0.9741$	$R^2 = 0.9927$	$R^2 = 0.9839$	$R^2 = 0.9977$	$R^2 = 0.9994$	$R^2 = 0.9915$	
	IC_{50} in $mg.l^{-1}$	576.30 ± 3.62	388.76 ± 2.64	364.87 ± 2.74	153.21 ± 2.95	77.32 ± 1.24	54.12 ± 0.95	89.76 ± 1.12	8.84 ± 0.69	
MTT (MCF-7)	Regression equation	$y = 0.1668x - 12.545$	$y = 0.1487x + 33.9687$	$y = 0.0443x + 8.6699$	$y = 0.1446x + 39.4702$	$y = 0.0401x + 3.1385$	$y = 0.0715x + 19.0155$	$y = 0.1038x + 32.2793$		$y = 6.4961x + 27.9133$
		$R^2 = 0.9184$	$R^2 = 0.9049$	$R^2 = 0.918$	$R^2 = 0.9163$	$R^2 = 0.9564$	$R^2 = 0.917$	$R^2 = 0.9188$		$R^2 = 0.9796$
	IC_{50} in $mg.l^{-1}$	374.97 ± 31.80	107.81 ± 6.40	932.96 ± 58.63	72.82 ± 1.30	> 1,000	433.35 ± 25.83	170.72 ± 14.12	3.40 ± 0.25	

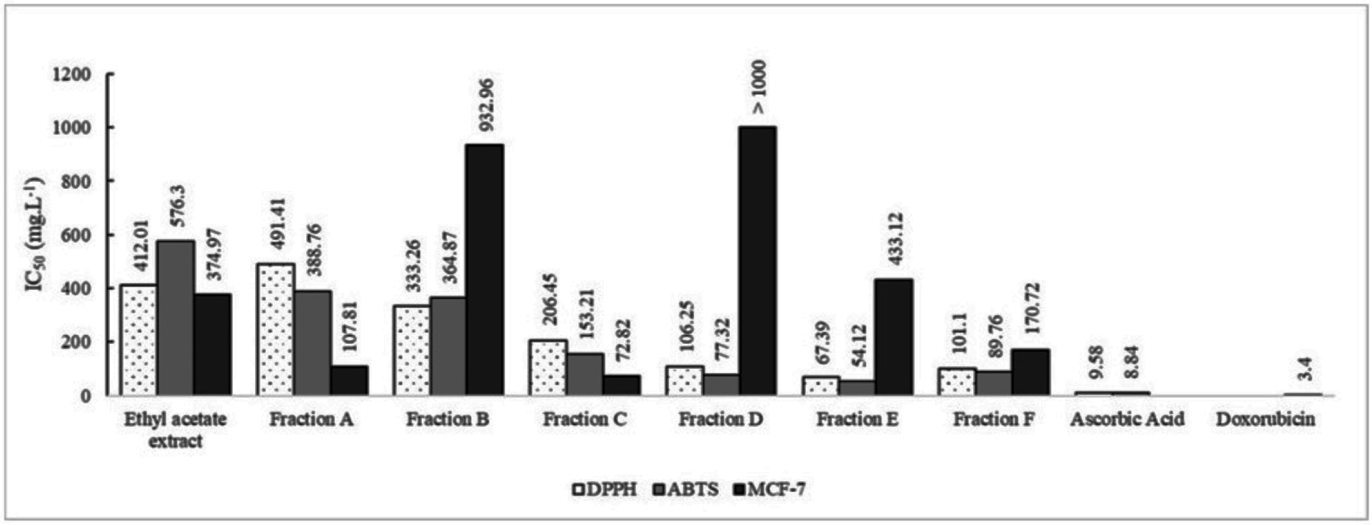

Figure 2. Bwwiological activities of the samples.

Table 3. Chemical profile of ethylacetat extract, fraction C and F based on LC-MS/MS data.

