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RESEARCH ARTICLE

Screening of NO Inhibitor Release Activity from Soft Coral Extracts Origin Palu Bay, Central Sulawesi, Indonesia

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Abstract: ***Background*:** As a marine organism, soft corals can be utilized to be various bioactive substances, especially terpenoids and steroids. The soft corals family which produces bioactive generally come from clavulariidae, alcyoniidae, nephtheidae and xeniidae family.

Objective: To investigate the bioactivity of Nitric Oxide (NO) inhibitor release from soft coral crude extracts of *Sinularia* sp. (SCA), *Nephthea* sp. (SCB), *Sarcophyton* sp. (SCC), *Sarcophyton* sp. (SCD), *Sinularia* sp. (SCE) and *Sinularia* sp. (SCF).

***Materials and Methods*:** Soft coral is collected from Palu Bay (Central Sulawesi). NO inhibitory release activity measured according to the Griess reaction. Soft corals sample macerated with 1:2 (w/v). Then, Soft coral extracts with the best NO Inhibitor activity partitioned with Dichloromethane, Ethyl acetate, and n-butanol. The bioactive of all crude extracts were identified by GC-MS to find compounds with anti-inflammatory potential.

***Results*:** *Sarcophyton* sp. (SCC) and *Sinularia* sp. (SCF) able to inhibit NO concentrations of 0.22 ± 0.04 and 0.20 ± 0.04 μM at 20 mg/mL, respectively. The chemical constituents determined and showed the potential as anti-inflammatory in the crude of *Sinularia* sp. (SCA) were Octacosane (3.25%). In *Nephthea* sp., (SCB) were Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-,(S)- (0.55%); Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)- (0.53%); and 1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene (4.72%). In *Sarcophyton* sp, (SCC) were Eicosane (0.12%); Nonacosane (10.7%); 14(β)-Pregnane (0.87%); Octacosane 6.39%); and Tricosane (1.53%). In *Sarcophyton* sp. (SCD) were 14(β)-Pregnane (2.69%); and Octadecane (27.43%). In crude of *Sinularia* sp. (SCE) were Oleic Acid (0.63%); 7,10-Hexadecadienoic acid, methyl ester (0.54%); 14(β)-Pregnane (1.07%); 5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)- (4.60%); Octacosane (7.75%); and 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide (1.23%). In the crude of *Sinularia* sp., (SCF) were Oxirane, decyl- (1.38%); Nonacosane (0.57%); Cyclohexanol, 5-methyl-2-(1-methylethenyl)- (0.61%); 14B-Pregnane (0.76%); and Tetratriacontane (1.02%).

***Conclusion*:** The extract of *Sarcophyton* sp. (SCC) and *Sinularia* sp. (SCF) showed the best NO inhibitory release activity. This study is making soft corals from Central Sulawesi, Indonesia can become a potential organism in the discovery and development of bioactive substances anti-inflammatory.

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1. INTRODUCTION

Indonesian marine organisms are so diverse and have potential as bioactive producer. Soft corals are well-known as various bioactive substances source, especially terpenoids and steroids with exceptional functionality [1-6]. Soft corals are included in cnidarian phylum, anthozoa class, octocorallia subclass and 60% of bioactive substances reportedly have the potential to produce drug compounds [7-9].

Soft corals are soft-bodied sessile invertebrates, having no physical defense systems that rely on chemical defense system to survive [2]. The percentage of drug raw materials from soft coral found at 11-17% [10]. Soft corals become a source of biologically active molecules and model compounds used in drug material [11, 12].

The soft corals family which produces bioactive generally come from Clavulariidae, Alcyoniidae, Nephtheidae, Xeniidae family. Family Alcyoniidae most reported as bioactive producer [13]. Soft corals were reported to produce a variety of unique bioactive substances, including sesquiterpenoid, diterpenoid and steroids compounds [14]. The bioactive substances isolated from soft corals had anti-inflammatory activity [15, 16].

The anti-inflammatory properties often isolated and applied to the treatment of fever, pain and inflammatory conditions [17]. From the literature, it was reported that soft corals produce potentially anti-inflammatory compounds by inhibiting and reducing the expression of iNOS protein [18-25]. iNOS (inducible nitric oxide synthase) is one of the pro-inflammatory proteins, which can affect the development of acute to be chronic inflammatory responses and is closely related to the development of chronic human disease, including Alzheimer's [26], atherosclerosis, arthritis [27], diabetes [28], inflammatory bowel disease [29], and cancer [30].

The production of nitric oxide (NO) by iNOS involves in the pathogenesis of inflammatory response [31]. NO concentration by iNOS expressed in macrophage cells is related to phagocytic activity [32]. The inhibitor of NO release in macrophages is one way to study the potential of anti-inflammatory agents. Increased levels of NO in chronic inflammatory affect various palogical conditions [33]. The activity and expression of the iNOS enzyme are related to the production of NO. Therefore, one way of developing anti-inflammatory agents is by controlling the levels of NO in macrophages [34].

The oceans of eastern Indonesia hold great potential in the marine organism biodiversity [35]. The great potential of the marine organism is theoretically also a potential for the discovery of bioactive substances from the marine organism [36]. However, there is not so many exploration of soft coral potential bioactive substances. Only 19 new compounds were isolated from soft coral and ascidians and have the potential pharmacological [37]. Based on the description above, this research examines NO-Inhibitor of six soft corals origin Palu Bay, Central Sulawesi, Eastern Indonesia. NO inhibitory release potency was measured by observing the inhibition of LPS-induced NO release. The aim of this research is to investigate the NO inhibitor release of soft corals crude extract *Sinularia* sp. (SCA), *Nephthea* sp. (SCB), *Sarcophyton* sp. (SCC), *Sarcophyton* sp. (SCD), *Sinularia* sp. (SCE) and *Sinularia* sp. (SCF) from Central Sulawesi, Indonesia, which is one oceans with high biodiversity.

2. MATERIALS AND METHODS

2.1. Chemical and Reagents

Dichloromethane (DCM with p.a grade and purity of purchased from Merck), methanol (MeOH p.a, Merck), Ethyl acetate (EtOAc p.a, Merck), n-butanol (BuOH p.a, Merck), dimethyl sulfoxide p.a, (Merck), Dulbecco's modified Eagle's medium (DMEM/F12) powder Gibco Life Technologies, 10% Fetal Bovine Serum (FBS), 1% penisilin-streptomicin, Lipopolysaccharide LPS *E. coli* O111:B4 (List Biological Laboratory, Inc.), Griess Reagent Kit for Nitrite Determination G-7921 (Thermo Fisher Scientific).

2.2. Animal Materials

Soft coral samples were collected from the coastal of Kabonga Besar Village, Donggala District, Palu Bay, Central Sulawesi, Eastern Indonesia at coordinates 43.31 South Latitude and 119.46 East Longitude in December, 2016. Sampling was

done with equipment SCUBA at a depth of 3-5 m. Each soft coral sample was rinsed with seawater and immediately stored in ice. After arriving at the laboratory, samples of soft corals were cut into smaller sizes, put into containers and stored in a freezer immediately. This study was approved by Animal care and used committee with ethical clearance number 680-KEP-UB.

2.3. Extraction

A total of 350 g (wet weight) sample was macerated 1:2 w/v with methanol: dichloromethane (1: 1) for 48 hours [18, 38, 39]. Sample was filtered, evaporated (Rotary Vacuum Evaporator EYELA N-1100), dried, weighed and divided into several parts: SCA (4.08 g), SCB (5.21 g), SCC (2.99 g), SCD (8.59 g), SCE (7.12 g), and SCF (5.58 g).

The maceration was performed three times each of the soft coral samples. Soft coral extracts with the best NO inhibitor activity were partitioned with Dichloromethane, Ethyl acetate, and n-butanol for 24 hours. Then, evaporated and weighed, so obtained SCC DCM (0.93 g), SCC EtOAc (0.08 g), SCC BuOH (0.06 g), SCE DCM (2.32 g), SCE EtOAc (0.27 g), SCE BuOH (0.18 g), SCF DCM (1.64 g), SCF EtOAc (0.14 g), SCF BuOH (0.09 g). Each crude extracts were subjected to preliminary phytochemical screening and assays for NO inhibitory activity.

2.4. Phytochemical Screening

All crude extracts were subjected to initial phytochemical screening assay to check the presence of secondary metabolites using the standard conventional protocol described by Harborne [39].

2.5. *In Vitro* NO Inhibitory Release Activity

In vitro anti-inflammatory assay followed instructions of of B.-W with modification [40-42]. Macrophage cells isolated from mice BALB/c were obtained from the Biomedical Center Laboratory, Faculty of Medicine, Brawijaya University. Isolation of macrophages from mice following the instructions of Zhang *et al*. with modifications [43].

Mice were cervical-dislocated and then placed in supine position, abdominal skin opened and cleaned the peritoneum sheath with 70% alcohol. Then, injected ±10 mL cold DMEM medium into the peritoneal cavity (wait ± 1 minute while pressed-press slowly). After that, the peritoneal fluid aspirated from the peritoneal cavity by tapping with two fingers of internal organs, fluids aspirated by syringe injection, selected in a non-fat and distant part of the intestine. Aspirates collected in a centrifuge tube and centrifuged (Biosan Centrifuge LMC-3000) at 800-1000 rpm at room temperature, 8-10 minutes. Then, the supernatant was removed and added to 1 ml of medium DMEM complete (containing 10% FBS) in pellet obtained.

