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Research Article

Phytochemical Screening *n*-hexane Extract of Sponge *Xestospongia testudinaria* from Spermonde Archipelago

Septaria Yolan Kalalinggi[1]*, Nunuk Hariani Soekamto[2], Firdaus[2], Felycitae Ekalaya Appa[3], Muhammad Irmawan[1]

[1] Program Studi Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Palangka Raya, Kalimantan Tengah, Indonesia
[2] Departemen Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Hasanuddin, Makassar, Sulawesi Selatan, Indonesia
[3] Program Studi Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Cenderawasih, Jayapura, Indonesia

*Email: septariayolankl@mipa.upr.ac.id

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Abstract

One of the islands in Indonesia that is inhabited by sponges of various types is the Spermonde Archipelago, which is located in the southern part of the Makassar Strait. Sponge X. testudinaria is one of the sponges that dominates the Spermonde Islands. The difference in environmental conditions where the sponge lives causes the sponge X. testudinaria to have a different adaptation mechanism in producing secondary metabolites as self-defense. Research on the of compounds from n-hexane extract sponge X. testudinaria has been carried out using phytochemical tests. Extraction begins with maceration using n-hexane solvent then the solvent is moved using an evaporator. Identification of extract functional groups using IR spectra. The results of the phytochemical screening indicated the presence of alkaloids and steroids which were supported by IR spectrum data indicating the presence of aliphatic NH, C=O and CH groups.

Introduction

Spermonde archipelago is an archipelago in Indonesia with an area of approximately 926.241 km^2, making it a habitat for various types of marine life including sponges. One of the sponges that dominates more than 90% of sponge species in the Spermonde Islands is *Xestospongia testudinaria* (Marzuki, 2018). *X. testudinaria* is a sponge of the genus *Xestospongia*. Several groups of secondary metabolitesthat have been successfully isolated from the sponge *Xestospongia* genus, namely sterols, alkaloids, quinones, and brominated aliphatic hydrocarbons. Isolated sterol compounds are (22E, 24S)-24- methylcholesta4,7,22,25-tetraene-3-one (He et al, 2016), aragusterol I, 21-O-octadecanoyl-xestokerol A, and petrosterol (Nguyen et al, 2013), cholesterol, 24-hydroperoxy-24-vinyl, saringosterol, 24-methylcholest-5-ene-3,25- diol (Zhou et al, 2011), langcosterol A, dan 24-hydroperoxy-24-vinylcholesterol (Nguyen et al, 2018). Other Sterol compounds: xestosterol palmitate, 18'-bromooctadeca-7'E, 9'E-diene-7',15'-diynoic acid, l6'-bromo- (7'E,11'E, l5'E)-hexa deca-7',11', l5'-triene-5',13'-diynoic acid (Gamal et al, 2016) and 18-bromooctadeca- (9E,17E)-diene- 7,15-diynoic acid (Ayyad, 2015).

Some alkaloid compounds: aaptamin, isoaptamin, dimetil (oxi) aaptamin, dimetil ketal (Calcul et al, 2003), *N-methylniphatyne A* (Arai et al, 2016), hachijodin A or 3-[12-(N-metoksiamino) dodecanil] piridin (Tsukamoto et al, 2000), and Manzamine C (Agustina et al,

2018). Quinone compounds: Xestoadociaquinone A (He et al, 2015), Eight pentacyclic compounds are xestosaprols F-M (Dai et al, 2010) and xestolactone (Centko et al, 2014). Brominated aliphatic hydrogen compounds: methyl (7E,9E,13E,15Z)-14,16-dibromo hexadeca-7,9,13,15-tetraen-5-triynoate (Yang et al, 2018), methyl (E, E)-14,14-dibromo-4,6,13-tetradecatrienoate (He et al, 2015), Metafuran H, xestospongic acid (Zhou et al, 2011), and xestospongiamide (Ayyad et al, 2015).

The presence of secondary metabolite compounds makes the sponge *X. testudinaria* have a certain bioactivity, one of which is toxic to *A. salina* shrimp. Research related to the toxicity of sponge *X. testudinaria* against *A. salina* has been conducted by Swantara & Rita (2018), it is known that the sponge methanol extract has an LC_{50} value of 31,62 ppm which is categorized active (Swantara and Rita, 2018). The extract also had an active activity against Hela cells in the very active category with IC_{50} value of 1,327 ppm (Swantara & Rita, 2018). In addition, three sterol compounds namely 24-hydroperoxy-24-vinylcholesterol, saringosterol, and 29-hydroperoxystigmasta-5,24(28)-dien-3β-ol extracted with alcohol and partitioned with petroleum ether, ethyl acetate, and butanol have LC_{50} values were significant against *A. salina* L. larvae of 0,56 – 6,99 µM (Zhou et al, 2011).

