Phytochemical screening and toxicity analysis of South Pacific palm (*Biophytum umbraculum*) ethanolic extract for potential utilization in aquaculture

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Abstract. The South Pacific palm *Biophytum umbraculum*, known as kebar grass, has several bioactive compounds that have the potential to improve reproductive performance, nutrition and health of mammals, thought to have potential for aquatic animals. The aims of this study were to determine the potential of several bioactive compounds in *B. umbraculum* and to evaluate the toxicity level of *B. umbraculum* ethanolic extract (SPEE) for its potential utilization in aquaculture. The research method is maceration and extraction of *B. umbraculum* with 80% ethanol solvent, in order to determine the potential of SPEE's bioactive compounds by phytochemical analysis. The analysis of SPEE toxicity used the Brine Shrimp Lethal Test method at concentrations of 0, 10, 100, 300, 500, 1,000, and 2,000 mg L^{-1}. Qualitative phytochemical analysis showed the dominating compounds in SPEE, such as alkaloids, steroids, triterpenoids, saponins, flavonoids, total phenols, and tannins, with an ethanol extract yield of 16.47%. Quantitative analysis reveals the presence of sitosterol (26.80 mg g^{-1}), stigmasterol (24.60 mg g^{-1}), saponins (17.10 mg g^{-1}), total flavonoids (2.50 mg QE 100 g^{-1}), total phenol (531.87 mg GAE 100 g^{-1}), and tannins (15.37 mg g^{-1}), having potential on reproductive, nutrition and fish health aspects. Based on an antioxidant analysis, the SPEE value of half maximal inhibitory concentration (IC_{50}) was 51.84±2.2, while the BSLT analysis for 24 hours of observation showed that the 50% lethal concentration (LC_{50}) of SPEE was of 903.964 mg L^{-1}.
Key Words: bioactive compounds, fish health, kebar grass, nutrition, reproductive.

Introduction. The development of phytochemical herbs or herbal plants utilization in aquaculture field is widely spread across the world. Herbal plants can possibly improve the cultured animal, in addition of their role as immunity system inductor, appetite-stimulant, feed intake enhancer, gonad maturity inductor, antimicrobial and anti-stress agents (Citarasu 2010; Farizah et al 2018). Plants may avoid infertility due to bioactive compositions that can affect folliculogenesis and steroidogenesis (Telefo et al 2012). Herbal plants contain various compounds, such as phenols, polyphenols, alkaloids, quinons, triterpenoids, steroids, lectin, and polypeptides (Telefo et al 2012; Sembiring & Darwati 2016; Gharaei et al 2020). More than 50 herbal plants have been reported to have biological effects as growth promoters, immunostimulants, anti-stress, antibacteria, antifungi, antiviral and appetitestimulant, and aphrodisiacs (Citarasu 2010; Bertha et al 2016; Farizah et al 2018; Sudrajat & Rasyid 2020; Jatiswara et al 2020). These bioactive compounds have antioxidative characteristics able to regulate several enzymes in steroidogenesis and to scavenge reactive oxygen species (ROS) or as a hormon production regulator in ovary (Telefo et al 2012). Spirulina is a microalga which contains 60-70% protein, vitamins (B1, B2, tocopherol), essential amino acids, and essential fatty acids such as g-linolenic acid (GLA) and can be used as additional feed for broodstock to enhance reproductive performance and fish growth (Nainggolan et al 2015). Furthermore, natural additives have been popularly used in the general population for wellbeing and health, as well as possible therapy for some diseases (Abdelnour et al 2019).