The macrophage cells placed in TC plate 96 well (1×10^6 cell/well) were suspended in a DMEM medium containing 10% FBS, at 5% CO_2 incubator, 37ºC for 2 hours. Inflammation in macrophages was induced by incubating them for 24 h in a medium containing LPS (0.01 µg/mL) without the presence of test extracts. The crude extracts SCA-SCF (5, 10, 20 mg/mL) were added to the cells 5 min before LPS challenge, respectively. The culture supernatant was collected to calculate the concentration of NO by Griess reaction and read using a microplate reader (Bio-Rad 550) absorbance of 570 nm.

2.6. Gas Chromatography and Mass Spectrometry (GC/MS)

For a quantitative analysis of bioactive profiles from all crude exatracts, Hewlett-Packard (HP) 6890 GC MS was used with Agilent 19091S-433 HP-5MS column having 30 m length and 250 µm id. Helium was used as carrier gas at flow rate of 1 mL/min and oven temperature was set at 325°C. The initial oven temperature was 150°C which was held at 1°C/min. It ran for 10°C/min and was later increased to 240°C hold time for 2 min. The total run time was 22 minutes. The scan range was 50 - 550 amu. Structural assignments were based on analysis of fragmentation pattern of mass spectra and direct comparison of mass spectra with profiles in the National Institute of Standards and Technology (NIST) and Wiley library.

2.7. Statistical Analysis

All experimental measurement data were performed in three replicates and expressed as Mean

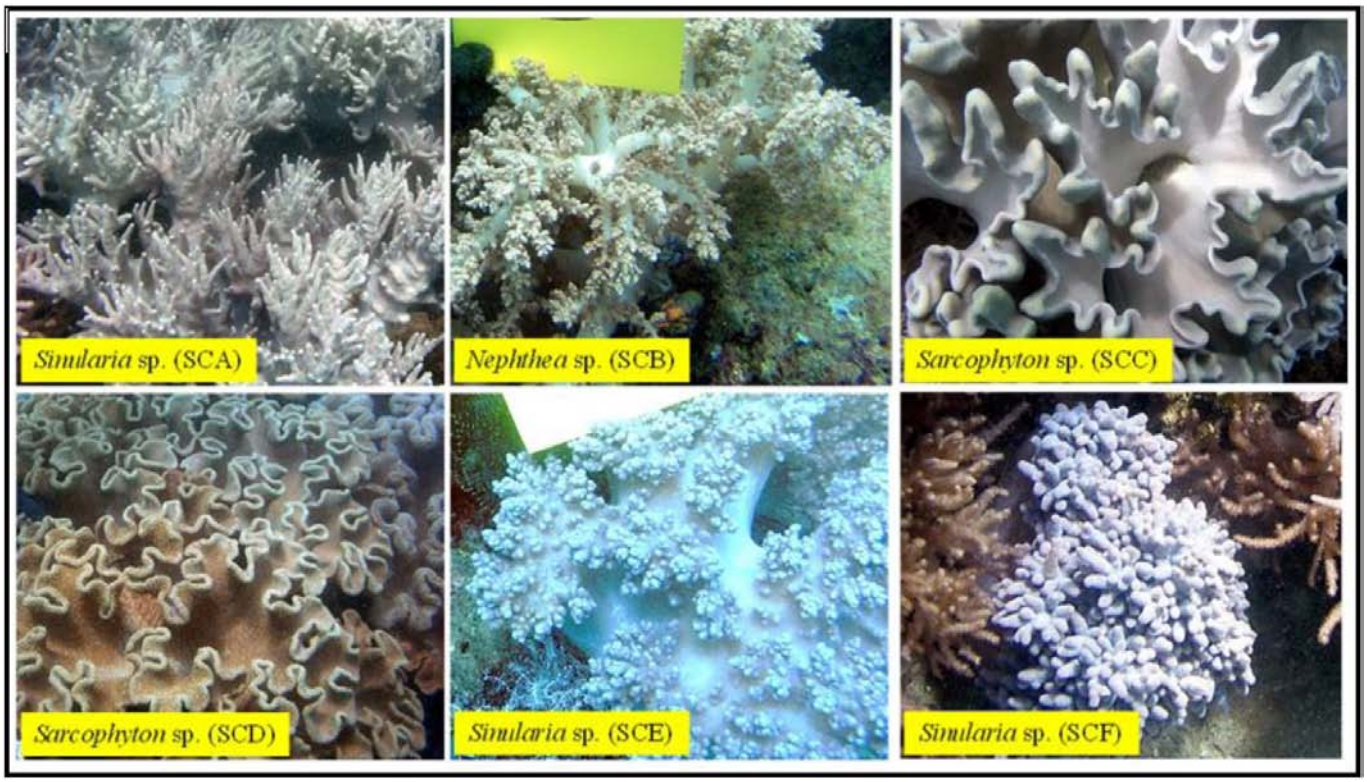

Fig. (1). Soft corals from Palu Bay, Central Sulawesi.

± SD (n = 3). The results of research were tested for statistical significance with One-way ANOVA. Differences were considered statistically significant at $P < 0.05$. Statistical analysis was completed using The Microsoft Excel 2013 data processing program.

3. RESULTS

The procedure of identification of soft corals samples follows the instructions of Fabricus & Alderslade (2001). Based on monomorphic colony color, interior and surface sclerites, soft coral samples were identified as *Sinularia* sp. (SCA), *Nephthea* sp. (SCB), *Sarcophyton* sp. (SCC), *Sarcophyton* sp. (SCD), *Sinularia* sp. (SCE) and *Sinularia* sp. (SCF) as presented in Fig. (**1**).

The phytochemical analysis of all soft corals crude extracts was presented in Table **1**. The crude extracts by using solvents dichloromethane: methanol of soft corals were assayed to detect secondary metabolites. The chemical constituent analysis of all crude extracts indicated the presence of saponins, polyphenols (tannins), steroids, triterpenoids, alkaloids, and flavonoids.

Six soft corals crude extract activity had analyzed as an inhibitor of NO release in macrophages. NO inhibitor activity was then evaluated by LPS-induced NO production on peritoneal mice macrophage cells, as presented in Fig. (**2**).

Fig. (**2**) illustrates all soft coral crude extracts, showed the ability to inhibit NO production. *Sarcophyton* sp. (SCC) and *Sinularia* sp. (SCF) extracts showed the best NO inhibition activity with the concentration of NO, 0.22 ± 0.04 and 0.20 ± 0.04 μM at 20 mg/mL, respectively. On the other hand, NO concentration on negative control was 9.12 ± 0.07 μM. *Sinularia* sp. (SCA), *Nephthea* sp. (SCB) and *Sarcophyton* sp. (SCD) extracts increases NO concentration at 20 mg/mL. The results of statistical analysis obtain that the different types of soft corals did not affect the concentration of NO, while the difference of extract concentration affected. The different types of soft corals and concentration of the extract did not show any interaction on the concentration of NO. The results of analysis of variance presented in Table **2**.

Furthermore, soft coral extract *Sarcophyton* sp. (SCC), *Sinularia* sp. (SCE) and *Sinularia* sp.

Table 1. Phytochemical analysis of all soft corals crude extracts.

Chemical Constituents	Soft Corals Species						Standard
	Sinularia sp. (SCA)	*Nephthea* sp. (SCB)	*Sarcophyton* sp. (SCC)	*Sarcophyton* sp. (SCD)	*Sinularia* sp. (SCE)	*Sinularia* sp. (SCF)	
Saponins	+	-	-	+	+	+	Stable foam formed for 15 minutes
Polyphenols (tannins)	+	+	+	+	-	+	Brown precipitate formed
Steroids	-	+	-	-	+	-	Green or blue colour produced (Lieberman-Buchard)
Triterpenoids	+	-	+	+	-	+	Brown or reddish-brown colour produced (Lieberman-Buchard)
Alkaloids	+	+	+	+	+	+	Orange precipitate formed (Dragendorff)
Flavonoids	+	-	-	-	+	-	Orange, pink or red colour produced

+: Present; -: Absent.

