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# Phytochemicals Screening and Antioxidant Activity Test of *Isis Hippuris* Methanol Extract

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**Abstract :** *Isis hippuris* (Sea bamboo) had been well-known by the East Indonesian and usually used as jewelry and sold as an export commodity. This research aims to understand phytochemicals screening results and antioxidant activity of *Isis hippuris* also to discover antioxidant content of *Isis hippuris* different parts and maceration times. Extraction method used was maceration technique with methanol as solventfor 24, 48, and 72 hours (1:8 of sample : solvent). Qualitative phytochemicals test were done by measured alkaloid, flavonoid, steroid/ triterpenoid, saponin, phenol hydroquinon dan tannin. The antioxidant test was done using 1,1-*diphenyl*-2-*picrylhydrazil* (DPPH) 0,2 mM method with 1:4 of DPPH and sample ratio. $IC_{50}$ (*inhibitory concentration*) value was used as a parameter to interpret antioxidant activity. The results of phytochemicals screening of *Isis hippuris* skin showed that alkaloid, flavonoid, phenol, steroid, and saponin were founded while from axial parts contained alkaloid, flavonoid, phenol and steroid. DPPH $IC_{50}$ results of *Isis hippuris*skin part with 24, 48 and 72 hours maceration times, respectively were 635,26 ppm, 635,61 ppm dan 663,40 ppm, while on the axial part were 870,34 ppm, 887,74 ppm, and 899,52 ppm. DPPH $IC_{50}$ value either on the skin or axial parts were higher than 200 ppm which means chemical compounds founded on Sea Bamboo were less active but still have potential as an antioxidant.
**Keywords:** phytochemical, sea bamboo, *Isis hippuris*, antioxidant, DPPH.

## Introduction

Marine organism potency as a new bioactive source is widely studied in recent years. Long evolution history of marine organisms leads to their very high molecular diversity. As the second highest diversity after Brazil, Indonesia has numerous islands with vast ocean compared to Brazil land. It makes Indonesia as the highest marine mega biodiversity in the world. New bioprospecting of many new natural ingredient compounds came from the marine environment[1]. It emphasizes that marine organisms are an important source for research and development of new medicines[2]. Most of the new marine natural compound have bioactivity as antibacterial, antiviral, antitumor, antituberculosis, antioxidant, antidiabetic and anti-inflammatory[3].

Among the variety of marine organisms, the sponge is the richest source of bioactive materials[4], such as antiviral[5,6], antibacterial[7], and antifungal[8]. In addition, some extracts of marine organisms have antioxidant such as phenol, more effective flavonoids and safer than synthetic antioxidants, like *butylated hydroxytoluene.* Phenolic acid antioxidant, polyphenol, flavonoid inhibit radical peroxide, hydroperoxideor *lipid peroxyl*, inhibits oxidative mechanism, thus preventing degenerative diseases, also useful as an anti-tumor and has a preventive effect on liver damage. Flavonoids has anti-inflammatory and antioxidant capabilities to inhibit the process of oxidative stress on cardiovascular disease and neurodegenerative. Antioxidants are substance that

able to counteract the effects of free radicals. Negative effects caused by free radicals include premature aging, coronary heart disease and cancer[9].

**Figure 1**. **Sea Bamboo (Isis hippuris) from Biak Sea, Papua**

Other than a sponge, gorgonian also proved to contain various bioactive materials such as anticancer and anti-inflammatory[10] and one of the gorgonian species which widely studied is Sea Bamboo (*Isis hippuris*). Sea Bamboo contain *hippuristanol*which has the properties as antiviral. *Hippuristanol* able to inhibit virus replication process, and as anticancer compound[11]. *Isis Hippuris* also contain hydrocarbon and fatty acids such as Naphthalene, Xylene, Phenylacetonitrile, 1,2 Benzenedicarboxylic and phenol derivatives[12].

However, the antioxidant activity of Sea Bamboo has not been documented until now. Therefore, this study aims to determine the outcome of phytochemical screening and antioxidant activity of Sea Bamboo which expected to be utilized in pharmaceutical, food, industry, and others fields.

## Materials and Methods

### Sample Preparation

Sea Bamboo samples were collected from Biak Sea, Papua. Samples were cleaned and aired for 7 days. Dried samples were separated between the skin and axial(inside) parts. Each part were finely crushed using the machine and sieved by 65 mesh sieve and stored for further tests.