Indonesia has diversity of biological resources of around 1,300 plant species that can be potentially be developed as medicines (herbs) (Aminudin et al 2020; Citarasu 2010; Farizah et al 2018; Jatiswara et al 2020). South Pacific palm, *Biophytum umbraculum* Welwitsch, 1859, also known as kebar grass in Indonesia, is a popular herbal plant used for medical or veterinary purposes, as well as to increase human or animal fertility (Mambrasar et al 2021). *B. umbraculum* is an endemic plant of Papua in Kebar District, Tambrauw Regency, West Papua (Aminudin et al 2020). The papua community, especially Kebar District community, often utilizes this plant as fertility enhancer, malaria medicine, a therapy for wounds, stomach pain, and kidney problems. South Pacific palm contains vitamin E that can inhibit oxidative reactions and bind free radicals (Tethool & Sambodo 2015; Aminudin et al 2020; Wakhidah 2020). *B. umbraculum* has long been used as medicinal plant for fertility problem (curative act) and functional food as antioxidative resources to prevent decreased reproduction performance (preventive) (Aminudin et al 2020). In addition to vitamin E, *B. umbraculum* also contains flavonoids as powerful antioxidant to prevent and medicate cancer attack. Flavonoids can neutralize cancer by activating carcinogenic, inhibiting cell cycle, and inducing apoptosis. Flavonoids can also bind to α-estrogen receptor (REα) in testis and epididimis that can replace estrogenic function and cooperate with testosterone for spermatozoa maturation (Unitly & Inara 2011; Laratmase et al 2020). Furthermore, Wakhidah (2020) explained that *B. umbraculum* contains phosphorus to sustain sexual arousal and calcium for spermatozoa motility.

B. umbraculum contains chemical compounds from alkaloids, saponins, tannins, phenols, flavonoids, triterpenoids, steroids, and glycosides (Sembiring & Darwati 2016; Wakhidah 2020). Steroids in body has roles in cellular protein synthesis, besides steroidal hormone precursors. Reproductive organs are steroidal hormone target (Aminudin et al 2020). Furthermore, vitamin E in a certain level is known to normalize tubulus seminiferus epithelial cells and improve epithelial cells that can contribute to testis mass increase. In addition, vitamin E is an antioxidative agent. An analysis by DPPH method *(*2,2-diphenyl-1-picrylhydrazyl) was performed to determine the amount of antioxidative properties in *B. umbraculum* ethanolic extract. Compounds analysis of simplicial or herbal plant extracts can use the Brine Shrimp Lethality Test (BSLT) method. *Artemia* sp. nauplii are used as test organisms for the BSLT method (Primahana et al 2015). This method is used to determine the toxicity level of steroid-composed materials identified as stigmasterols (Rohmawati & Sutoyo 2018). BSLT analysis calculates total nauplli that presents 50% mortality from several material concentrations (LC_{50} or Lethal Concentration 50) (Sari et al 2016; Hanafi et al 2020). Based on the herbal material utilization potential, a further study regarding the bioactive compound potentials in *B. umbraculum* as a reproduction performance enhancer in fish with series of analyses is necessary to evaluate *B. umbraculum* potential as an alternative natural hormone. This study aimed to determine the potential of several bioactive compounds in *B. umbraculum* and to evaluate the toxicity level of South Pacific palm ethanolic extract (SPEE) and its potential utilization in aquaculture, by studying its influence on reproduction, nutrition and fish health.

Material and Method

Study area. All plant parts, including roots, stem, and leaves were sampled from Kebar District, Tambrauw Regency, West Papua Province, Indonesia (Figure 1). Plant samples were all identified by the Indonesian Institute of Sciences (LIPI) (No. B-645/IV/DI.01/6/2021), Cibinong. Based on the identification results, the plant samples were identified as South Pacific palm under the species name *B. umbraculum* Welwitsch, 1859 (Mambrasar et al 2021).