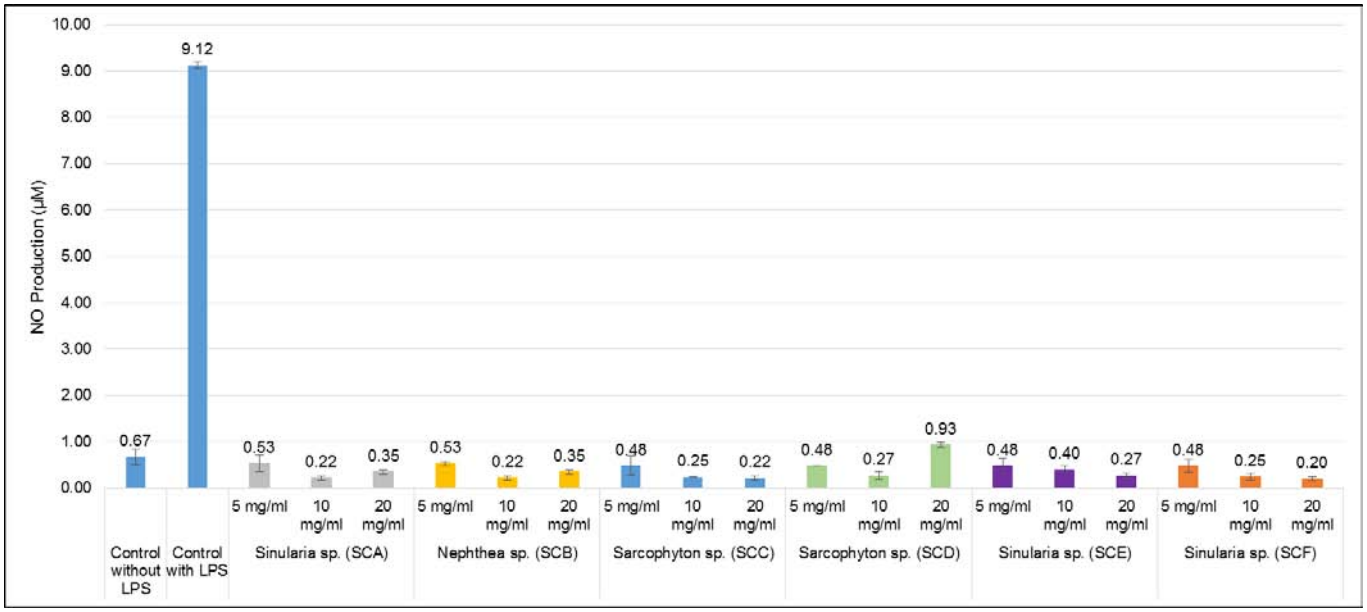

Fig. (2). Inhibitory effects of all soft corals crude extracts on LPS-induced NO production in Mice Peritoneal Macrophages cell. Extracts were tested at 5 - 20 mg/ml. Data are presented as the means ± SD of three replications. *Sarcophyton* sp. (SCC) and *Sinularia* sp. (SCF) extracts showed the best NO inhibition activity. Based on statistical analysis, the difference of extract concentration affects the NO concentration, not the different types of soft corals.

(SCF) was partitioned by polarity. Then, evaluated by LPS-induced NO production on peritoneal mice macrophage cells, as presented in Table **3**.

Table **3** illustrates SCC, SCE, and SCF partitioned extracts showed the ability to inhibit NO production. *Sarcophyton* sp. (SCC) DCM and *Sinularia* sp. (SCF) EtOAc extracts showed the best NO inhibition activity with the concentration of NO 4.85 ± 0.17 and 5.07 ± 0.50 µM at 5 mg/mL, respectively.

Table 2. Analysis of variace two-factor with replication ANOVA.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.221	9	0.025	1.194	0.351	2.393
Columns	0.978	1	0.978	47.476	0.000	4.351
Interaction	0.109	9	0.012	0.585	0.794	2.393
Within	0.412	20	0.021	-	-	-
Total	1.720	39	-	-	-	-

Table 3. Inhibitory effects of SCC, SCE, and SCF partitioned extracts on LPS-induced NO production in Mice Peritoneal Macrophages cell. Extracts were tested at 5 - 20 mg/ml. Data are presented as the means ± SD.

Fractions	**NO Production (μM)**		
	5 mg/ml	**10 mg/ml**	**20 mg/ml**
Control with LPS	163.38 ± 3.79		
Control without LPS	6.00 ± 0.92		
SCC DCM	4.85 ± 0.17	6.31 ± 0.21	6.08 ± 0.01
SCC EtOAc	6.48 ± 0.25	6.46 ± 0.38	6.62 ± 0.34
SCC BuOH	8.46 ± 0.17	5.15 ± 0.21	4.77 ± 0.21
SCE DCM	6.54 ± 0.32	6.38 ± 0.17	6.77 ± 0.17
SCE EtOAc	6.38 ± 0.32	6.92 ± 0.17	7.08 ± 0.21
SCE BuOH	4.62 ± 0.17	4.54 ± 0.01	6.38 ± 0.17
SCF DCM	6.08 ± 0.01	7.23 ± 0.27	6.54 ± 0.17
SCF EtOAc	5.07 ± 0.50	5.84 ± 0.66	7.97 ± 0.28
SCF BuOH	6.23 ± 0.34	6.62 ± 0.21	5.69 ± 0.27

The study was extended by GC-MS analysis of soft coral crude extracts (SCA-SCF). The mass spectrum of each compound compared with that in the NIST and Wiley library. In the crude extract *Sinularia* sp. (SCA) there were 68 peaks, but only 52 peaks were detected with qualities above 85% and identified in 25 compounds.

Crude extract *Nephthea* sp. (SCB) there were 64 peaks, but only 35 peaks with qualities above 85% and identified in 33 compounds.

Crude extract *Sarcophyton* sp. (SCC) there were 31 peaks, but only 23 peaks with quality min 85% and identified in 18 compounds.

In the crude extract *Sarcophyton* sp. (SCD) there were 33 peaks, but only 26 peaks were detected with qualities above 85% and identified in 15 compounds.

Crude extract *Sinularia* sp. (SCE) there were 56 peaks, but only 44 peaks with qualities above 85% and identified in 35 compounds. In the crude extract *Sinularia* sp. (SCF) there were 29 peaks, but only 25 peaks with quality min 85% and identified in 22 compounds.

Based on GC-MS analysis and literature studies, there were 148 compounds identified in all crude extracts (SCA-SCF), and there were 22 compounds that have the potential to be anti-inflammatory. The chemical constituents identified by GC-MS analysis of all crude extracts that indicated as potentially anti-inflammatory presented in Table **4**.

Table 4. The chemical constituents probabilities identified by GC-MS analysis of all crude extracts that were indicated as potentially anti-inflammatory.

Compound	Molecular Formula	RT (min)	Area (%)	Quality (min 85)	Library	References
***Sinularia* sp. (SCA)**						
Octacosane	$C_{28}H_{58}$	11.466	3.25	91	NIST	[44]
***Nephthea* sp. (SCB)**						
Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	$C_{15}H_{24}$	4.745	0.55	90	NIST	[45]
Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)-	$C_{15}H_{24}$	5.957	0.53	93	NIST	[46]
1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	$C_{12}H_{18}$	6.334	4.72	90	NIST	[47]
***Sarcophyton* sp. (SCC)**						
Eicosane	$C_{20}H_{42}$	7.357	0.12	86	NIST	[48]
Nonacosane	$C_{29}H_{60}$	13.352	6.78	98	NIST	[48]
		21.525	3.92	96	NIST	
14(β)-Pregnane	$C_{21}H_{36}$	14.398	0.87	95	Wiley	[49]
Octacosane	$C_{28}H_{58}$	17.450	6.39	99	NIST	[44]
Tricosane	$C_{23}H_{48}$	18.250	0.32	95	NIST	[48]
		19.227	1.21	93	NIST	
***Sarcophyton* sp. (SCD)**						
14(β)-Pregnane	$C_{21}H_{36}$	6.997	0.11	99	Wiley	[49]
		15.758	0.77	96	Wiley	
		15.953	0.98	96	Wiley	
		17.302	0.83	97	Wiley	
Octadecane	$C_{18}H_{38}$	10.798	13.06	95	NIST	[44]
		18.273	9.27	97	NIST	
		21.977	5.10	95	NIST	
***Sinularia* sp. (SCE)**						
Oleic Acid	$C_{18}H_{34}O_2$	7.351	0.63	86	NIST	[50]
7,10-Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	8.740	0.54	90	NIST	[51]
14(β)-Pregnane	$C_{21}H_{36}$	10.752	0.36	97	Wiley	[49]
		17.296	0.71	95	Wiley	
5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	$C_{22}H_{36}O_2$	12.381	4.60	95	NIST	[51]

(Table 4). contd…

Compound	Molecular Formula	RT (min)	Area (%)	Quality (min 85)	Library	References
Octacosane	$C_{28}H_{58}$	18.256	7.75	91	NIST	[44]
1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	$C_{13}H_{16}N_2O_2S$	18.759	1.23	91	NIST	[48]
***Sinularia* sp. (SCF)**						
Oxirane, decyl-	$C_{12}H_{24}O$	10.003	1.38	92	NIST	[52]
Nonacosane	$C_{29}H_{60}$	10.363	0.13	94	NIST	[48]
		12.346	0.44	90	NIST	
Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	$C_{10}H_{18}O$	10.906	0.61	86	NIST	[53]
14(β)-Pregnane	$C_{21}H_{36}$	11.060	0.26	90	Wiley	[49]
		11.969	0.50	86	Wiley	
Tetratriacontane	$C_{34}H_{70}$	13.678	1.02	95	NIST	[44]

The results also showed an increase in NO production at the concentration of 20 mg/ml; this indicated that there were cytotoxic compounds. The chemical constituents of the GC-MS analysis in Table **5** showed compounds that indicated as cytotoxic.