### Tools and Materials

Tools used in this research were 65 mesh sieve, blender, analytic scale, aluminum foil, WhatmannNo.42 filter paper, UV-Vis Spectrophotometer, test tube, beaker glass, oven, pipette, evaporator, vortex, incubator, Erlenmeyer flask and tube racks.

Other than Sea Bamboo samples, chemical materials used were ethanol 95%, concentrated H2SO4 solution, Mayer and Dragendorff reagent, chloroform, ammonia, concentrated HCl, HCl I N, FeCl3 1%, Mg powder, anhydride acetic acid, 1,1-*difenil*-2-*pikrilhidrazil* (DPPH), and aquadest.

### Extraction

Samples extraction were done by maceration technique. Each sample (skin and axial parts) of Sea Bamboo measured for 40 g, and soaked in 320 mL methanol 95%, left in a shaker for 24, 48 and 72 hours, then filtered with Whatmann No.42 filter paper. The filtrate extractevaporated by a rotary evaporator in $40^0$C until the solution becomes concentrated. Crude extract was weighed to determine rendement based on solvent types using following formula:

Rendement (b/b) = $\frac{\text{Dry extract weight (g)}}{\text{Initial sample weight (g)}}$ x 100%

**Phytochemical Tests**[13]

Qualitative phytochemicaltests are the first stage in determining chemical compounds in simplicityand crude extract of Sea Bamboo (*Isis hippuris*). The objective of qualitative phytochemical testing is to determine chemical compounds and bioactive components of Akar Bahar, as well as provide additional information regarding the existence of particular compound by color examination. Qualitative phytochemicalstest consists ofalkaloid, flavonoid, steroid/triterpenoid, saponin, phenol hydroquinone and tannin.

**Alkaloid Test**

Forty milligrams extracts were added with 2 mL chloroform and 2 mL ammonia then filtered. The filtrates were added with 3-5 drops of concentrated H2SO4 then shaken until two layers formed. Acid fractions were taken and added with 4-5 drops of Mayer and Dragendorff reagent. If sediment formed, it means samples contain alkaloid. Mayer reagent gives white colored sediment while Dragendorff reagent gives yellow-red colored sediment.

**Phenolic Test**

Forty milligrams extracts were added with 10 drops of FeCl3 1%. If the extracts positively contain phenol, the solution color will turn green, red, purple, blue or pitch-black.

**Flavonoid Test**

Forty milligrams extracts were added into 100 mL hot water, boiled for 5 minutes, and then filtered. Five milliliters filtrates were added with 0.05 mg of Mg powder and 1 mL concentrated HCl, then shaken well. Positive test results are shown by the color changes to red, yellow or orange.

**Saponin Test**

Forty milligrams extracts were added into 10 mL aquadest while shaken for 1 minutes, then 2 drops of HCl 1 N were added. If the foam formed remain stable for ± 7 minutes, then the extracts positively contain saponin.

**Steroid/Triterpenoid**

Forty milligrams extracts were added with 10 drops of CH3COOH glacial and 2 drops of H2SO4. The solution then slowly shake and settled for few minutes. Steroid indicated by the change in blue or green while triterpenoid gives red or purple.

**Tannin**

Twenty milligrams fine extracts were added with ethanol until completely submerged. One milliliters solutions moved into test tube and 2-3 drops of FeCl3 1% were added. The positive result indicated by color change into bluish-black or green.

**Antioxidant activity test using DPPH method**[14]

Sea Bamboo extracts (skin and axial part) from 24, 48 and 72 hours maceration times, each treatment were dissolved into methanol pro-analysis solution with a concentration of 100, 150, 200, 250 and 300 ppm. DPPH reagent solution was made by dissolving DPPH into 0,2 mMmethanol pro-analysis. Four milliliters of each concentration solution were added with 1 mL 0,2 mM DPPH. The solutions were homogenized with vortex and settled down for 30 minutes. Then, the solutions absorbance against methanol were measured at 517 nm using Spectrophotometer UV-Visible[13]. Inhibition percentage ($IC_{50}$) was counted using following formula:

$$\% \text{ Inhibition} = \frac{\text{Blank abs.- Sample abs.}}{\text{Blank abs.}} \times 100\ \%$$

Description:

Blank abs. = Solvent without sample absorbance value
Sample abs. = Extract absorbance value

The calculation results were included into regression equation with extract concentration (µg/mL) as abscissa (X axis) and inhibition percentage of antioxidant as ordinate (Y axis). $IC_{50}$ value was counted when inhibition percentage at 50% using y = ax + b equation.

