INTRODUCTION

Plants have been used for such a long time in various aspects of human life, including for their primary health care. Utilization of plants as herbal medicine is related to their biologically active compounds, because plants are capable of producing various chemicals capable of fighting diseases categorized as secondary metabolites. Kong et al. [1] indicated that plants are rich source of secondary metabolites, such as alkaloids, flavonoids, saponins, steroids, terpenoids, polysaccharides, and tannins. Many of these secondary metabolites have pharmacological and biological activities [2,3] such as free radical scavengers or antioxidant.

Free radicals (superoxide and hydroxyl radicals) are produced in the body during metabolic processes, and in a state of excess will lead to negative effects, including cell death and tissue damage [4]. Atherosclerosis, coronary heart disease, cancer, and premature aging [5]. On the other hand, the body does not have large amounts of antioxidant reserves, so that the body needs exogenous antioxidants to protect it more efficiently against oxidative stress caused by free radicals [6].

Antioxidants are components that can donate an electron to a free radical, and neutralize it [7]. The exogenous antioxidant can be divided into synthetic and natural antioxidants. Natural antioxidants have advantages over synthetic antioxidants which can be obtained easily and economically and have a slight or negligible side effect [8]. Recently, the interests in natural products and environment-friendly antioxidants are looking for as a substitute and various plants contained a massive pool of bioactive compounds [9].

Some species of Santalaceae have been reported to have biological activities. *Exocarpos lattifolius* has yielded exocarpic acid and its analogs as antimycobacterial compounds [10]. Some species of Santalaceae have been reported as potential free radical scavengers such as *Viscum album, Osyris quadripartita*, and *Quinchamali chilensis* which correlated with its total phenol [8,11,12].

*Exocarpos longifolius* is a small tree or shrub belongs to Santalaceae. *E. longifolius* distributed in Southeast Asia, Australia, and the Pacific Islands. Several plant species from Santalaceae have been known to be potential as an antioxidant. However, the biological potential of *Exocarpos* as free radical scavenger has not been studied widely. Therefore, this study focused on the chemical compounds and the free radical scavenging activity of various extracts of *E. longifolius*.

METHODS

Plant material

*Exocarpos longifolius* or Kayu Sulaeman (common name) was collected from Batudulang, Batu Lanteh District, West Sumbawa, West Nusa Tenggara. The plant was identified and authenticated at the Herbarium Bogoriense, Botany Division, Research Center for Biology, Indonesian Institute of Sciences.

Sample preparation and extraction

Twigs and leaves were separated, washed thoroughly under tap water to remove dirt. The samples were cut into small pieces and dried in the oven at 40°C. The dried samples were ground into powder.

Samples were subsequently extracted with different solvent polarity, namely hexane, chloroform, ethyl acetate, and methanol successively. Samples were macerated with hexane thrice. The filtrate was concentrated with the rotary evaporator (Heidolph WB2000). The
same steps follow for other solvents. Dried crude extract weighted and extraction yield were calculated as follows: (Weight of extract/weight of original sample) x 100%

**Phytochemical analysis**

Qualitative phytochemical screening was carried out on ether, chloroform, ethyl acetate, and methanol extract of *E. longifolius* for the identification of major phytochemical constituents such as saponins, tannins, alkaloids, terpenoids, and flavonoids [13].

**Saponins**
The extract is diluted with distilled water and shaken vigorously. The appearance of persistent froth for 1 min indicates the presence of saponins.

**Tannins**
The extract was added with distilled water and then added with ferric chloride (FeCl₃). The appearance of a dark green color indicates the presence of tannins.

**Alkaloids**
The extract was added with 2% sulfuric acid, stand for 2 min. A few drops of Dragendorff’s reagent were added. The appearance of orange precipitate indicates the presence of alkaloids.

**Terpenoids**
The extract was added with chloroform and added with concentrated sulfuric acid from the side of the test tube. The appearance of the reddish brown rings at the junction of two layers indicates the presence of terpenoids.