4. DISCUSSION

The analysis of the bioactive substances component of all soft coral crude extracts showed the presence of saponins, polyphenols (tannins), steroids, triterpenoids, alkaloids, and flavonoids. Phytochemical analysis of *Sinularia* sp. and *Lobophytum* sp. also reported the presence of alkaloids, Flavonoids, tannins, steroids, triterpenoids and saponins [38, 66]. Previous research has reported the terpenoid and steroids derivative bioactive compounds of the soft coral genus *Lobophytum*, *Sarcophyton*. *Nephthea* and *Sinularia*, showing potential anti-inflammatory biological activity. This research also gives information on anti-inflammatory of the soft coral extract of *Nephthea* sp., *Sarcophyton* sp., and *Sinularia* sp.

NO is a potential mediator of physiological processes, with functions related to cell signaling and vasodilation, protect the organs from ischemic damage, and also shows antimicrobial and anti-tumor activity [67]. NO is a mediator synthesized by the enzyme NO-synthase (NOS) [68], which can be divided into two types: constitutive isoform (NOS endothelial and neuronal NOS) and an inducible isoform (iNOS). iNOS is regulated by inflammatory mediators (LPS, cytokines) and increased levels of NO by iNOS was directly involved in the pathogenesis of inflammatory response [31].

NO endothelial-derivate induce vascular relaxation (vasodilation) and platelet aggregation and adhesion inhibitors [69]. Some research results report the importance of anti-inflammatory agents that can control NO, as cardioprotective and hypotensive agents [70, 71]. In the chronic inflammatory stage, iNOS produces NO as an inflammatory mediator, causing vasodilation and endema at the site of inflammation [72]. Thus, by inhibiting NO production directly it also inhibits the expression of the iNOS enzyme and this is one way in the treatment of inflammation.

Chemical constituents of the six crude extracts showed the potential of each extract which could be developed as a potential anti-inflammatory agent. In the extract of *Sinularia* sp. (SCA) there are 3.25% compounds that have the potential as anti-inflammatory agents; *Nephthea* sp. (SCB) of 5.80%; *Sarcophyton* sp. (SCC) of 19.61%; *Sarcophyton* sp. (SCD) of 30.12%; *Sinularia* sp. (SCE) of 15.82%; and *Sinularia* sp. (SCF) of 4.34%.

Table 5. The chemical constituents Probabilities identified by GC-MS analysis of all crude extracts that were indicated as cytotoxic.

Compound	Molecular Formula	RT (min)	Area (%)	Quality (min 85)	Library	References
***Sinularia* sp. (SCA)**						
Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	10.969	1.60	93	NIST	[54]
1-Hexacosene	$C_{26}H_{52}$	11.792	2.52	97	NIST	
		13.346	0.75	92	NIST	
Tricosane	$C_{23}H_{48}$	12.089	2.16	90	NIST	[55]
		12.232	3.55	91	NIST	
		13.581	1.45	91	NIST	
		14.175	0.82	95	NIST	
		14.838	9.62	95	NIST	
		15.044	0.44	96	NIST	
		15.947	0.47	90	NIST	
***Nephthea* sp. (SCB)**						
Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)- [1S-(1.alpha.2.beta.,4.beta.)]-	$C_{15}H_{24}$	3.836	1.30	90	NIST	[56]
(-)-Aristolene	$C_{15}H_{24}$	4.185	0.68	99	NIST	[57]
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	9.060	1.22	97	NIST	[58]
.beta.-caryophyllene	$C_{15}H_{24}$	9.226	0.34	92	Wiley	[2]
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	10.969	0.52	97	NIST	[59]
Octadec-9-enoic acid	$C_{18}H_{34}O_2$	11.106	0.97	99	NIST	[60]
5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	$C_{22}H_{36}O_2$	12.386	3.31	94	NIST	[61]
***Sarcophyton* sp. (SCD)**						
9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	8.334	0.14	92	NIST	[62]
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	9.054	1.06	95	NIST	[58]
1-Hexacosene	$C_{26}H_{52}$	12.781	0.86	94	NIST	[63]
Tricosane	$C_{23}H_{48}$	12.935	1.53	96	NIST	[55]
		13.164	1.87	92	NIST	
***Sinularia* sp. (SCE)**						
Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)- [1S-(1.alpha.2.beta.,4.beta.)]-	$C_{15}H_{24}$	3.836	0.71	98	NIST	[56]
		9.654	1.01	89	NIST	
7-Pentadecyne	$C_{15}H_{28}$	9.134	0.94	98	NIST	[64]

(Table 5) contd…

Compound	Molecular Formula	RT (min)	Area (%)	Quality (min 85)	Library	References
Tricosane	$C_{23}H_{48}$	12.986	2.86	91	NIST	[55]
1-Hexacosene	$C_{26}H_{52}$	14.061	0.76	94	NIST	[63]
		17.181	1.50	90	NIST	
		20.302	0.18	95	NIST	
***Sinularia* sp. (SCF)**						
Acetamide, N-methyl-N-[4-[4-methoxy-1-hexahydropyridyl]-2-butynyl]-	$C_{13}H_{22}N_2O_2$	9.060	0.25	91	Wiley	[65]
n-Eicosane	$C_{20}H_{42}$	10.980	0.41	93	Wiley	[55]
Tricosane	$C_{23}H_{48}$	11.415	0.35	91	NIST	[55]
		18.250	0.89	95	NIST	
1-Hexacosene	$C_{26}H_{52}$	11.792	3.44	95	NIST	[63]

However, there was increased NO concentrations at 20 mg/ml, indicating the presence of substances as NO activators and cytotoxic effects if at high levels (Wanzola *et al*., 2010). Table **5** shows the chemical constituents GC-MS analysis indicated cytotoxic. There are 23.38% of SCA extracts which are cytotoxic; SCB has 8.34%; SCD has 5.46%; SCE has 7.96%; while SCF has 5.34%. This data confirms that crude extracts of *Sarcophyton* sp. (SCC) are best developed as an anti-inflammatory agent because from the compound profile the results of a GC-MS analysis are not obtained by compounds that have cytotoxic properties. Compounds with cytotoxic properties can damage cells and can increase NO concentration as an immune response in its role to kill tumor or cancer cells. In the inflammatory response, by inhibiting NO level, it is associated with inhibition of iNOS expression, in this case it can prevent the acute inflammatory response from becoming chronic.

The soft coral genus *Sarcophyton* is a rich source cembraneterpen [73-76]. About 100 references have reported the results of research on secondary metabolites with various biological variations of the genus *Sarcophyton* [77]. Previous research has isolated seven compounds from *Sarcophyton crassocaule* which indicate efficacy as an anti-inflammatory because it can inhibit the expression of iNOS protein in RAW267.7 macrophage cells were stimulated LPS [19]. Six compounds were isolated from *S. ehrenbergii* also able to reduce the expression of iNOS protein [78]. Soft corals *S. pauciplicatum* also reportedly able to reduce the expression of iNOS [79].

In the octocorallia subclass of the genus *Nephthea*, there are a variety of species, which produce various sesquiterpenes, diterpenes, and steroids compounds [15, 80, 81]. Genus *Nephthea* is a famous coral reef organisms with a wonderful source of terpenoids with various biological activities and widespread throughout the world, especially in the Indo-Pacific region [61, 82]. Previous research has presented the genus *Nephthea* proven to produce compounds capable of inhibiting the accumulation of iNOS protein expression, such as *N. columnaris* produces Columnariols A and B [18]; *N. erecta* produces erectathiol [25]; and *N. chabroli* produces 4-methylated steroids, nebrosteroids A-E, G-H [83], nebrosteroids M, 19-oxygenatedsteroids, nebrosteroids I-J, K-L [25].

Sinularia genus has proven to be a rich source of bioactive steroids [1, 4]; diterpenoids [84-86], sesquiterpenoids [87-89] and cembranoids [90, 91] with various biological activities [92]. The many previous studies have reported that compounds isolated from soft coral genus *Sinularia* have been shown to inhibit or reduce the pro-inflammatory protein expression of iNOS and NO production in

macrophages, among others Cembrane-based diterpenoids compound were isolated from *S. triangular* [84]; Secosterol isolated from *S. granosa* [23]; Sinularioside from *Sinularia* sp. origin of Bunaken Marine Park North Sulawesi [93]; Crassarosterosides A and C were isolated from *S. crassa* [94]; Eight diterpenoid compounds from *S. flexibilis* [95]; Crassarines F and H were isolated from *S. crassa* which significantly inhibited iNOS protein [96]; 11-dehydrosinulariolide, sinulariolide and 11-epi-sinulariolide acetate from *S. discrepans* [97]; Thioflexibilolide A was isolated from *S. flexibilis* [98]; Gyrosanolides A-C and gyrosanin A were isolated from *S. gyrosa* [16, 99]. Flexibilisolide A and flexilarin from *S. granosa* and *S. querciformis* [100]; (+)-11,12-epoxysarcophytol A potentially anti-inflammatory of *S. gibberosa* [101]. Grandilobatins D was isolated from *S. grandilobata* [102].

Chemical constituents from the results of GC-MS analysis showed variations in chemical compounds with bioactivity potential from soft coral extracts. These compounds are thought to interact with each other to provide biological effects. Bioactive substances from natural products can work in synergy between compounds with one another [103]. Natural products can work through multi-compound and multi-target synergistic modes [104].