## Results and Discussion

### Phytochemicals Screening

Based on phytochemicals screening the result of Sea bamboo skin and axial parts, positive results were found on several secondary metabolite (Table 1).

**Table 1. Phytochemicals Screening Result of Sea Bamboo Skin and Axial Parts**

| Phytochemicals test | Sea Bamboo part | | Standard |
|---|---|---|---|
| | Axial | Skin | |
| Alkaloid | | | |
| - Alkaloid Meyer | + | + | Little yellowish sediment formed |
| - Alkaloid Dragendroff | + | + | Orange sediment formed |
| Flavonoid | + | + | Color change from green to orange |
| Phenol | + | + | Color change from green to blackish green |
| Steroid | + | + | Color turned into bluish green |
| Tannin | - | - | Color turned into blackish green |
| Saponin | - | + | Stable foam formed |
| Triterpenoid | - | - | Brownish ring formed |

(+) detected; (-) not detected

Based on phytochemicals screening result, the methanol extract of Sea Bamboo skin contained an alkaloid, flavonoid, phenol, steroid, and saponin, while in axial part contained an alkaloid, flavonoid, phenol, and steroid. Secondary metabolite components found between the skin and axial were relatively same.Secondary metabolite components of Sea Bamboo skin was founded on spicula of coenzyme layer while hard axial allegedly made from stockpiled metabolism results. Akar Baharis rich of essential nutrients elements such as protein, fat, and carbohydrates which become a valuable food source for predators. Life sustainability of Sea Bamboo leads to metabolite compounds production for predator protection. The active compound contained in octocoral animals are used as colony protection, formation and rapid expansion for colony habitat. Allelopathy also used as a strategy to reclaim habitat from new coral[15].

Wagner and Mayer's test showed that alkaloid was founded on ethanol extract of Kelor skin bark. Wagner test leads to sedimentation of reacted chemical compounds. The positive alkaloid result of Wagner test indicated by light brown to yellow sediment which expected as Potassium-alkaloid. On Wagner test, K+ ion will bind to nitrogen creating a coordinate covalent bond of alkaloid resulted in Potassium -alkaloid sediment. On alkaloid test using Mayer reagent, nitrogen will react with K+ ion of Potassiumtetraiodomercurate(II) creating Potassium-alkaloid sediment[16]. In the healthcare sector, alkaloids serve as an analgesic, changing cardiac activity, affects blood circulation and respiration, anti-malarial, uterine stimulant and local anesthetic[17].

Flavonoid test showed a positive result by solution color change into yellow. Flavonoids are included in phenolic compounds which have many -OH bonds with high electronegativity difference (polar compound). This compound easily extracted into ethanol solvent which has polar properties because of its hydroxyl groups

and form hydrogen bonds[18]. Flavonoids are compounds usually found in fruits, vegetables, and beverages with various benefits of biochemical and antioxidant effects. Flavonoid compounds have an antihypertensive effect. Flavonoids also detected as plant's pigment to produce red or blue flowers with a yellow pattern to attract pollinator.

Flavonoid as secondary metabolite was expected from the color of Sea Bamboo colony. The color of colony affected by unicellular algae zooxanthellae pigment that lives symbiotically inside its coenzyme tissue[15]. Flavonoids are polyphenol compound which known has the abilityto seize free-radical particles, hydrolysis, and oxidative enzyme inhibitor, and anti-inflammatory[19]. Flavonoids control growth, photosynthesis, antimicrobial and antiviral. Flavonoids are helpful to protect cell structure, improve the effectiveness of vitamin C, anti-inflammatory, preventing osteoporosis and antibiotic[20-21]. Flavonoid test using Wilstater reagent is done by adding Mg and concentrated HCl for kelor stem bark ethanol extract. The addition of concentrated HCl is used to hydrolyze flavonoid to be its aglycone, by hydrolyzing O-glycosyl. Glycosyl will be replaced by H+ acid because of its electrophilic reaction. Reduction by Mg and concentrated HCl will result in red or orange complex compounds of flavonol, flavanone, flavanonol and xanthone[20].

Soft coral *Sinularia capillosa*contained *capilloquinol, a* phenolic compound which extracted using acetone[22]. Positive phenol phytochemical test indicated by color changes from green to blackish green. A phenolic compound commonly used as antibacterial, because it's has the ability to change bacteria cytoplasmic permeability membrane which leads to cell's nutrients leakage and bacteria's cell death or inhibited growth and protein precipitate. Phenol is acidic, due to -OH which easily break away. Phenol also has the ability to form chelate compounds with metal, easily oxidized and form polymers that cause dark colors. The emergence of dark colors of cut or die plant's part due to this reaction, also simultaneously inhibits plant growth[23-24].