**Flavonoids**
The extract added with methanol and heated at 50°C. After that, it added with magnesium and 4 drops of hydrochloric acid. Appearance of orange-red color indicates the presence of flavonoids.

**Bioautographic analysis for 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity**

Ten microliters of hexane, chloroform, ethyl acetate, and methanol extracts (10 mg/ml) and (–)-catechin as standard were transferred on thin-layer chromatography (TLC) plate (Silica gel GF₄₅₄₅). The TLC plate was air-dried and then sprayed with 0.2% DPPH in methanol [14]. Observations of DPPH free radical scavenging activity were examined at 30 min after DPPH spraying. The yellowish-white spot around the extract against a purple background on TLC-plate indicating DPPH free radical scavenging activity.

Active extracts with free radical scavenging activity were further analyzed. 10 µL of ethyl acetate or chloroform extract was transferred on TLC plate, then developed in mobile phase dichloromethane: methanol:water (6:4:1). The TLC plates were subsequently dried then sprayed with 0.2% DPPH in methanol. The plates were incubated at room temperature for 30 min. The chemical compounds with DPPH free radical scavenging activity were detected as yellow spots against a purple background.

**Determination of IC₅₀**
The extracts with DPPH free radical scavenging activity were further analyzed for their IC₅₀ values by serial dilution method in a 96 well microplate according to the modified method recommended by Zhou et al. [15]. The test was conducted in triplicates. The working solution of extract was prepared at a concentration of 1000 µg/ml in methanol. Catechin was used as positive control and prepared at a concentration of 1000 µg/ml. The DPPH solution in methanol (0.1 mmol) was freshly prepared. Each well within the first row (row A) was added with 100 µL of working solution (1000 µg/ml). The following rows were added with 50 µL methanol. Then, 50 µL of solution in the first row was taken out and transferred into the second row and homogenized. After homogenized, 50 µL was taken out and transferred into the next row resulting in serial dilution. In the past row, 50 µL was taken out and discarded. After the serial dilution is completed, each well was added with 80 µL 0.1 mmol DPPH. The microplate was incubated for 30 min at 37°C in the dark condition. After the incubation period was completed, the absorbance of each sample was measured at 517 nm (Varioskan Flash, Thermo Scientific). The value of IC₅₀ was calculated using a linear curve between the concentration of the test sample and the percentage of inhibition.

**Gas chromatography-mass spectrometry (GC-MS) analysis**
The chemical compounds of ethyl acetate extract of twigs were identified by GC-MS analysis. It was carried out using a GC-MS (Shimadzu 2010), equipped with Rtx-5MS capillary column (5% diphenyl/95% dimethylpolysiloxane, 30 m, 0.25 mm ID, 0.25um df, max. 350°C, RESTEK, USA). The carrier gas was helium at a flow rate of 2.0 ml/min. The volume of the injected sample was 5 µL. The injector temperature was 250°C, and the interface temperature was 300°C. The initial temperature of the column was 100°C, and the final temperature was 270°C. The temperature program: initial temperature 100°C, held for 3 min and then ramping at the rate of 10°C/min up to 270°C and held for 18 min [16]. Identification of chemical components in the extract based on interpretation on mass spectrum of GC-MS using the database of NIST 11 (National Institute Standard and Technology, US) and WILEY 8.

**RESULTS**

**Qualitative phytochemical analysis**
The qualitative phytochemical analysis was carried out on different solvent from leaves and twigs of *E. longifolius* (Table 1). Phytochemical screening revealed similar chemical compounds of the leaves and the twigs of *E. longifolius*. Tannins and terpenoids were present in the hexane and chloroform extracts, while ethyl acetate extracts revealed the presence of tannins, alkaloids, terpenoids, and flavonoids (Table 1).