No	Rt (minute)	Observed m/z	Neutral mass	Formula	Compound name	Ethyl acetate extract	Fraction C	Fraction E
1	10.42	293.1745	292.16746	$C_{17}H_{24}O_4$	2a-Acetoxycostic acid	√	-	-
2	10.04	263.1277	262.12051	$C_{15}H_{18}O_4$	Artemisin	√	-	-
3	8.04	197.1169	196.10994	$C_{11}H_{16}O_3$	Digiprolactone	√	-	-
4	10.68	219.1740	218.16707	$C_{15}H_{22}O$	Nootkatone	√	-	-
5	10.22	295.1538	294.14672	$C_{16}H_{22}O_5$	Rengyolester	√	-	√
6	9.74	251.0893	250.08214	$C_{13}H_{14}O_5$	3-Acetyl-3,4-dihudro-5,6-dimethoxy-2(1) H-benzopyrone	-	√	-
7	10.23	233.1142	210.12559	$C_{12}H_{18}O_3$	Oxyphyllenone B	-	√	-
8	10.31	352.1909	329.19909	$C_{20}H_{27}NO_3$	Piperolactam-C9:1(8E),	-	-	√
9	1.86	118.0858	117.07898	$C_5H_{11}NO_2$	Valine	-	-	√
10	10.35	232.2585	231.25113	$C_{15}H_{21}NO$	Candidate mass	√	√	-
11	10.52	3325852.	331.25113	$C_{21}H_{33}NO_2$	Candidate mass	√	√	-
12	9.22	311.1490	31014164	$C_{16}H_{22}O_6$	Candidate mass	√	-	-
13	8.34	266.1748	265.16779	$C_{15}H_{23}NO_3$	Candidate mass	√	√	-
14	13,07	871.5738	870.56458	$C_{54}H_{78}O_9$	Candidate mass	√	-	-
15	8.34	232.1694	231.16231	$C_{15}H_{21}NO_2$	Candidate mass	-	√	-
16	9.24	264.1595	263.15214	$C_{15}H_{21}NO_3$	Candidate mass	-	√	-
17	8.29	871.5753	848.58611	$C_{45}H_{84}O_{14}$	Candidate mass	-	√	-
18	10.15	800.6053	777.61723	$C_{52}H_{79}N_3O_2$	Candidate mass	-	-	√
19	9.94	562.3747	561.36655	$C_{32}H_{51}NO_7$	Candidate mass	-	-	√

When compared to other samples, the biological activity of fraction C revealed that it had the strongest cytotoxic capability against MCF-7 breast cancer cells lines (Table 2 and Fig. 2). Based on the cytotoxicity level (Sadarun *et al*., 2022), the extract is categorized as *very active* (IC_{50} < 10 mg.ml^{-1}), *active* (10–100 mg.ml^{-1}), and *moderately active* (100–500 mg.ml^{-1}). Thus, the ethyl acetate extract and fraction E were categorized as moderately active, while fraction C was categorized as active against MCF-7 breast cancer cells lines. The component concentration of fraction C, which is primarily nitrogen compounds, is assumed to be the cause (Table 3). Doxorubicin, a nitrogen molecule with the chemical formula $C_{27}H_{29}NO_{11}$, is used as a positive control. Tamoxifen is a nitrogen molecule with the chemical formula $C_{26}H_{29}NO$ or 2-[4-[(Z)-1,2-diphenylbut-1-enyl]phenoxy]-N,N-