Phytochemical screening was carried out to identify secondary metabolites from plant extracts and marine natural materials such as sponges. Phytochemical screening is characterized by specific color changes after the addition of several reagents to the sample. The color change that occurs in phytochemical screening is caused by compatible binding between each of the secondary metabolites in the sample (Apriliani et al, 2020). The difference in the environmental conditions in which the sponge lives cause sponge *X. testudinaria* has different adaptation mechanisms in producing secondary metabolites as self-defense. Based on the description above, phytochemical screening was carried out on the n-hexane extract of *X. testudinaria* sponge and identification of functional groups with IR spectra.

Materials and Methods

Materials

Xestospongia testudinaria sponge obtained from the Island of Badi, Pangkep Regency, South Sulawesi. The materials used in this study were *Xestospongia testudinaria* sponge powder, several organic solvents such as technical n-hexane, chloroform pa, technical ethyl acetate, technical acetone, phytochemical reagent Liebermann-Buchard, Dragendorff, Wagner, iron (III) chloride, H2SO4 10%, TLC plate (Kieselgel 60 F254 0.25 mm brand), silica gel 60 (Merk, catalog number 7733), silica gel60 (Merk, catalog number 7734), silica gel 60 (Merk, catalog number 7730), Marine NaCl (Sigma, catalog no. S-9883), DMSO (Merck, catalog no. 802912), and *Artemia salina* Leach shrimp eggs.

Instruments

The tools used in this study are glassware, glassware, blenders, funnels, separating funnels, Buchner funnels, rotary evaporators, digital scales, Vigreux distillation devices, micropipets, microplates, eppendorf tubes, crystal filters, containers droplets, thin layer chromatography (TLC) (chambers, capillary tubes, pencils, cutters and ruler), and UV lamps, microscopes. While for spectrometryanalysis are used IR spectrometers with the FTIR 8501 Shimadzu variant.

Procedure Isolation

5 kg of powder sponge *X. testudinaria* macerated using n-hexane solvent for 1x24 hours several times, then filtered to produce maserat (n-hexane extract). The extract obtainedwas subsequently evaporated to remove the solvent. The evaporation process obtained thick n-hexane extract with a deep yellow color. After that, extract analyzed by phytochemical assay.

Phytochemical Test

n-hexane extract was tested for phytochemical using several reactions including steroid and terpenoid testing using 10 drops of acetic anhydride and 3 drops of H_2SO_4. Flavonoid testing using 1% iron (III) chloride solution in water. Alkaloid testing carried out several reagents namely Dragendorf reagent (0,8 gram $Bi(NO_3)$ added CH_3COOH 10 mL and 40 mL water, then mixed

with a solution made from 8 grams KI in 10 mL of water), Wegner reagent (2,5 grams of iodine added 2 grams of KI and 10 mL of water and then diluted with distilled water to 200 mL), Meyer reagent (1,36 grams of $HgCl_2$ added 0,5 gram of KI was dissolved then diluted with diluted distilled water to 100 mL).

Identification of Functional Groups

Identification of functional groups is done by measuring the spectrum using a spectrometer FT-IR.

Results and Discussion

Phytochemistry assay

n-hexane exract of sponge *X. testudinaria* is brownish yellow. Phytochemical assay of extractn-hexane sponge *X. testudinaria* (Table 1).

Table 1. Phytochemical assay of extract n-hexane sponge *X. testudinaria*

No	Test	Reagent	Test Results
1	Steroid	Lieberman-Burchard	+
2	Terpenoid	Lieberman-Burchard	-
3	Alkaloid	Dragendrof	-
		Wegner	+
		Meyer	-
4	Flavonoid	Mg + amil alcohol	-

Based on Table 1. Alkaloid and steroid compounds detected in n-hexane extract by giving a brick red color change after the addition of a Wegner reagent (Alkaloids Wegner) and green color afterthe addition of a Lieberman-Burchard reagent (steroid). Thus, steroid and alkaloid compounds are dominant in n-hexane extract. There may also be other compounds in the extract but not detected because the components are still complex.

