Secondary metabolites compound of sea plumes gorgonian (Rumphella, Isis and Ellisella) from Maumere Water- East Nusa Tenggara Indonesia

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Abstract. The abundance and diversity of gorgonian sea plumes in the aquatic of Maumere - East Nusa Tenggara lead us to undertake research of their secondary metabolites. The objective of this study was to identify the species sea plumes gorgonian and to characterize its secondary metabolites that had biological activity. Identification of genus was based on morphology characteristics: sclerite forms; while phytochemical tests was undertaken on gorgonian extract of sea plumes. The phytochemical test showed that secondary metabolites of gorgonian extract contained alkaloids, flavonoids, quinone, steroid/triterpenoid, tannin and saponin. The identification results were found three gorgonian genera, namely Genus Rumphella, Isis and Ellisella. The highest yield of gorgonian extract was Rumphella sp. 7.84%. Components of secondary metabolites identified were alkaloids, flavonoids, quinone, steroids, triterpenoids, tannins/ phenolics and saponin.

1. Introduction
One of the richest and most complex ecosystems is marine environment. The extreme chemical and physical conditions in environment have stimulated the production of various important molecules with unique structural features. Molecules derived from the sea display various types of biological activities [1]. Discovery of novel compounds from marine ecosystem is increasingly diverse and rapidly growing. The exploration of new compounds from marine organisms, especially invertebrates has been widely reported. Study on Rumphella antipathies, Carijoa sp., Octocoral Carijoa multiflora gorgonian showed potential biological properties as antibacterial [2,3,4,5], as antiprotozoal [6]. Octocoral Dendronephthya griffin and Soft Coral Scleronephthya gracillimum have anti-inflammatory properties [7,8]; gorgonian Rumphella antipathies exhibited inhibition of superoxide anion and the release of elastase by human neutrophils [9,10]. Gorgonian Isis hipirus, Rumphella sp. and Hicksonella sp. were toxic against Artemia salina Leach [11,12]. The abundance of sea plumes gorgonian in the aquatic of East Nusa Tenggara is one of the potentials that should be further researched. Tuti [13] reported on sea plumes gorgonian identified 16 genera from 8 families.
of gorgonian in the water of Maumere East Nusa Tenggara. This research was limited to identification of gorgonian. Study on secondary metabolites in the gorgonian Rumphella sp. and Hicksonella sp. by Teffu et al. [14] revealed that simplicia and sea plumes extract contain several active compounds namely saponin, triterpenoids, steroids, alkaloids, and flavonoids as anti-inflammatory and antioxidant. Early research on secondary metabolites compound needs to be done due to the potential existence of gorgonian diversity dispersed in the aquatic of East Nusa Tenggara. The diversity of aquatic characteristics in East Nusa Tenggara allows sea plumes gorgonian has different secondary metabolites compounds from the same sea plumes in other aquatic. The objective of this research was to identify sea plumes gorgonian and its secondary metabolite compound having potential biological activities as anti-bacterial, anti-fouling, anti-viral, anti-cancer, anti-inflammatory and anti-tumor.

2. Material and Method

2.1. Material
Raw material of this research was sea plumes gorgonian, ethyl acetate, phytochemical reagents and gorgonian identification books gorgonian in Indonesian waters [15]. The tools used such as scuba diving, pH meter, salinometer, flow/current meter, sechi disk, Global Positioning System (GPS Garmin etrex), C23 binocular microscope (Olympus), micropipette (corning), rotary evaporator (Eyela N-1200BS-W).

2.2. Method

2.2.1. Sampling. Sampling was carried out in the water of Wailiti Maumere East Nusa Tenggara at 3-20 m depths using diving equipment. Gorgonian than dried by aerated for 6-7 days. Identification of the gorgonian morphology characteristics: colony form, sclerite needles, location of needle in the body, tentacle by observing the colony form and sclerite needles morphological shape located in the body or tentacles according to Tuti [13] method. Samples were taken approximately 15 cm at the tip, middle and base of the sample cut into 1 cm. Furthermore, separated sample tissue by 5-7 drops of bleach. Waited for 5-10 minutes. Colony central axis would separate with sclerite forming flour, then washed of sclerite by clean water. Observed the morphological of clean sclerite with a microscope. The result of sclerite photo was compared with references to determine the form of polyps, surfaces and subsurface using Fabricius and Alderslade identification books [16].

2.2.2. Extraction. Extraction was carried out using maceration method based on Wikanta et al. [17] modified using a single solvent. Dried sea plumes separated into skin and axial than mushed up. Weighed 600 g of skin sample for Rumphella sp., 130 g of Isis sp., ad 95 g of Ellisella sp. The solvent used was ethyl acetate with a ratio between sample and solvent 1:3. Shaken and stored the mixture in the dark room for 24 H. Thereafter, filtered using Whatman paper no 42. The first filtrate saved into refrigerator. Extraction was done until the solution becomes clear. The filtrate was collected and combined then evaporated using a rotary evaporator with a temperature of 28°C till become concentrate. The concentrated extract was transferred into a vial bottle. The results of crude extract were weighed to determine the yield.