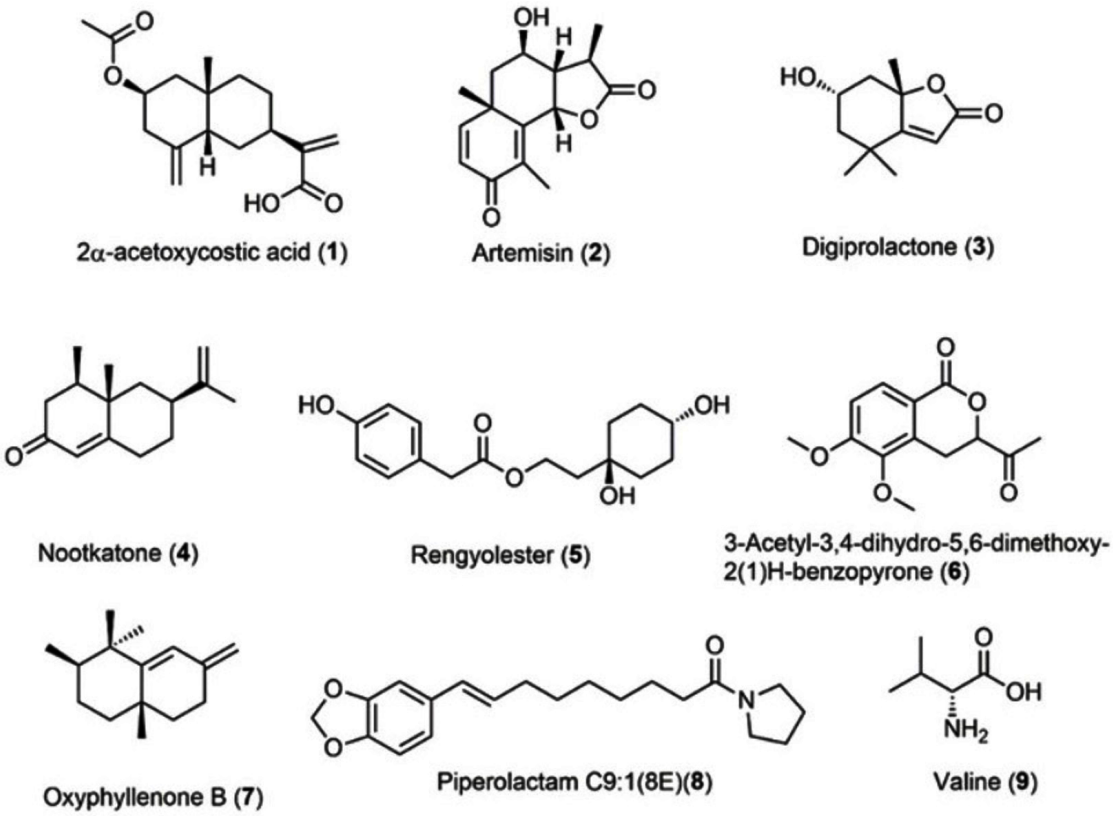

Figure 3. Identified compound structures of ethyl acetate extract, fraction C and E.

dimethylethanamine, which is often used by breast cancer patients. Tamoxifen works by preventing estrogen from acting on the breast. The hormone estrogen is required for the development of several kinds of breast cancer. This mechanism of action can be employed in both the treatment and prevention of breast cancer in high-risk women (Thayyeb *et al*., 2020).

In comparison to the other samples, fraction E had a maximum level of antioxidant capacity. The IC_{50} value of the DPPH and ABTS test findings is demonstrated in Table 2, and Figure 2 shows the classification of the antioxidant potential proposed by Blois, where Blois distinguishes it into four levels, namely *very strong* if $IC_{50} < 50$ mgml^{-1}, *strong* if IC_{50} is between 50 and 100 _etl^{-1}, *moderate* if IC_{50} is between 100 and 150 _etl^{-1}, and *weak* if IC_{50} is more than 150 _orl^{-1} (Nur *et al*., 2021). According to the Blois criteria, the ethyl acetate extract and fraction C were categorized as weak antioxidants, while fraction E was included in the category of strong antioxidants. The quantity of phenolic compounds in fraction E influenced the strength of the antioxidant activity of the fraction quantitatively. The TPC value of fraction E was the greatest compared to the other samples, and the presence of flavonoids was detected in fraction E as well, as evidenced by the TFC value (Table 1). In terms of quality, phenolic compounds have conjugated double bonds with hydroxyl groups, allowing them to neutralize free radicals via resonance (Sahidin *et al*., 2014). Vitamin C (ascorbic acid), for example, has four hydroxyl groups, a conjugated double bond with a carbonyl unit, and an ester group, making it an excellent antioxidant substance. Rengyolester as the owner of this condition has a phenolic unit, an ester group, and two hydroxyl units that make up this chemical. Fraction E is regarded to have greater antioxidant effects than other samples due to the presence of this molecule. The ethyl acetate extract had rengyolester as well; however, fraction E had a larger mole fraction of rengyolester than the ethyl acetate extract.

CONCLUSION

The diversity of chemicals and biological activities of soft coral *Nepthea* sp. was clearly observed after fractionation. Fractionation of the ethyl acetate extract of soft coral *Nepthea* sp. produced six fractions (fractions A–F). Fraction C revealed that it had the strongest cytotoxic capability against MCF-7 breast cancer cells lines compared to other samples, which has an active category that is thought to be due to the high amount of nitrogenous chemicals. Meanwhile, the most antioxidant potential was shown by fraction E, which has a strong category that is caused by the content of phenolic compounds. As a result, the ethyl acetate extract and its subfraction from *Nepthea* sp., especially fractions C and E, can be used as a source of raw materials for anticancer agents and antioxidants, respectively.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest in this study.

AUTHORS' CONTRIBUTIONS

The idea was developed by I. Sahidin, B. Sadarun, and A. Fristiohady. Sample collection was arranged by B. Sadarun and A. W. M. Yodha. I. Sahidin, B. Sadarun, and A. W. M. Yodha worked on sample preparation, extraction, and fractionation. Evaluations of biological activities were done by A. Fristiohady and N. S. Rahmatika. A. Sundowo and I. Sahidin worked on the LC-MS/MS experiment and interpretation of the data. I. Sahidin, B. Sadarun, and A. Fristiohady contributed to manuscript preparation and revision.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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