**Detection of DPPH free radical scavenging activity**
The results of DPPH free radical scavenging activity assay of *E. longifolius* twigs and leaves were presented in Fig. 1. Fig. 1 showed that all of the extracts had active compounds as DPPH free radical scavenger indicated by a yellowish white band. The active

### Table 1: Qualitative phytochemical analysis of twigs and leaves of *E. longifolius*

| Compounds | Twigs | Leaves |
|-----------|-------|--------|
|           | Hexane | Chloroform | Ethyl acetate | Methanol | Hexane | Chloroform | Ethyl acetate |
| Saponin   | –      | –        | –            | +        | –      | –        | –            |
| Tannin    | +      | +        | +            | +        | –      | –        | –            |
| Alkaloid  | –      | –        | +            | +        | –      | –        | –            |
| Terpenoids| +      | +        | +            | +        | +      | +        | +            |
| Flavonoids| –      | –        | ++           | +        | –      | –        | ++           |

*–: Secondary metabolites not detected, ++: Secondary metabolites detected, E. longifolius: Exocarpus longifolius*
extracts were further analyzed to determine their potential as free radical scavengers. $IC_{50}$ values of extracts were presented in Table 2. Three extracts exhibited in vitro strong free radical scavengers ($IC_{50}$ value $<100 \mu g/ml$) [17], which were ethyl acetate and methanol extracts of the twigs and ethyl acetate extract of the leaves.

**GC-MS profiling of ethyl acetate extract of twigs**

The GC-MS analysis of ethyl acetate extract of the twigs *E. longifolius* was shown in Fig. 2.

**DISCUSSIONS**

Phytochemical screening is important to ascertain the chemical components in the extract and also as the first step in predicting the potential of active compounds in plant [24]. Priyanga et al. and Sowmya et al. indicated that the chemical compounds such as alkaloids, tannins, flavonoids, steroids, terpenoids, and phenolic in plants are natural bioactives [25,26]. Saponins are sedimenting and thickened red blood cells, may bind cholesterol [27], and overcome inflammation [28]. Alkaloids might be used as an analgesic, antispasmodic, and antibacterial [29]. Terpenoids increase glutathione-S-transferase and apoptotic against cancer cells [30], antibacterial [29,31], and sebacic acid, ethyl methyl ester); steroids (1-dehydrotestosterone, methyloxime, trimethylsilyl ether; Androst-11-en-17-one, 3-formyloxy-, (3.alpha., 5.alpha.)-; and stigmast-4-en-3-one); acyclic diterpene (phytol, acetate); alcohol (tetracontane-1,40-diol); benzofuran derivative (2-isopropenyl-4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-benzofuran-6-ol); acetamide derivative (N1-(8-Methyl-8-azabicyclo[3.2.1]oct-2-yl)-2-(2-methoxyphenyl)acetamide); and thiophene derivative (4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[cthiophene-1-carboxylic acid methyl ester). Some of these compounds have been reported by other researchers to have biological activity (Tables 3 and 4).

**Table 2: $IC_{50}$ of twigs and leaves *E. longifolius* extracts**

| No | Part of the plant | Solvent | $IC_{50}$ |
|----|------------------|---------|----------|
| 1  | Twigs            | Hexane  | >385     |
| 2  | Twigs            | Chloroform | >385      |
| 3  | Twigs            | Ethyl acetate | 15.65     |
| 4  | Twigs            | Methanol | 67.24    |
| 5  | Leaves           | Hexane  | >385     |
| 6  | Leaves           | Chloroform | >385      |
| 7  | Leaves           | Ethyl acetate | 78.59     |