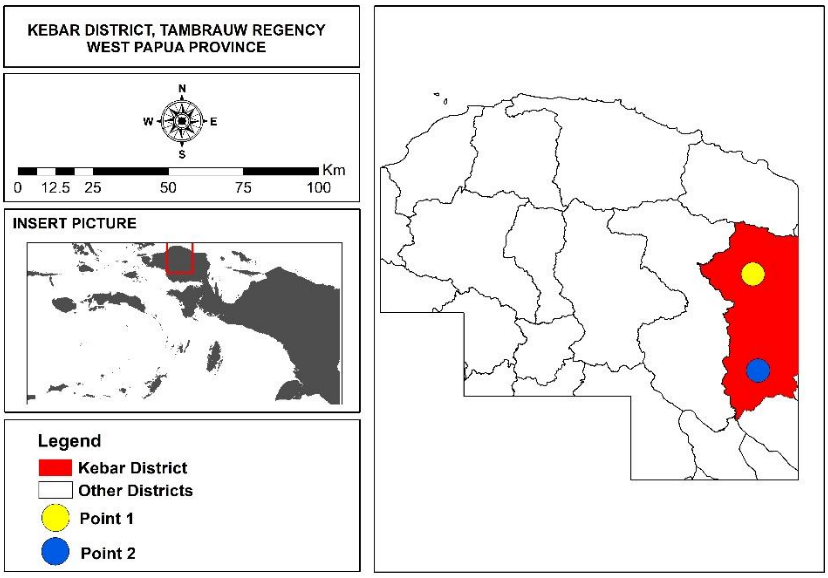

Figure 1. South Pacific palm *Biophytum umbraculum* sampling map in Tambrauw Regency at Point 1: (0º46'54.73"S, 132º23'37.81"E) and Kebar District at Point 2: (1º3'20.59"S, 133º15'6.98"E).

B. umbraculum* *ethanolic extract (SPEE). Extraction aims to select chemical components contained in natural materials. *B. umbraculum* simplicia was extracted and its action on the genetic and molecular aspects of fish reproduction was teste in laboratory at the IPB University's Department of Aquaculture, Faculty of Fisheries and Marine Sciences. Maceration procedure was modified from Balamurugan et al (2013) with ethanol 80% as a solvent (1:5). Simplicia *B. umbraculum* was dried in the open air (24^{o}C), being protected from direct sunlight, obtaining a quantity of 500 g, then grinded roughly,and dissolved in ethanol 80% at 2.5 L. Maceration procedure was repeated three times (3x24 hours) using new ethanol solvent at room temperature in dark condition. Extract was filtered with a Whatman paper 0.2, before concentrating in a rotary evaporator at 45^{o}C. *B. umbraculum* extract ethanol (SPEE) was the end result of this extraction. Extraction process results are measured by the following equation (Nuryanti et al 2021):

Yieid (%) = [Initial sample weight (g) / Extract weight (g)] x 100

SPEE qualitative phytochemical analysis. Qualitative phytochemical analysis was performed in the Laboratory of Analytical Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University. Using the methods of Iqbal et al (2015) and Thouri et al (2017), a qualitative phytochemical analysis of SPEE was performed to determine the presence of secondary metabolites in *B. umbraculum*. The analysis results were observed in symbols (+ and -). Alkaloids presence was occupied after SPEE was added with chloroform 2 mL and three drops of concentrated NH3, then shaken and stood to form two layers. The upper layer was taken with a pippette onto a splot-plate, before adding Meyer, Wagner, and Dragendoff reactants, thus emerging colored precipitates (white precipitate in Mayer reactant, chocolate precipitate in Wagner, and orange precipitate in Dragendorff reactant), were qualified as a positive alkaloid. The SPEE sample was added with heated-aquadest (1:10), magnesium powder, and amyl-alcohol, then shaken and stood to form two layers. The upper layer with a more concentrated color than the lower layer was positive to the flavonoids presence. Phenol hydroquinon was obtained after SPEE was added with methanol, then shaken and pippetted onto a spot plate, before adding NaOH 10%. The color change from cream to red indicated a positive phenol hydroquinon. To identify the existence of steroids/triterpenoids, SPEE was shaken and evaporated until remaining a residue in a reaction tube, before being added with diethyl ether, pippetted onto a spot plate, and added with Lieberman Burchard reagent containing acetic anhydrate and concentrated sulfuric acid (3:1). A positive reaction was marked by a green ring (positive steroids) or a red ring (positive triterpenoids) outside the solution mixture. Tannins were checked in SPEE by adding 1-2 drops of hot aquadest and shaking it together with SPEE, before pippetting onto a spot plate and adding $FeCl_3$ 1% at 1-2 drops. Thus, a blue green color occurred in the mixture, indicating a positive tannin. Meanwhile, the presence of saponins in the SPEE was determined by adding hot aquadest, shaking for about 30 counts spontaneously, and allowing to stand for 2-10 minutes. A stable foam formed indicated a positive saponins.