The chemical compounds identified with the GC-MS analysis in soft coral extracts also show potential as antioxidants. *Sarcophyton* sp. (SCC) there are 13.39%, *i.e*. 1-Hexadecanethiol [105]; 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexame-thyl-, (all-E)- [106]; 2-Dodecen-1-yl(-) succinic anhydride [107]; and Octacosane [44]. *Sinularia* sp. (SCF) has 19.81%, *i.e*. Cyclohexanol, 5-methyl-2-(1-methylethenyl)- [53]; Nonadecane [108]; Bacchotricuneatin c [59]; Tetrapentacontane, 1,54-dibromo-; Hexatriacontane [109]; and Longifolenaldehyde [110]. These compounds can become a natural anti-inflammatory-mechanism of action of bioactive antioxidants, namely by providing electrons, so that free radical molecules are unstable. Bioactive antioxidants can prevent oxidative stress through the scavenging of free radicals. Inflammation can be inhibited by preventing stress oxidative in cells [111]. The author also determined the antioxidant potential (Scavenging DPPH Method) of the six soft coral extracts in other research. The results showed that all six extracts could scavenge DPPH radicals. This research has presented at the International Conference on Fisheries and Marine, Airlangga University October 6th, 2018 (The manuscript is also temporary the process of publishing proceedings).

The NO is a signaling molecule that has an important role in the pathogenesis of inflammation. Excessive NO production by iNOS was detected in several inflammatory diseases [112]. NO is considered a pro-inflammatory mediator that induces inflammation because of excessive production under abnormal conditions [113]. Therefore, one pathway discovery of an anti-inflammatory agent by observing inhibition of NO levels produced by iNOS pro-inflammatory proteins [114-116].

CONCLUSION

Based on the assay results, extract of *Sarcophyton* sp. (SCC) and *Sinularia* sp. (SCF) showed the best NO inhibitory release activity. Purification and characterization of compounds from both can then be done so that the compounds that act as anti-inflammatory can be known. This study is exclusively making soft corals from Central Sulawesi, Indonesia can become a potential organism in the discovery and development of bioactive substances anti-inflammatory.

LIST OF ABBREVIATIONS

iNOS = Inducible Nitric Oxide Synthase

NO = Nitric Oxide

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the reported experimental procedures on animals were approved by the Animal Care and Used Committee with ethical clearance number 680-KEP-UB, Indonesia.

HUMAN AND ANIMAL RIGHTS

No humans were used in the study. All the reported experiments on animals were in accordance

with the Committee for the update of the Guide for the Care and Use of Laboratory Animals.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the [Universitas Brawijaya, Doctoral Dissertation: Wendy Alexander Tanod, supervised by Yenny Risjani] at [http://ub.ac.id], reference number [In press, 2019].

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Hu, L.C.; Yen, W.H.; Su, J.H.; Chiang, M.Y.N.; Wen, Z.H.; Chen, W.F.; Lu, T.J.; Chang, Y.W.; Chen, Y.H.; Wang, W.H.; Wu, Y.C.; Sung, P.J. Cembrane derivatives from the soft corals, *Sinularia gaweli* and *Sinularia flexibilis*. *Mar. Drugs,* **2013**, *11*(6), 2154-2167.

[2] Chen, W.; Li, Y.; Guo, Y. Terpenoids of Sinularia soft corals: Chemistry and bioactivity. *Acta Pharm. Sin. B,* **2012**, *2*(3), 227-237.

[3] Fattorusso, E.; Romano, A.; Taglialatela-Scafati, O.; Janib Achmad, M.; Bavestrello, G.; Cerrano, C. Lobozoanthamine, a new zoanthamine-type alkaloid from the Indonesian soft coral Lobophytum sp. *Tetrahedron Lett.,* **2008**, *49*(14), 2189-2192.

[4] Lu, Y.; Huang, C.Y.; Lin, Y.F.; Wen, Z.H.; Su, J.H.; Kuo, Y.H.; Chiang, M.Y.; Sheu, J.H. Anti-inflammatory cembranoids from the soft corals *Sinularia querciformis* and *Sinularia granosa. J. Nat. Prod.,* **2008**, *71*(10), 1754-1759.

[5] Duh, C.Y.; El-Gamal, A.A.H.; Chu, C.J.; Wang, S.K.; Dai, C.F. New cytotoxic constituents from the Formosan soft corals *Clavularia viridis* and *Clavularia violacea*. *J. Nat. Prod.,* **2002**, *65*(11), 1535-1539.

[6] Duh, C.Y.; Wang, S.K.; Weng, Y.L. Brassicolene, a novel cytotoxic diterpenoid from the Formosan soft coral *Nephthea brassica*. *Tetrahedron Lett.,* **2000**, *41*(9), 1401-1403.

[7] Sheu, J.H.; Ahmed, A.F.; Shiue, R.T.; Dai, C.F.; Kuo, Y.H. Scabrolides A-D, four new norditerpenoids isolated from the soft coral *Sinularia scabra. J. Nat. Prod.,* **2002**, *65*(12), 1904-1908.

[8] Higa, T.; Tanaka, J.; Ohtani, I.I.; Musman, M.; Roy, M.C.; Kuroda, I. Bioactive compounds from coral reef invertebrates. *Pure Appl. Chem.,* **2001**, *73*(3), 589-593.

[9] Coll, J.C. The chemistry and chemical ecology of octocorals (Coelenterata, Anthozoa, Octocorallia). *Chem. Rev.,* **1992**, *1002*, 613-631.

[10] Leewis, R.J.; Janse, M. *Advances in coral husbandry in public Aquariums.,* **2008**, *2*.

[11] Carté, B.K. Biomedical potential of marine natural products. *BioSci.,* **1996**, *46*(4), 271-286.

[12] Coll, J.C.; Bowden, B.F.; Tapiolas, D.M.; Willis, R.H.; Djura, P.; Streamer, M.; Trott, L. Studies of Australian soft corals-XXXV. The terpenoid chemistry of soft corals and its implications. *Tetrahedron,* **1985**, *41*(6), 1085-1092.

[13] Putra, M.Y. Bioactive marine natural products from the Indonesian soft coral *Sinularia* sp. (order Alcyonacea, family Alcyoniidae). **2012**, PhD Thesis.

[14] Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.,* **2013**, *30*(2), 237-323.

[15] Wei, W.C.; Sung, P.J.; Duh, C.Y.; Chen, B.W.; Sheu, J.H.; Yang, N.S. Anti-inflammatory activities of natural products isolated from soft corals of Taiwan between 2008 and 2012. *Mar. Drugs,* **2013**, *11*(10), 4083-4126.

[16] Cheng, S.Y.; Chuang, C.T.; Wen, Z.H.; Wang, S.K.; Chiou, S.F.; Hsu, C.H.; Dai, C.F.; Duh, C.Y. Bioactive norditerpenoids from the soft coral *Sinularia gyrosa*. *Bioorg. Med. Chem.,* **2010**, *18*(10), 3379-3386.

[17] Rainsford, K.D. Anti-inflammatory drugs in the 21st century. Inflammation in the pathogenesis of chronic diseases. *Subcell Biochem*., **2007**, *42*, 3-27.

[18] Hsiao, T.H.; Sung, C.S.; Lan, Y.H.; Wang, Y.C.; Lu, M.C.; Wen, Z.H.; Wu, Y.C.; Sung, P.J. New anti-inflammatory cembranes from the cultured soft coral *Nephthea columnaris*. *Mar. Drugs,* **2015**, *13*(6), 3443-3453.

[19] Lin, W.Y.; Chen, B.W.; Huang, C.Y.; Wen, Z.H.; Sung, P.J.; Su, J.H.; Dai, C.F.; Sheu, J.H. Bioactive cembranoids, sarcocrassocolides P-R, from the Dongsha Atoll soft coral *Sarcophyton crassocaule*. *Mar. Drugs,* **2014**, *12*(2), 840-850.

[20] Thao, N.P.; Luyen, B.T.T.; Ngan, N.T.T.; Song, S.B.; Cuong, N.X.; Nam, N.H.; Kiem, P.V.; Kim, Y.H.; Minh, C.V. New anti-inflammatory cembranoid diterpenoids from the Vietnamese soft coral *Lobophytum crassum*. *Bioorg. Med. Chem. Lett.,* **2014**, *24*(1), 228-232.

[21] Roy, P.K.; Roy, M.C.; Taira, J.; Ueda, K. Structure and bioactivity of a trisnorditerpenoid and a diterpenoid from an Okinawan soft coral, *Cespitularia* sp. *Tetrahedron Lett.,* **2014**, *55*(8), 1421-1423.

[22] Yin, J.; Zhao, M.; Ma, M.; Xu, Y.; Xiang, Z.; Cai, Y.; Dong, J.; Lei, X.; Huang, K.; Yan, P. New casbane diterpenoids from a South China Sea soft coral, *Sinularia* sp. *Mar. Drugs,* **2013**, *11*(2), 455-465.

[23] Huang, C.Y.; Su, J.H.; Duh, C.Y.; Chen, B.W.; Wen, Z.H.; Kuo, Y.H.; Sheu, J.H. A new 9,11-secosterol from the soft coral *Sinularia granosa*. *Bioorg. Med. Chem. Lett.,* **2012**, *22*(13), 4373-4376.

[24] Lin, W.Y.; Su, J.H.; Lu, Y.; Wen, Z.H.; Dai, C.F.; Kuo, Y.H.; Sheu, J.H. Cytotoxic and anti-inflammatory cembranoids from the Dongsha Atoll soft coral *Sarcophyton crassocaule*. *Bioorg. Med. Chem.,* **2010**, *18*(5), 1936-1941.