2.2.3. Qualitative phytochemical analysis. Qualitative phytochemical analysis was carried out according to Harborne [18] consist of alkaloid, steroid / triterpenoid, flavonoid, saponin, phenol hydroquinone and tannin tests. Alkaloid test was 1 mg of extract from of each solvent was dissolved in a few drops of sulfuric acid 2 N, after that will be tested with several alkaloid reagents including Dragendorff, Meyer and Wagner. The presence of alkaloid indicated by the appearance of brown precipitate for Wagner solution, cream-colored precipitate for Meyer solution and red to orange colored precipitate for Dragendorff solution. Meyer's reagent was made by adding 1 mL of HgCl₂
with 0.5 g of KI then dissolved and diluted with distilled water to 100 mL with a volumetric flask. This reagent was colorless. Wagner reagent was made of 10 mL distilled water added 2.5 g of iodine and 2 g of potassium iodide, then dissolved and diluted with distilled water to 200 mL in a volumetric flask. This reagent was brown color. Dragendorff reagents were prepared by dissolving 0.8 g of bismuth subnitrate in 10 mL of acetic acid and 40 mL of water. This solution mixed with a solvent made from 8 g of potassium iodide in 20 mL of water. Before used, 1 volume of this mixture was diluted with 2.3 volume mixture of glacial acetic acid 20 mL and 100 mL of water. This reagent was orange color. Steroid/triterpenoid test was 1 mg of extract was dissolved into 2 mL of chloroform in the test tube. Then, added 10 drops of anhydrous acetate and 3 drops of concentrated sulfuric acid into the mixture. The appearance of red color solution and turns into blue-grown indicates the presence of steroid/triterpenoid. Flavonoid test was 1 mg extract of each solvent, added 0.1 mg of magnesium, 0.4 mL of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol in the same volume) and 4 mL of alcohol. The mixture was shaken. The appearance of red, yellow or orange color in the lining of amyl alcohol indicates the presence of flavonoid. Saponin (foam test) was 1 mg of extract dissolved in hot water and shaken. The formation of stable foam for 30 minutes and did not disappear if added 1 drop of HCl 2N indicates the presence of saponin. Phenol hydroquinone test (FeCl₃ reagent) was 1 mg of sample extracted with 20 mL ethanol 70%. Taken 1 mL of the resulting solution, then added 2 drops of 5% FeCl solution. The appearance of green and green-blue color solution indicates the presence of phenol hydroquinone. Tannin test was 1 mg of sample added with FeCl₃ then, the mixture was homogenized. The appearance of green-black color indicates the presence of tannin.

3. Result

3.1. Identification of Sea Plumes Gorgonian

The results of identification of sea plumes gorgonian have led to identification of Rumphella sp. Isis sp. and Ellisella sp. genus gorgonian. Identification based on sclerite needle shape commonly used to identify the type of gorgonian. The sclerite shape shown in the samples visually similar Rumphella sp. genus sclerite had sharper tip. Isis sp. sclerite had round shape, the sclerite form of Ellisella sp. was similar to Rumphella sp. Similar forms of sclerite, often difficult to identify. According to Teffu et al. (2015) described sea plumes gorgonian Rumphella sp. genus from Raijua Island-East Nusa Tenggara found bulges gada-like and spindle shape, similar sclerite form with the results of the research conducted. Visualization of the sclerite forms and sea plumes gorgonian shown in Figure 1.
Morphologically, the axis of *Rumphella* sp. and *Isis* sp. genus looks different. *Rumphella* sp. had black and hard axis, while axis on *Isis* sp. looks like bamboo segments, bounded by black color between the segments. The morphology of *Ellisella* sp. was different from the two gorgonian genus. The axis had red color, like wood and easy to cut. Visualization of sclerite and axis shape from 3 genus of gorgonian shown in Figure 2.