SPEE quantitative phytochemical analysis. Thin layer chromatography (TLC) is a chromatographic method that uses a thin stationary phase supported by an inert backing to separate the components of a mixture. It can be done on an analytical scale to monitor the progress of a reaction or it can be done on a preparative scale to purify small amounts of a compound (Gogoi et al 2016). Quantitative phytochemical analysis was performed in the Laboratory of Center for Spices and Medicinal Plants, Agency for Agricultural Research, Development, and Laboratory Analysis, Center for Agricultural Postharvest Research and Development, Indonesian Ministry of Agriculture. Analysis was performed following the standard procedure of TLC Scanner (Thin Layer Chromatography - Scanner) and spectrophotometer.

Gas cromatography and mass spectroscopy (GC-MS) characterization. The goal of GC-MS analysis is to identify hundreds of compound components present in plant cells that cannot be identified by conventional phytochemical screening (Awaludin et al 2020). GC-MS analysis was carried out at the Forensic Laboratory, National Police Headquarters, Sentul, Bogor. The presence of compounds from SPEE was identified using the Gas Chromatography and Mass Spectroscopy (GC-MS) at 50-290°C, with interface 0 (290°C), in control mode (split), at a pressure of 20.8 psi, with a total flow of 23.7 mL min^{-1}, a split ratio of 200:1, a split flow of 199 mL min^{-1}, using He gas and an MSD detector.

Antioxidant analysis with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The remaining DPPH violet radicals can be measured using a UV-Vis spectrophotometer configured at around 515–520 nm, to determine antioxidant activity. Antioxidant analysis procedure was performed based on Setiawan et al (2018) and Sirivibulkovit et al (2018). Several SPEE sample solutions were prepared at concentrations of 25, 50, 75, 100, 125, 150 and 200 ppm and Trolox were prepared at concentrations of 20, 40, 60, 80 and 100 ppm. The sample was pipetted and added to DPPH solution (200 ppm) at 1:4 ratio, into a 96-well clear polystyrene microplate, until homogenous. The mixture was incubated for 30 minutes at 37°C, then the absorbance was measured with a microplate reader at 520 nm wavelength. Trolox, as a positive control, was treated the same way as the sample.

Brine Shrimp Lethality Test (BSLT). This method is used to determine the toxic effect of an active ingredient in *Artemia* nauplii. The analysis was carried out in the laboratory of fish molecular genetics and reproduction at the Department of Aquaculture, IPB University, Faculty of Fisheries and Marine Sciences. The SPEE analysis was performed using seawater media at 0 mg L^{-1} as a control, then at 10, 100, 300, 500, 1,000 and 2,000 mg L^{-1} for each treatment. Each concentration was produced in four replications. The 0.1 g of *Artemia* sp. was incubated for 48 hours using seawater in a conical container equipped with aeration under a 40 watt TL lamp at 28-30°C. Nauplii of *Artemia* sp. were harvested by separating them from the shells. About 20 individuals were dropped using a pipette in vial bottles filled with 4 mL of seawater and various SPEE concentrations. The number of dead and alive nauplii was calculated for 24 hours (Fikayuniar et al 2022).

Data analysis. Data from phytochemical tests (qualitative and quantitative) and GC-MS characterization were descriptively analyzed, presented in figures and tables. Meanwhile, data from DPPH and BLST analyses were explained descriptively and analyzed with SPSS 22.0.

Results

SPEE production with maceration method. 20 g of dried *B. umbraculum* simplicia was used. After the maceration process, 3.29 g of ethanolic extract were produced, at a yield of 16.47%. The SPEE was used further for phytochemical (qualitative and quantitative), GC-MS, DPPH, and BSLT analyses.

SPEE qualitative phytochemical analysis. Phytochemical analyses (qualitative) display the presence of certain phytochemical compounds (Table 1). Based on the analysis results, *B. umbraculum* contains various bioactive compounds. SPEE has alkaloids with positive visualization results. Steroids were also positive, like triterpenoids and saponins. Flavonoids were present, with a strongly positive mark. Total phenols presented a strong positive mark. An extremely positive mark was displayed for the presence of tannins.