Taxonomy and Morphology Sponge X. testudinaria

Classification of sponge *X. testudinaria* which is the object of this study is as follows (Van Soest,2008; WORMS, 2019):

Kingdom : Animalia
Phylum : Porifera
Class : Demospongiae
Order : Haplosclerida
Family : Petrosiidae
Genus : *Xestospongia*
Species : *Xestospongia testudinaria*

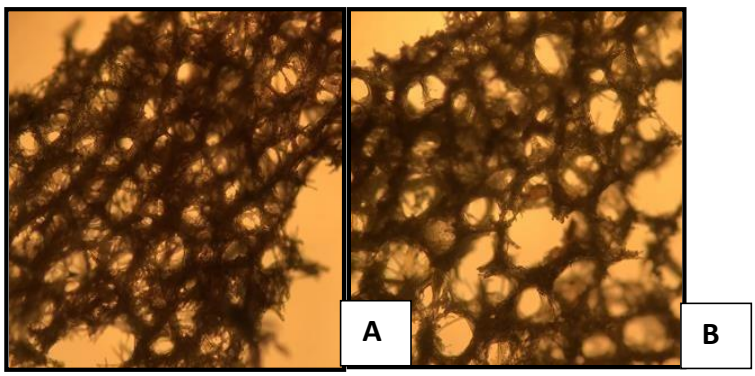

Figure 1. Skeleton of *Xestospongia testudinaria*: (A) ectosomal; (B) choanosomal

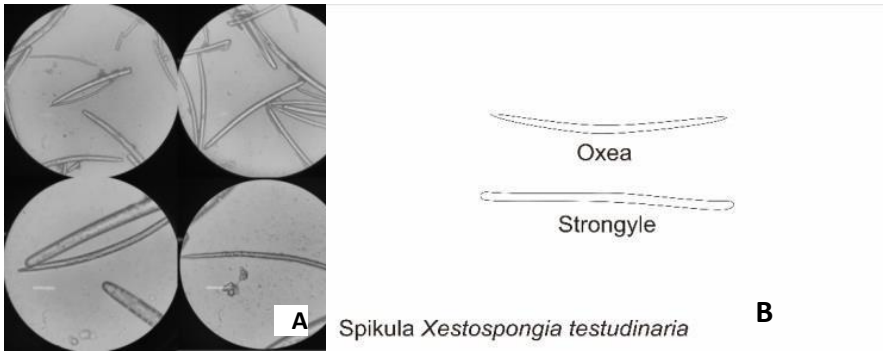

Figure 2. Spicules of *Xestospongia testudinaria*: (A) and (B) (Ackers & Moss, 2007)

The morphology of sea sponges is strongly influenced by physical, chemical and biological environmental factors. Specimens that are in an open environment and have big waves tend to have short growth or also propagate. On the other hand, specimens of the same type in a protected environment or in deeper waters and with calm currents tend to grow upright and tall and have a symmetrical and larger body as a result of a more stable environment when compared to the same type in shallow waters. (Marzuki, 2018).

X. testudinaria is found in the lagoon reef area, in front and behind the reef with a depth of 5-15 meters. The color of the sponge is peacock brown. The sponge is shaped like an upright cup which has been described as a volcanic sponge with a pattern of vertical worms or ridges on its outer surface. The ridge may be highly prominent and extend at right angles to 5 cm from the surface or more flat and form a rounded knob-like shape. The texture of the sponge body is hard, springy, the body tissue is very compact and is interspersed with channels with a diameter of 0,5 (Fromont, 1990; Hatami *et al.,* 2022).

Figure 3. Sponge *Xestospongia testudinaria* from Spermonde Archipelago, Indonesia

Structure Elucidation FTIR Analysis

IR spectrum data of n-hexane extract showed the presence of NH groups (3417 cm^{-1}) which were supported by the absorption of C-N groups (1261 cm^{-1}), supported also by the absorption of1095 cm^{-1} indicating the presence of aliphatic amine groups. Uptake of 2926 and 2854 cm^{-1} shows aliphatic C-H groups supported by the presence of CH_2 (1462 cm^{-1}) and CH_3 (1377 cm^{-1}) groups. There is also an absorption of 1651-1732 cm^{-1} which shows the presence of an aromatic group withthe substitution of para amplified with a signal at the wave number 804 cm^{-1}. Furthermore, the absorption of 1712 cm^{-1} indicates the presence of C = O ketone groups, C = C olefin groups are found in wavenumber 1651 cm^{-1}. Wavenumber 1556 cm^{-1} shows the aromatic C = C group. The Haloalkana C-Br group is also found in wavenumber 393 cm^{-1}, besides that 2320-2355 cm^{-1} is the wavenumber forfatty acid compounds. Based on the interpretation of FTIR data, n-hexane extract

showed that the classof compounds contained in the extract included alkaloids and steroids. This is positively correlated with the phytochemical results obtained.

Conclusions

Phytochemical screening of the n-hexane extract of the sponge *X. testudinaria* showed that the positive n-hexane extract contained alkaloid and steroid compounds which were supported by the presence of aliphatic NH, CN, C=O, and C-H groups in the IR spectrum.

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