[25] Cheng, S.Y.; Huang, Y.C.; Wen, Z.H.; Chiou, S.F.; Wang, S.K.; Hsu, C-H.; Dai, C.F.; Duh, C.Y. Novel sesquiterpenes and norergosterol from the soft corals *Nephthea erecta* and *Nephthea chabroli*. *Tetrahedron Lett.,* **2009**, *50*(7), 802-806.

[26] Newcombe, E.A.; Camats-Perna, J.; Silva, M.L.; Valmas, N.; Huat, T.J.; Medeiros, R. Inflammation: The link between comorbidities, genetics, and Alzheimer's disease. *J. Neuroinflammation,* **2018**, *15*(1), 276.

[27] McCartney-Francis, N.L.; Song, X.; Mizel, D.E.; Wahl, S.M.; Sharon, M.W. Selective inhibition of inducible nitric oxide synthase exacerbates erosive joint disease. *J. Immunol.,* **2001**, *166*(4), 2734-2740.

[28] Kaplanski, G.; Marin, V.; Montero-Julian, F.; Mantovani, A.; Farnarier, C. IL-6: A regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends Immunol.,* **2003**, *24*(1), 25-29.

[29] Hu, G.P.; Yuan, J.; Sun, L.; She, Z.G.; Wu, J.H.; Lan, X.J.; Zhu, X.; Lin, Y.C.; Chen, S.P. Statistical research on marine natural products based on data obtained between 1985 and 2008. *Mar. Drugs,* **2011**, *9*(4), 514-525.

[30] Marris, E. Marine natural products: Drugs from the deep. *Nature,* **2006**, *443*(7114), 904-905.

[31] Ianaro, A.; O'Donnell, C.A.; Di Rosa, M.; Liew, F.Y. A nitric oxide synthase inhibitor reduces inflammation, down-regulates inflammatory cytokines and enhances interleukin-10 production in carrageenin-induced oedema in mice. *Immunology,* **1994**, *82*(3), 370-375.

[32] Risjani, Y.; Yunianta, ; Couteau, J.; Minier, C. Cellular immune responses and phagocytic activity of fishes exposed to pollution of volcano mud. *Mar. Environ. Res.,* **2014**, *96*, 73-80.

[33] Hinz, B.; Brune, K. Cyclooxygenase-2-10 years later. *J. Pharmacol. Exp. Ther.,* **2002**, *300*(2), 367-375.

[34] Kwon, T.H.; Yoon, I.H.; Shin, J.S.; Lee, Y.H.; Kwon, B.J.; Lee, K.T.; Lee, Y.S. Synthesis of indolyl-3-acetonitrile derivatives and their inhibitory effects on nitric oxide and PGE2 productions in LPS-induced RAW 264.7 cells. *Bioorg. Med. Chem. Lett.,* **2013**, *23*(9), 2571-2574.

[35] Tapilatu, Y.H. Status of drug discovery research based on marine organisms from Eastern Indonesia. *Proc. Chem.,* **2015**, *14*, 484-492.

[36] Yunianta, Y.; Risjani, Y. Methanolic extract of *Sargassum crystaefolium* induce the apoptosis of human mammary carcinoma cells. *Phycologia,* **2017**, *56*(4), 838.

[37] Chasanah, E. Marine biodiscovery research in indonesia : Challenges and rewards. *J. Coastal Dev.,* **2008**, *12*(1), 1-12.

[38] Putra, M.Y.; Murniasih, T.; Swasono, R.T.; Wibowo, J.T.; Saputri, A.N.C.; Widhiana, M.R.; Arlyza, I.S. Secondary metabolites and their biological activities in Indonesian soft coral of the genus *Lobophytum*. *Asian Pac. J. Trop. Biomed.,* **2016**, *6*(11), 909-913.

[39] Harborne, J.B. *Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis. Published in the USA by Chapman and Hall in association with Methuen, Inc.,* **1998**, *Vol. 3.*

[40] Chen, B.W.; Wang, S.Y.; Huang, C.Y.; Chen, S.L.; Wu, Y.C.; Sheu, J.H. Hirsutalins I-M, eunicellin-based diterpenoids from the soft coral *Cladiella hirsuta*. *Tetrahedron,* **2013**, *69*(10), 2296-2301.

[41] Ravipati, A.S.; Zhang, L.; Koyyalamudi, S.R.; Jeong, S.C.; Reddy, N.; Bartlett, J.; Smith, P.T.; Shanmugam, K.; Münch, G.; Wu, M.J.; Satyanarayanan, M.; Vysetti, B. Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. *BMC Complement. Altern. Med.,* **2012**, *12*, 173-187.

[42] Fattorusso, E.; Luciano, P.; Putra, M.Y.; Taglialatela-Scafati, O.; Ianaro, A.; Panza, E.; Bavestrello, G.; Cerrano, C. Chloroscabrolides, chlorinated norcembranoids from the Indonesian soft coral *Sinularia* sp. *Tetrahedron,* **2011**, *67*(41), 7983-7988.

[43] Zhang, X.; Goncalves, R.; Mosser, D.M. The isolation and characterization of murine macrophages. *Curr. Protocols Immunol.,* **2008**, *14*, 1-18.

[44] Bakr, R.O.; El-Naa, M.M.; Zaghloul, S.S.; Omar, M.M. Profile of bioactive compounds in *Nymphaea alba* L. leaves growing in Egypt: Hepatoprotective, antioxidant and anti-inflammatory activity. *BMC Complement. Altern. Med.,* **2017**, *17*(1), 52.

[45] Komalavalli, T.; Packia Lincy, M.; Muthukumarasamy, S.; Mohan, V.R. Determination of bioactive components of *Asystasia travancorica* Bedd (Acanthaceae) by GC-MS Analysis. *Int. J. Pharm. Clin. Res.,* **2014**, *6*(2), 155-158.

[46] Sahi, N.M. Evaluation of insecticidal activity of bioactive compounds from *Eucalyptus citriodora* against *Tribolium castaneum*. *Int. J. Pharmacogn. Phytochem. Res.,* **2016**, *8*(8), 1256-1270.

[47] Okagu, I.U.; Ngwu, U.E.; Odenigbo, C.J. Bioactive constituents of methanol extract of *Xylopia aethiopica* (UDA) fruits from Nsukka, Enugu State, Nigeria. *OAlib,* **2018**, *5*(3), 1-11.

[48] Kim, J.E.; Park, K.M.; Lee, S.Y.; Seo, J.H.; Yoon, I.S.; Bae, C.S.; Yoo, J.C.; Bang, M.A.; Cho, S.S.; Park, D.H. Anti-inflammatory effect of *Allium hookeri* on carrageenan-induced air pouch mouse model. *PLoS One,* **2017**, *12*(12), e0190305.

[49] Chao, C.H.; Wen, Z.H.; Su, J.H.; Chen, I.M.; Huang, H.C.; Dai, C.F.; Sheu, J.H. Further study on anti-inflammatory oxygenated steroids from the octocoral *Dendronephthya griffini*. *Steroids,* **2008**, *73*(14), 1353-1358.

[50] Folmer, F.; Jaspars, M.; Solano, G.; Cristofanon, S.; Henry, E.; Tabudravu, J.; Black, K.; Green, D.H.; Küpper, F.C.; Aalbersberg, W.; Feussner, K.; Dicato, M.; Diederich, M. The inhibition of TNF-α-induced NF-kappaB activation by marine natural products. *Biochem. Pharmacol.,* **2009**, *78*(6), 592-606.

[51] Hassan, M.H.A.; Mohammed, R.; Hetta, M.H.; Abdelaziz, T.A.; El-Gendy, A.O.; Sleim, M.A. Biological and chemical investigation of the soft coral *Lobophytum pauciflorum* collected from the Egyptian Red Sea. *Int. J. Pharmacogn. Phytochem. Res.,* **2016**, *8*(6), 906-911.

[52] Pandiyan, R.; Subbiah, L.; Palanisamy, S.; Ariyamuthu, S.; Muthusamy, T.; Velu, R.K. A wide array on anti-inflammatory study in an ethanolic extract of *Pupalia lappaceae* juss. (amaranthaceae) by using wistar rats. *Arch. Appl. Sci. Res.,* **2009**, *1*(2), 150-158.

[53] Salem, M.Z.M.; Elansary, H.O.; Ali, H.M.; El-Settawy, A.A.; Elshikh, M.S.; Abdel-Salam, E.M.; Skalicka-Woźniak, K. Bioactivity of essential oils extracted from *Cupressus macrocarpa* branchlets and *Corymbia citriodora* leaves grown in Egypt. *BMC Complement. Altern. Med.,* **2018**, *18*(1), 23.

[54] Saravanakumar, K.; Vivek, R.; Sithranga Boopathy, N.; Yaqian, L.; Kathiresan, K.; Chen, J. Anticancer potential of bioactive 16-methylheptadecanoic acid methyl ester derived from marine trichoderma. *J. Appl. Biomed.,* **2015**, *13*(3), 199-212.