Table 1

Qualitative phytochemical screening on *Biophytum umbraculum* ethanolic extract

Compound	*SPEE*
Alkaloids	+ +
Steroids	+ +
Triterpenoids	+ +
Saponins	+ +
Flavonoids	+ + +
Total phenol	+ + +
Tannins	+ + + +

negative (-); weakly positive (+); positive (++); strongly positive (+++); extremely positive (++++).

SPEE quantitative phytochemical analysis. The SPEE quantitative phytochemical analysis results are presented in Table 2. From this table, SPEE successively showed steroids consisting of sitosterol (26.80 mg g^{-1}) and stigmasterol (24.60 mg g^{-1}). Furthermore, saponins were 17.10 mg g^{-1}, total flavonoids in SPEE were 2.50 mg QE 100 g^{-1}, total phenols were 531.87 mg GAE 100 g^{-1}, and tannins were 15.37 mg g^{-1}. As a result of the presence of phytochemical compounds like as steroids (sitosterol and stigmasterol), saponins, flavonoids, total phenols and tannins, *B. umbraculum* has the potential to be used in aquaculture to improve reproduction, nutrition, and fish health.

GC-MS characterization. Based on the GC-MS analysis method, SPEE has certain phytochemical components as presented in Table 3. 1,2-benzenediol (pyrocatechol), homovanillic acid (HVA), 9,12,15 octadecatrienoic acid, linoleic acid and palmitic acid are five compounds with the potential to be used in aquaculture for improving reproduction, nutrition and fish health. 1,2-benzenediol (pyrocatechol) is a steroid with an area percentage of 3.35% of the chromatogram total area and a retention time of 5.434 min, with the potential to improve fish reproduction. Homovanillic acid (HVA) is a dopamine hormone with a retention time of 10.907 min and an area percentage of 3.65 of the chromatogram total area, which has the potential to improve fish reproduction. 9,12,15-octadecatrienoic acid as part of the fatty acid group (omega-3) with a retention time of 15,805 and an area percentage of 4.63% of the chromatogram total area, which has the potential to improve reproductive activity and fish nutrition. Linoleic acid as part of the fatty acid group (omega-6), with a retention time of 15.745 min and an area percentage of 5.82% of the chromatogram total area, which has the potential to improve fish nutrition. Palmitic acid as part of the fatty acid group with a retention time of 14,113 min and an area percentage of 7.63% of the chromatogram total area, which has the potential to improve fish health. Furthermore, quinic acid, a tannin, has a retention time of 11.373 min and an area percentage of 7.63% of the chromatogram total area, and the potential to improve fish health.

Table 2

Quantitative phytochemical properties of *Biophytum umbraculum* ethanolic extract, compared to those of several phytochemical herbs

Phytochemical herbs	*Compounds*						*Methods extract*	*References*
	Sitosterol ($mg\ g^{-1}$)	*Stigmasterol ($mg\ g^{-1}$)*	*Saponins ($mg\ g^{-1}$)*	*Flavonoids ($mg\ QE\ 100\ g^{-1}$)*	*Total phenols ($mg\ GAE\ 100\ g^{-1}$)*	*Tannins*		
SPEE *Biophytum umbraculum*	26.80	24.60	17.10	2.50	531.87	15.37 $mg\ g^{-1}$	Maceration with ethanol 80%	This research
Panax spp.	7.35-59.09	2.22-23.04	3.09	59.74-137.30	10.46-95.98	-	Maceration with ethanol 70%	Zhu et al (2004) Kim (2016) Farizah (2022)
Melastoma malabathricum	160.80	-	114.60	1.63-60.29	2.47-431.69	0.0068 $mg\ g^{-1}$	Maceration with ethanol 80%	Danladi et al (2015) Noviyanty et al (2020) Awaludin et al (2020)
Sauropus androgynus	6.90	11.00	20.07	148.94	276.86	28.51 $mg\ TAE\ g^{-1}$	Maceration with ethanol 70%	Subekti (2007) Andari et al (2022) Laveena & Chandra (2018) Savitri & Februyani (2013)
Pimpinella alpine	2.00	1.12	6.00-10.00	3.91-5.04	3.17-4.32	158.83 $mg\ TAE\ g^{-1}$	Maceration with acetone 80%	Rahardjo et al (2006) Rebey et al (2019) Sofiane et al (2017)