*E. longifolius: Exocarpos longifolius*

**Table 3: Identified chemical compounds of ethyl acetate twigs extract of *E. longifolius***

| Retention time (min) | Area     | Area percentage (%) | Compounds                                      |
|----------------------|----------|---------------------|------------------------------------------------|
| 14.18                | 56351    | 0.85                | Phytol, acetate                                |
| 15.11                | 107075   | 1.62                | Methyl palmitate                               |
| 16.79                | 86467    | 1.31                | Inoleic acid, methyl ester                     |
| 16.86                | 261350   | 3.96                | Alfa inoleic acid, methyl ester                |
| 18.20                | 57836    | 0.88                | Sebacic acid, ethyl methyl ester               |
| 18.56                | 49588    | 0.75                | 1-Dehydrotestosterone, methyloxime, trimethylsilyl ether |
| 19.55                | 45983    | 0.70                | Androst-11-en-17-one, 3-formyl-oxy, (3.alpha., 5.alpha.)- |
| 19.93                | 56051    | 0.85                | Tetracontane-1,40-diol                         |
| 20.55                | 160571   | 2.43                | N1-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-2-(2-methoxyphenyl)acetamide |
| 21.55                | 197451   | 2.99                | 4-Acetyloxyimino-6,6-dimethylsulfanyl-4,5,6,7-tetrahydro-benzo[c] thiophene-1-carboxylic acid methyl ester |
| 21.58                | 49929    | 0.76                |                                                 |
| 21.70                | 1449611  | 21.91               |                                                 |

*E. longifolius: Exocarpos longifolius*
Table 4: Bioactivity of compounds in *E. longifolius* ethyl acetate extract

| No. | Name of compound                                      | Activity                          | Ref.  |
|-----|-------------------------------------------------------|-----------------------------------|-------|
| 1   | Phytol, acetate                                       | Antimycobacterial                 | [18]  |
| 2   | Methyl palmitate                                      | Acaricidal activity               | [19]  |
| 3   | Linoleic acid, methyl ester                           | Increase zinc availability, acidifier, inhibit production of uric acid | [20]  |
| 4   | Alpha-linolenic acid, methyl ester                    | Anti-cancer                       | [21]  |
| 5   | Sebamic acid, ethyl methyl ster                        | Acidifier, inhibit production of uric acid | [20]  |
| 6   | 2-Isopropenyl-4,4,7a-trimethyl-2,4,5,6,7a-hexahydrobenzofuran-6-ol | Oligosaccharide provider | [20]  |
| 7   | 1-Dehydrotestosterone, methylxolime, trimethylsilyl ether | -                               | -     |
| 8   | Androst-11-en-17-one, 3-formyloxy-, (3.alpha., 5.alpha.- | Alpha-amylase inhibitor, alpha-glucosidase inhibitor | [20]  |
| 9   | Tetracontane-1,40-diol                                 | -                                 | -     |
| 10  | N1-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-2-(2-methoxyphenyl) acetamide | Antitumor, anaphylactic, inhibit production of tumor necrosis factor | [20]  |
| 11  | 4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c][ | Acidifier, inhibit production of uric acid | [20]  |
| 12  | Stigmaster-4-en-3-one                                  | Hypoglycemic effect               | [23]  |

in the extract. Flavonoids are able as a free radical scavenger [39], act as hydrogen donor [40] and inhibit the enzyme responsible for free radicals generation [41]. Tannins in the ethyl acetate extracts might also contribute to free radical scavenging activity and antibacterial activity [42]. The DPPH radical scavenger activity of the extract serves as an important indicator of its potential as an antioxidant. An antioxidant prevents the oxidation process of the molecule by protecting the key cellular components from free radicals [43].

The active extract was analyzed further to identify its chemical compounds by GC-MS analysis. The compounds from the extract are identified based on interpretation on mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST11) and WILEY &. The twelve compounds were characterized and identified (Table 3).

**CONCLUSIONS**

Based on the result, it can be concluded that the ethyl acetate extract of *E. longifolius* twigs had strong free radicals scavengers’ activity, and the main compounds were Stigmaster-4-en-3-one. Further study needs to be done to fractionate, isolate and elucidate the potential natural free radicals scavengers from *E. longifolius*.

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**AUTHOR'S CONTRIBUTION**

Praptiwi designed the work and performed the laboratory analysis for extraction and bioassay, participated in drafting the article and contributed to the final manuscript. Ahmad Fathoni, performed the laboratory analysis in phytochemical analysis and GC-MS, performed data analysis and interpretation, and contributed to the final manuscript.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interests.

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