[55] Ahmed, H.H.; Abd-Rabou, A.A.; Hassan, A.Z.; Kotob, S.E. Phytochemical analysis and anti-cancer investigation of *Boswellia serrata* bioactive constituents *in vitro*. *Asian Pac. J. Cancer Prev.,* **2015**, *16*(16), 7179-7188.

[56] Barrero, A.F.; Herrador, M.M.; Quílez del Moral, J.F.; Arteaga, P.; Meine, N.; Pérez-Morales, M.C.; Catalán, J.V. Efficient synthesis of the anticancer β-elemene and other bioactive elemanes from sustainable germacrone. *Org. Biomol. Chem.,* **2011**, *9*(4), 1118-1125.

[57] Akaberi, M.; Iranshahy, M.; Iranshahi, M. Review of the traditional uses, phytochemistry, pharmacology and toxicology of giant fennel (*Ferula communis* L. subsp. communis). *Iran. J. Basic Med. Sci.,* **2015**, *18*(11), 1050-1062.

[58] Byju, K.; Anuradha, V.; Rosmine, E.; Sankar, H.S.H.; Gopinath, A.; Peter, K.J.P.; Kumar, T.R.G.; Vasundhara, G.; Kumar, N.C.; Nair, S.M. DPPH scavenging property of active principles from soft coral *Sarcophytonf flexuosum* Tixier-Durivault. *Pharm. Chem. J.,* **2015**, *49*(3), 178-182.

[59] Ashraf, A.; Sarfraz, R.A.; Anwar, F.; Shahid, S.A.; Alkharfy, K.M. Chemical composition and biological activities of leaves of *Ziziphus mauritiana* L. Native to Pakistan. *Pak. J. Bot.,* **2015**, *47*(1), 367-376.

[60] Joel, E.L.; Valentin Bhimba, B. Evaluation of secondary metabolites from mangrove associated fungi *Meyerozyma guilliermondii*. *Alexandria J. Med.,* **2013**, *49*(3), 189-194.

[61] Hu, J.; Yang, B.; Lin, X.; Zhou, X.; Yang, X.; Long, L.; Liu, Y. Chemical and biological studies of soft corals of the Nephtheidae family. *Chem. Biodivers.,* **2011**, *8*(6), 1011-1032.

[62] Mathan, S.; Smith, A.A.; Kumaran, J.; Prakash, S. Anticancer and antimicrobial activity of *Aspergillus protuberus* SP1 isolated from marine sediments of South Indian coast. *Chin. J. Nat. Med.,* **2011**, *9*(4), 286-292.

[63] Lavilla, C.A., Jr; Uy, M.M.; Ohta, S. Cytotoxic long-chain alkene and terpene isolated from the methanol extract of the air-dried leaves of *Pipturus arborescens* C.B. *Rob. J. Multidiscip. Stud.,* **2014**, *3*(1), 16-26.

[64] Sianipar, N.F.; Purnamaningsih, R. Enhancement of the contents of anticancer bioactive compounds in mutant clones of rodent tuber (*Typhonium flagelliforme* Lodd.) based on GC-MS analysis. *Pertanika, J. Trop. Agric. Sci.,* **2018**, *41*(1), 305-320.

[65] Ahmad, B.; Khan, I.; Bashir, S.; Azam, S. Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal and antibacterial activities of the essential oils of *Acacia modesta*. *J. Med. Plants Res.,* **2012**, *6*(31), 4653-4659.

[66] Apri, R.; Zamani, N.P.; Effendi, H. Exploration of soft coral as antioxidant at Pongok Island, South Bangka. *J. Fish. Mar. Technol.,* **2013**, *4*(2), 211-217.

[67] Balboa, E.M.; Conde, E.; Moure, A.; Falqué, E.; Domínguez, H. *In vitro* antioxidant properties of crude extracts and compounds from brown algae. *Food Chem.,* **2013**, *138*(2-3), 1764-1785.

[68] Moncada, S.; Palmer, R.M.; Higgs, E.A. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.,* **1991**, *43*(2), 109-142.

[69] Bhardwaj, A.; Huang, Z.; Kaur, J.; Knaus, E.E. Rofecoxib analogues possessing a nitric oxide donor sulfohydroxamic acid (SO_2NHOH) cyclooxygenase-2 pharmacophore: Synthesis, molecular modeling, and biological evaluation as anti-inflammatory agents. *ChemMedChem,* **2012**, *7*(1), 62-67.

[70] El-Gamal, M.I.; Lee, W.S.; Shin, J.S.; Oh, C.H.; Lee, K.T.; Choi, J.; Myoung, N.; Baek, D. Synthesis of new tricyclic and tetracyclic fused coumarin sulfonate derivatives and their inhibitory effects on LPS-induced nitric oxide and PGE_2 productions in RAW 264.7 Macrophages: Part 2. *Arch. Pharm. (Weinheim),* **2016**, *349*(11), 853-863.

[71] Bhardwaj, A.; Batchu, S.N.; Kaur, J.; Huang, Z.; Seubert, J.M.; Knaus, E.E. Cardiovascular properties of a nitric oxide releasing rofecoxib analogue: Beneficial anti-hypertensive activity and enhanced recovery in an ischemic reperfusion injury model. *ChemMedChem,* **2012**, *7*(8), 1365-1368.

[72] Yun, H.Y.; Dawson, V.L.; Dawson, T.M. Neurobiology of nitric oxide. *Crit. Rev. Neurobiol.,* **1996**, *10*(3-4), 291-316.

[73] Eltahawy, N.A.; Ibrahim, A.K.; Radwan, M.M.; El-Sohly, M.A.; Hassanean, H.A.; Ahmed, S.A. Cytotoxic cembranoids from the Red Sea soft coral, *Sarcophyton auritum*. *Tetrahedron Lett.,* **2014**, *55*(29), 3984-3988.

[74] Hegazy, M.E.F.; Mohamed, T.A.; Abdel-Latif, F.F.; Alsaid, M.S.; Shahat, A.A.; Paré, P.W. Trochelioid A and B, new cembranoid diterpenes from the Red Sea soft coral *Sarcophyton trocheliophorum*. *Phytochem. Lett.,* **2013**, *6*(3), 383-386.

[75] Blunt, J.W.; Copp, B.R.; Hu, W.P.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.,* **2008**, *25*(1), 35-94.

[76] Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Yamakado, T.; Matsuzaki, T.; Hirata, Y. Ca-antagonistic substance from soft coral of the genus Sarcophyton. *Experientia,* **1983**, *39*(1), 67-69.

[77] Liang, L.F.; Guo, Y.W. Terpenes from the soft corals of the genus Sarcophyton: Chemistry and biological activities. *Chem. Biodivers.,* **2013**, *10*(12), 2161-2196.

[78] Cheng, S.Y.; Wen, Z.H.; Chiou, S.F.; Tsai, C.W.; Wang, S.K.; Hsu, C.H.; Dai, C.F.; Chiang, M.Y.; Wang, W.H.; Duh, C.Y. Ceramide and cerebrosides from the octocoral *Sarcophyton ehrenbergi*. *J. Nat. Prod.,* **2009**, *72*(3), 465-468.

[79] Thao, N.P.; Luyen, B.T.T.; Sun, Y.N.; Song, S.B.; Thanh, N.V.; Cuong, N.X.; Nam, N.H.; Kiem, P.V.; Kim, Y.H.; Minh, C.V. NF-κB inhibitory activity of polyoxygenated steroids from the Vietnamese soft coral *Sarcophyton pauciplicatum*. *Bioorg. Med. Chem. Lett.,* **2014**, *24*(13), 2834-2838.

[80] Wang, S.K.; Puu, S.Y.; Duh, C.Y. New steroids from the soft coral *Nephthea chabrolii*. *Mar. Drugs,* **2013**, *11*(2), 571-580.

[81] Ishii, T.; Matsuura, H.; Zhaoqi, Z.; Vairappan, C.S. A new 4alpha-methylated sterol from a *Nephthea* sp. (Nephtheidae) Bornean soft coral. *Molecules,* **2009**, *14*(9), 3360-3366.

[82] Amir, F.; Koay, Y.C.; Yam, W.S. Chemical constituents and biological properties of the marine soft coral Nephthea : A review (Part 1). *Trop. J. Pharm. Res.,* **2012**, *11*(3), 485-498.

[83] Huang, Y.C.; Wen, Z.H.; Wang, S.K.; Hsu, C.H.; Duh, C.Y. New anti-inflammatory 4-methylated steroids from the Formosan soft coral *Nephthea chabroli*. *Steroids,* **2008**, *73*(11), 1181-1186.

[84] Su, J.H.; Wen, Z.H. Bioactive cembrane-based diterpenoids from the soft coral *Sinularia triangular*. *Mar. Drugs,* **2011**, *9*(6), 944-951.

[85] Su, J.H.; Ahmed, A.F.; Sung, P.J.; Chao, C.H.; Kuo, Y.H.; Sheu, J.H. Manaarenolides A-I, diterpenoids from the soft coral *Sinularia manaarensis*. *J. Nat. Prod.,* **2006**, *69*(8), 1134-1139.

[86] Rudi, A.; Aknin, M.; Gaydou, E.M.; Kashman, Y.; Kashman, Y. Several new isoprenoids from the soft coral *Sinularia erecta*. *J. Nat. Prod.,* **1998**, *61*(7), 872-875.