Isolate four pregnane steroids compounds from gorgonian *Carijoa* sp. using ethanol as solvent[25]. The research reported that steroid also produced from a variety of soft corals, such as *Sarcophyton* sp.[26]; *Nephthea chabrolii*[26-27]extracted using acetone. Steroidal compounds found in plants can act as protector. These compounds not only works to reject but also attract some other insects[20]. Some types of steroid compounds used as drugs, included estrogen, a type of steroid sex hormones used for contraception as ovulation inhibitor, progestins are synthetic steroids used to prevent miscarriage and pregnancy testing, glucocorticoids as anti-inflammatory, allergy, fever, leukemia, and hypertension and cardenolide is a cardiac glycosides steroid used as diuretics and cardiac reinforcement[28].

Saponin test of Sea Bamboo skin had the positive result. It was shown by foam formation after reagent addition. Saponin content also founded on extracted Sea Cucumber using ethanol-water solvent[29]. successfully isolate thirteen new saponin compounds which havepregnane derived aglycone using methanol-chloroform solvent of gorgonian octocoral *Euniceapinta*[30]. Saponins are active surfactant compound which has polar and nonpolar response with water when shaken and creating micelles[31]. When micelles formed then the polar group will face outward and nonpolar groups facing inwards and this state create a form-like foam.

**Antioxidants activitity of Sea Bamboo Extract**

Comparation results between Sea bamboo skin and axial parts inhibition percentage with extract concentration and maceration times resulted into various interactions (figure 2 and 3).