**Figure 1.** Sclerite form of sea plumes gorgonian. a1) Sclerite of *Rumphella*; a2) *Rumphella* sp; b1) Sclerite of *Isis*; b2) *Isis* sp; c1) Sclerite of *Ellisella*; c2) *Ellisella* sp

**Figure 2.** Axis form of sea plumes a) *Rumphella* sp.; b) *Isis* sp.; c) *Ellisella* sp.

### 3.2. Extract yield

The extraction result was carried out on sea plumes gorgonian using ethyl acetate solvent. The yield of three gorgonian was presented in Table 1.
Table 1. The yield of sea plumes gorgonian extract

| Gorgonian   | Extract yield (%) |
|-------------|-------------------|
| *Rumphella* sp. | 7.84              |
| *Isis* sp.     | 6.95              |
| *Ellisella* sp. | 1.93              |

There were significantly different between three extraction yield. The highest yield obtained from *Rumphella* sp. Different result gained from Teffu *et al.* [12] study, with the same sample and solvent, the yield result for both *Rumphella* sp. and *Hicksonella* sp. gorgonian sample was 0.15% and 0.74% respectively. Ethyl acetate solvent used to take out the same characteristic which the same semi polar compound. The difference in extract yield results was caused by many factors. Factors that affecting include sample size, sample number, extraction methods used. Solvent used affected the yield result. Higher yield commonly used methanol due to the polar properties of solvent, able to take out the same secondary metabolite characteristic or semi-polar. Different from ethyl acetate had semi-polar properties.

3.3. Phytochemical of Sea Plumes Gorgonian

Phytochemical analysis obtained the three gorgonian genera used showed different outcomes from previous studies. Phytochemical properties of three gorgonian genera were summarized in Table 2.

Table 2. Phytochemical characteristic of three gorgonian genera

| Phytochemical test | Sea plumes gorgonian genera | Standard (color)                  |
|--------------------|------------------------------|-----------------------------------|
|                    | *Rumphella* sp | *Isis* sp | *Ellisella* sp |                                                |
| Alkaloid           | +              | -         | -              | Present of brown precipitate                    |
| Flavonoid          | +              | +         | -              | Present of yellow to red color                  |
| Quinone            | -              | +         | +              | Present of yellow                               |
| Steroid            | -              | -         | -              | Present of red                                  |
| Triterpenoid       | +              | +         | +              | Present of blue                                 |
| Tannin /phenolik   | +              | -         | -              | Present of green                                |
| Saponin            | +              | +         | -              | Foam formed on the filtrate                      |

Note: (+ indicates present, - indicates absent)

The results of phytochemical analysis on three gorgonian, terpenoid secondary metabolite components present in all gorgonian extract samples. *Ellisella* sp. genera only detected secondary metabolites of quinone and triterpenoid. Shen *et al.* and Qi *et al.* [19,20] reported that *Juncella fragilis* and *Juncella junceae* gorgonian from the Elliselidae family contained terpenoid compounds and diterpenoids as potential anti-inflammatory and antifouling properties. Sheu *et al.* [21] found secondary metabolites of terpenoids as antitumor drugs in *Isis hippurus* gorgonian. Sesquiterpenoid compounds also found in *Rumphella antipathies* Gorgonian coral potentially inhibited superoxide anion and the release of elastase by human neutrophils [9,10]. The secondary metabolite component of steroids was not detected, this was in contrast with Teffu *et al.* [14] detected the presence of a secondary metabolic component of steroids from both *Rumphella* sp. and *Hicksonella* sp. extract in ethyl acetate solvents. Steroids were also found in *Isis hippurus* gorgonian as antitumor drugs [22,23,24]. The secondary metabolite component of saponin was not detected in Teffu *et al.* [14] on the combined extracts of *Rumphella* sp. and *Hicksonella* sp., however saponins were detected in this research using ethyl acetate solvent in *Rumphella* sp. and *Isis* sp. by forming foam in the extract. Flavonoids compound were detected in this research and Teffu *et al.* [14] on combined *Rumphella* sp. and *Hicksonella* sp. extract. The secondary metabolites of alkaloids detected in gorgonian *Rumphella* sp. in ethyl acetate solvents, in contrast to Teffu *et al.* [14] secondary metabolite of
alkaloids were detected using methanol and n-hexane as solvents. The difference between secondary metabolites compounds may be influenced by environmental factors where the sea plumes gorgonian live. Sea plumes gorgonian that lives in conservation environments had dissimilar type and a number of secondary metabolites from the secondary metabolite compound of sea plumes live in open ocean environment condition. The difference in amount and type of secondary metabolite compounds determines the potential of these to be utilized compounds. Manuputty [25] stated active compounds in the body tissues of octocoral animals were used as a defense, the rapid formation and expansion of the colony or in an effort to compete for a place to live for their extension. Another characteristic of animal life was allelopathic used as a strategy to seize land from new corals.

4. Conclusion

Sea plumes gorgonian sample used in the research was identified as Gorgonian Genus *Rumphella* sp., *Isis* sp., and *Ellisella* sp. based on its sclerite form. The highest yield of extracts was obtained from gorgonian *Rumphella* sp. phytochemical test showed the presence of secondary metabolites of alkaloids, flavonoids, quinones, steroids, triterpenoids, tannins/phenolics and saponins.

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