Table 3

Biophytum umbraculum ethanolic extract characterization based on GC-MS analysis

Compound	*Chemical formula*	*Compound group*	*Potential activity utilization on cultivation*	*RT*	*Relative area (%)*	*Reference*
1,2-Benzenediol (Pyrocatechol)	$C_6H_5NaO_2$	Steroids, Saponins	Reproduction fish	5.434	3.35	Timmers & Lambert (1989)
Homovanillic acid (HVA)	$C_9H_{10}O_4$	Total phenols (methoxyphenols)	Reproduction fish	10.907	3.65	Hernandes-Rauda et al (1996)
9,12,15 Octadecatrienoic acid	$C_{18}H_{30}O_2$	Fatty acid (omega-3)	Reproduction fish and fish nutrition	15.805	4.63	Awaludin et al (2020)
Linoleic acid	$C_{18}H_{32}O_2$	Fatty acid (omega-6)	Fish nutrition	15.745	5.82	Awaludin et al (2020); Kolb et al (2018)
Palmitic acid	$C_{16}H_{32}O_2$	Fatty acid	Fish health	14.113	7.63	Librán-Pérez et al (2019); Rebey et al (2019)
Quinic acid	$C_7H_{12}O_6$	Tannins	Fish health	11.373	7.22	Zhang et al (2012)

DPPH analysis. DPPH analysis of SPEE showed a concentration of half the maximal inhibitory value (IC50), namely 51.84±2.20. This value was lower than the IC50 value reported by Aminudin et al (2020), for the ethyl acetate fraction and the crude extract of *B. umbraculum*, at 85.94±5.75 and 257.75±4.90, respectively, however, this value presents a high antioxidative activity. Antioxidants inhibitors of free radicals, which can damage human cells due to oxidation reactions, resulting in an excess of reactive oxygen species (ROS), that can cause cell metabolism disturbances. Free radicals are unstable reactive oxygen species due to unpaired electrons, which capture electrons from biological macromolecules.

BSLT analysis. The BSLT analysis results are presented in Figure 2. Various SPEE concentrations (0, 10, 100, 300, 500, 1,000, and 2,000 mg L^{-1}) after probability unit test using *SPSS* obtained a lethal concentration (LC_{50}) value of 903.964 mg L^{-1}, which was higher than the ethanolic extract of katuk leaves *S. androgynus* (552.208 mg L^{-1}). Different concentration levels of SPEE showed a different effect on the death of nauplii of *Artemia* sp.