[87] Chen, D.; Cheng, W.; Liu, D.; van Ofwegen, L.; Proksch, P.; Lin, W. Capillosananes S-Z, new sesquiterpenoids from the soft coral *Sinularia capillosa*. *Tetrahedron Lett.,* **2014**, *55*(19), 3077-3082.

[88] Chao, C.H.; Hsieh, C.H.; Chen, S.P.; Lu, C.K.; Dai, C.F.; Sheu, J.H. Sinularianins A and B, novel sesquiterpenoids from the Formosan soft coral *Sinularia* sp. *Tetrahedron Lett.,* **2006**, *47*(33), 5889-5891.

[89] Chao, C.H.; Hsieh, C.H.; Chen, S.P.; Lu, C.K.; Dai, C.F.; Wu, Y.C.; Sheu, J.H. Novel cyclic sesquiterpene peroxides from the Formosan soft coral *Sinularia* sp. *Tetrahedron Lett.,* **2006**, *47*(13), 2175-2178.

[90] Lai, D.; Li, Y.; Xu, M.; Deng, Z.; van Ofwegen, L.; Qian, P.; Proksch, P.; Lin, W. Sinulariols A-S, 19-oxygenated cembranoids from the Chinese soft coral *Sinularia rigida*. *Tetrahedron,* **2011**, *67*(33), 6018-6029.

[91] Duh, C.Y.; Wang, S.K.; Tseng, H.K.; Sheu, J.H.; Chiang, M.Y. Novel cytotoxic cembranoids from the soft coral *Sinularia flexibilis*. *J. Nat. Prod.,* **1998**, *61*(6), 844-847.

[92] Liang, C.H.; Wang, G.H.; Hung, W.J.; Lin, R.J.; Cheng, D.L.; Chou, T.H. Apoptosis effect of *Sinularia leptoclados*, *S. depressan* and *S. inflate* extracts in human oral squamous cell carcinomas. *J. Taiwan Institute Chem. Eng.,* **2010**, *41*(1), 86-91.

[93] Putra, M.Y.; Ianaro, A.; Panza, E.; Bavestrello, G.; Cerrano, C.; Fattorusso, E.; Taglialatela-Scafati, O. Sinularioside, a triacetylated glycolipid from the Indonesian soft coral *Sinularia* sp., is an inhibitor of NO release. *Bioorg. Med. Chem. Lett.,* **2012**, *22*(8), 2723-2725.

[94] Chao, C.H.; Chou, K.J.; Huang, C.Y.; Wen, Z.H.; Hsu, C.H.; Wu, Y.C.; Dai, C.F.; Sheu, J.H. Steroids from the soft coral *Sinularia crassa*. *Mar. Drugs,* **2012**, *10*(2), 439-450.

[95] Shih, H.J.; Tseng, Y.J.; Huang, C.Y.; Wen, Z.H.; Dai, C.F.; Sheu, J.H. Cytotoxic and anti-inflammatory diterpenoids from the Dongsha Atoll soft coral *Sinularia flexibilis*. *Tetrahedron,* **2012**, *68*(1), 244-249.

[96] Chao, C.H.; Chou, K.J.; Huang, C.Y.; Wen, Z.H.; Hsu, C.H.; Wu, Y.C.; Dai, C.F.; Sheu, J.H. Bioactive cembranoids from the soft coral *Sinularia crassa*. *Mar. Drugs,* **2011**, *9*(10), 1955-1968.

[97] Lu, Y.; Su, H.J.; Chen, Y.H.; Wen, Z.H.; Sheu, J.H.; Su, J.H. Anti-inflammatory cembranoids from the Formosan soft coral *Sinularia discrepans*. *Arch. Pharm. Res.,* **2011**, *34*(8), 1263-1267.

[98] Chen, B.W.; Chao, C.H.; Su, J.H.; Huang, C.Y.; Dai, C.F.; Wen, Z.H.; Sheu, J.H. A novel symmetric sulfur-containing biscembranoid from the Formosan soft coral *Sinularia flexibilis*. *Tetrahedron Lett.,* **2010**, *51*(44), 5764-5766.

[99] Cheng, S.Y.; Chuang, C.T.; Wang, S.K.; Wen, Z.H.; Chiou, S.F.; Hsu, C.H.; Dai, C.F.; Duh, C.Y. Antiviral and anti-inflammatory diterpenoids from the soft coral *Sinularia gyrosa*. *J. Nat. Prod.,* **2010**, *73*(6), 1184-1187.

[100] Lu, Y.; Huang, C-Y.; Lin, Y.; Wen, Z.; Kuo, Y.; Chiang, M.Y.; Sheu, J.H. Anti-inflammatory cembranoids from the soft corals *Sinularia querciformis* and *Sinularia granosa*. *Chem. Pharm. Bull. (Tokyo),* **2010**, *58*(4), 464-466.

[101] Ahmed, A.F.; Tai, S.H.; Wen, Z.H.; Su, J.H.; Wu, Y.C.; Hu, W.P.; Sheu, J.H.A. A C-3 methylated isocembranoid and 10-oxocembranoids from a formosan soft coral, *Sinularia grandilobata*. *J. Nat. Prod.,* **2008**, *71*(6), 946-951.

[102] Ahmed, A.F.; Wen, Z.H.; Su, J.H.; Hsieh, Y.T.; Wu, Y.C.; Hu, W.P.; Sheu, J.H. Oxygenated cembranoids

from a Formosan soft coral *Sinularia gibberosa*. *J. Nat. Prod.,* **2008**, *71*(2), 179-185.

[103] Ulrich-Merzenich, G.; Panek, D.; Zeitler, H.; Vetter, H.; Wagner, H. Drug development from natural products: Exploiting synergistic effects. *Indian J. Exp. Biol.,* **2010**, *48*(3), 208-219.

[104] Long, F.; Yang, H.; Xu, Y.; Hao, H.; Li, P. A strategy for the identification of combinatorial bioactive compounds contributing to the holistic effect of herbal medicines. *Nature Publishing Group,* **2015**, 1-11.

[105] Faridha B.I.; Mohankumar, R.; Jeevan, M.; Ramani, K. GC-MS analysis of bio-active molecules derived from *Paracoccus pantotrophus* FMR19 and the antimicrobial activity against bacterial pathogens and MDROs. *Indian J. Microbiol.,* **2016**, *56*(4), 426-432.

[106] Su, J-H.; Ahmed, A.F.; Sung, P.J.; Wu, Y-C.; Sheu, J-H. Meroditerpenoids from a Formosan soft coral *Nephthea chabrolii*. *J. Nat. Prod.,* **2005**, *68*(11), 1651-1655.

[107] Rawal, J.R.; Sonawani, P.R. Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis & it's *in vitro* antimicrobial activity. *Int. J. Pharm. Life Sci.,* **2016**, *7*(1 (Jan)), 4880-4885.

[108] Bhardwaj, A.; Shakil, N.A.; Jha, V.; Gupta, R.K. Screening of nutritional, phytochemical, antioxidant and antibacterial activity of underutilized seeds of *Scirpus articulatus* the basis of Khubahi Ramdana Industry. *J. Pharmacogn. Phytochem.,* **2014**, *3*(4), 11-20.

[109] Zubair, M.; Hassan, S.; Rizwan, K.; Rasool, N.; Riaz, M.; Zia-Ul-Haq, M.; De Feo, V. Antioxidant potential and oil composition of *Callistemon viminalis* leaves. *Sci. World J.,* **2013**, *2013*489071.

[110] Al-Abd, N.M.; Nor, Z.M.; Mansor, M.; Zajmi, A.; Hasan, M.S.; Azhar, F.; Kassim, M. Phytochemical constituents, antioxidant and antibacterial activities of methanolic extract of *Ardisia elliptica*. *Asian Pac. J. Trop. Biomed.,* **2017**, *7*(6), 569-576.

[111] Arulselvan, P.; Fard, M.T.; Tan, W.S.; Gothai, S.; Fakurazi, S.; Norhaizan, M.E.; Kumar, S.S. Role of antioxidants and natural products in inflammation. *Oxidative Med. Cell. Longev.,* **2016**, 1-15.

[112] Hämäläinen, M. *Inducible Nitric Oxide Synthase as a Target of Treatment Modalities.,* **2008**,

[113] Sharma, J.N.; Al-Omran, A.; Parvathy, S.S. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology,* **2007**, *15*(6), 252-259.

[114] Momi, S.; Monopoli, A.; Alberti, P.F.; Falcinelli, E.; Corazzi, T.; Conti, V.; Miglietta, D.; Ongini, E.; Minuz, P.; Gresele, P. Nitric oxide enhances the anti-inflammatory and anti-atherogenic activity of atorvastatin in a mouse model of accelerated atherosclerosis. *Cardiovasc. Res.,* **2012**, *94*(3), 428-438.

[115] Wallace, J.L. Nitric oxide as a regulator of inflammatory processes. *Mem. Inst. Oswaldo Cruz,* **2005**, *100*(Suppl. 1), 5-9.

[116] Hämäläinen, M.; Lilja, R.; Kankaanranta, H.; Moilanen, E. Inhibition of iNOS expression and NO production by anti-inflammatory steroids. Reversal by histone deacetylase inhibitors. *Pulm. Pharmacol. Ther.,* **2008**, *21*(2), 331-339.