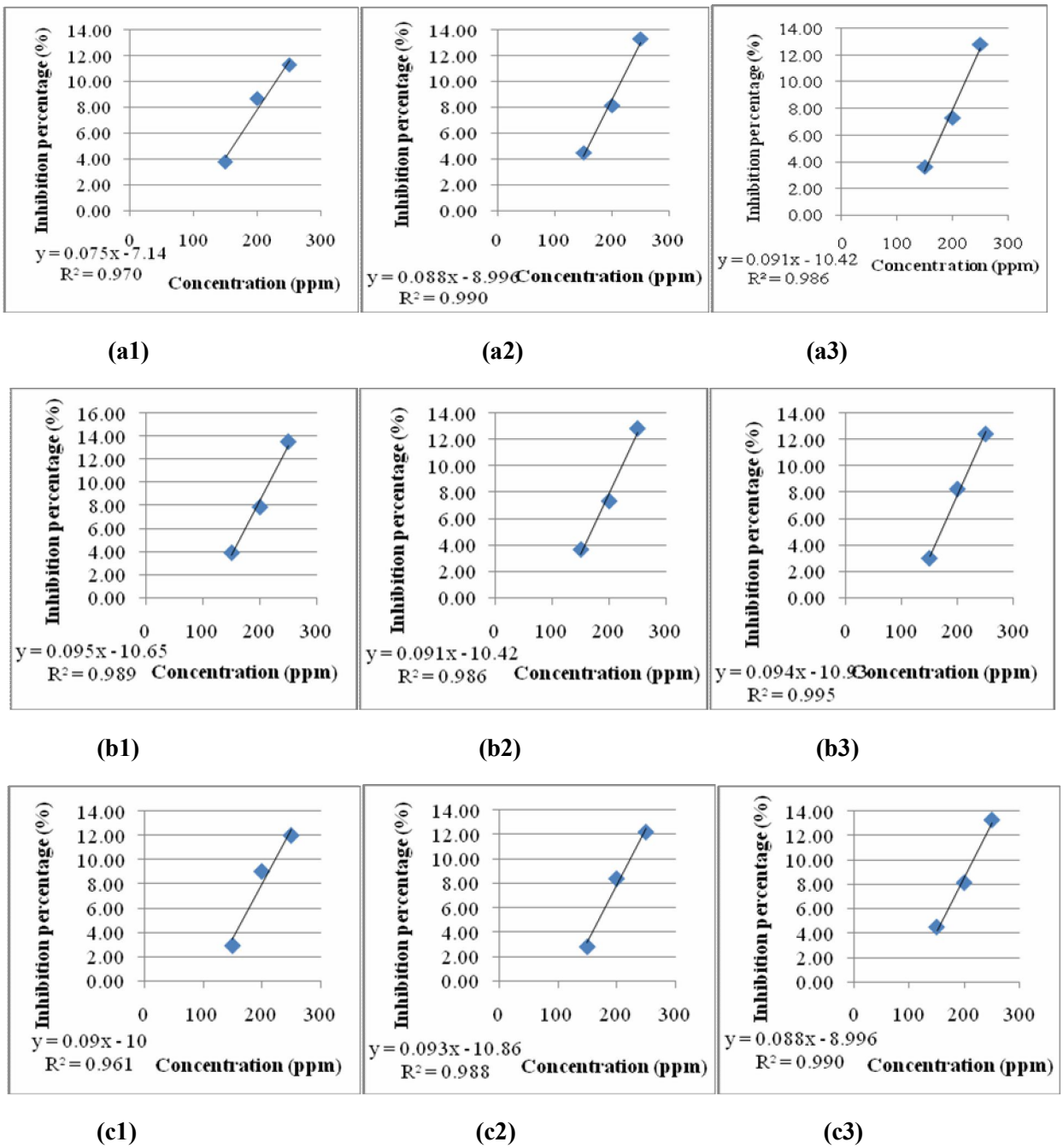


**Figure 2. Inhibition percentage of Sea Bamboo skin extracts toward DPPH (maceration times 24 hours: a1, a2, a3; 48 hours: b1, b2, b3; 72 hours: c1, c2, c3)**

Inhibition percentage results of each examination were used to determined DPPH $IC_{50}$ value. $IC_{50}$ value showed that free radical reduction (DPPH) by Sea bamboo extract was 50%. Rendement and DPPH test results of Sea bamboo skin and axial extracts compared with various maceration times stated in Table 2.

**Table 2. Extract rendements and DPPH test results of Sea Bamboo skin and axial part compared to various maceration times.**

| Body parts | Maceration times | Extract rendements | DPPH $IC_{50}$ value |
|---|---|---|---|
| Skin | 24 | 4,09 ± 0,015 d | 635,28 ± 55,34 a |
| | 48 | 4,23 ± 0,015 e | 635,61 ± 14,20 a |
| | 72 | 4,50 ± 0,053 f | 663,40 ± 13,04 a |
| Axial | 24 | 1,14 ± 0,051 a | 870,34 ± 35,84 b |
| | 48 | 1,24 ± 0,021 b | 887,74 ± 22,70 b |
| | 72 | 1,31 ± 0,042 c | 899,52 ± 10,25 b |

Means within treatment column followed by a common letter are not significantly different at 95% level of probability using LSD as post hoc test.

The extract rendements is supporting data to understand how much the extract obtained from each treatment. Variance analysis of Sea Bamboo extract rendements based on maceration times showed that body part and maceration times had a significant effect on a number of extract rendements. The amount of extract rendementsbetween the skin and axial parts also maceration time treatments were different (Table 2).

DPPH $IC_{50}$ results of Sea Bamboo skin parts with 24, 48 and 72 hours maceration times, respectively, were 635,26 ppm, 635,61 ppm and 663,40 ppm, while from axial parts were 870,34 ppm, 887,74 ppm and 899,52 ppm. Based on analysis of variance, the treatments were significantly affecting antioxidant activity. However, from two factors, only Sea Bamboo parts had significant effects towards IC50 value.

DPPH $IC_{50}$ values, whether skin or axial parts had more than 200 ppm, which means chemical compounds found were less active. Chemical compound has the antioxidant ability if the $IC_{50}$ value is less than 200 ppm[14]. The smaller IC50 value, the higher antioxidant activity. If $IC_{50}$ value ranged between 200-1000 ppm, then it means the compounds less active but still has its potential as antioxidant. Molyneux (2004) also classify antioxidant activity based on $IC_{50}$ value, i.e. very high ($IC_{50}$< 50 ppm), high (50 ppm < $IC_{50}$> 100 ppm), medium (100 ppm < $IC_{50}$>150 ppm), low (150 ppm < $IC_{50}$> 200 ppm), and very low ($IC_{50}$> 200 ppm).

In conclusion, based on the results of phytochemicals screening, Sea Bamboo skin contained secondary metabolite such as alkaloid, flavonoid, phenol, steroid, and saponin, while axial part contained an alkaloid, flavonoid, phenol, and steroid. From antioxidant activity test, Sea Bamboo had very low antioxidant because of $IC_{50}$ value > 200 ppm. Maceration time treatments had no significant effect on Sea Bamboo antioxidant activity.

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