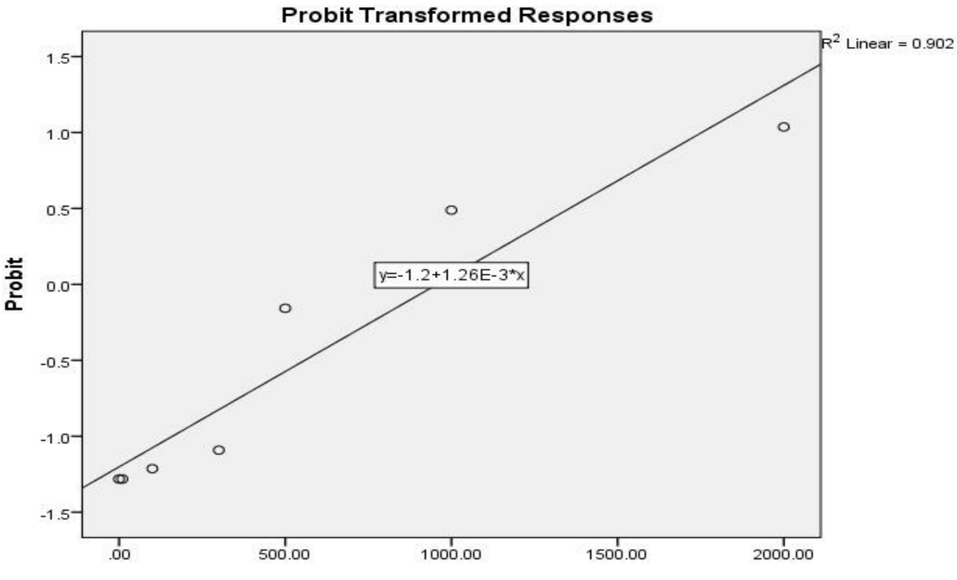

Figure 2. Concentration log of *Biophytum umbraculum* ethanolic extract.

Discussions

Plant sample identification and SPEE production. The 20 g dried *B. umbraculum* simplicia (80% ethanol) produced 3.29 g of concentrated ethanolic extract, with a extract yield value of 16.47%, by the maceration method. This extract yield value was higher than the methanolic extract yield of South Pacific palm *Biophytum sensitivum* at 14.17% (Lisangan 2014). However, this yield value was also slightly lower than the yield value after traditional extraction method at 18.32% (Aminudin et al 2021). This condition occurred as maceration method used a non-polar solvent, that usually produce less than 10% yield. According to Ngo et al (2017), extraction methods that use semi- or non-polar solvents will produce a yield of <10%, while using polar solvents will produce yield of >10%.

Different yields also indicate different species and types of solvents used (Aminudin et al 2020). The amount of yield obtained was thought to be due to the semi-polar ethanol solvent, which could attract semi- to non-polar compounds in *B. umbraculum*simplicia. Zhao & Zhang (2014) added that different solvents dissolved different compounds, depending on their polarity degree. The dielectric constant in ethanol solvent was greater (24.30), so it can attract more secondary metabolites from the *B. umbraculum* simplicia.

SPEE qualitative phytochemical analysis. Based on Table 1, the phytochemical screening (qualitative) of SPEE, alkaloids, steroids, triterpenoids, saponins, flavonoids, total phenol, and tannins are present in South Pacific palm with positive to extremely positive visualization.

SPEE quantitative phytochemical analysis. The highest SPEE fractions were obtained for the steroids stigmasterol (24.60 mg g^{-1}) and for the total phenols (531.87 mg GAE 100 g^{-1}). This condition indicates the potential of phytosterols in South Pacific palm that can be utilized to improve the fish reproduction performance. Based on Table 2, phytochemicals in *M. malabathricum* (160.80 mg g^{-1}) were composed mainly of sitosterol, which is a steroid compound. The SPEE value in *B. umbraculum* (26.80 mg g^{-1}) was slightly higher than in *Panax* spp. (7.35-59.09 mg g^{-1}), *S. androgynus* (6.90 mg g^{-1}) and *P. alpine* (2.00 mg g^{-1}). Common plant sterols are β-sitosterol, campesterol, and stigmasterol. Sitosterol has a similar chemical structure as cholesterol that will become the main ingredient for steroid hormone synthesis. Phytosterols are steroid-alcoholic group found in plants with similar chemical structure as cholesterol Farizah et al 2018; Sudrajat & Rasyid 2020; Aminudin et al 2020. In detail, sitosterol is a steroid synthesis substrate in adrenal gland. Consuming phytosterol can induce estradiol, estrone, and SHBG (sex hormone-binding globulin) concentrations in blood serum that will be metabolized as pregnenolone and dehydroepiandrosterone as steroid hormone precursors (Schulz et al 2008). It is also claimed that plants contain a variety of alkaloids, coumarins, triterpenoids, β-sitosterol, steroid lactones, and essential oils as potent bioactive compounds that can affect the digestive process by boosting enzyme activity, nutrient digestibility, and feed absorption, leading to increased fish growth (Chakraborty et al 2013).

Almost the same as the sitosterol, the stigmasterol, which is a steroid compound, has the potential to be used for increasing the reproductive ability of fish. Table 2 shows that the *B. umbraculum* SPEE's stigmasterol concentration is of 24.60 mg g^{-1}, higher than in other phytochemical herbs, such as *Panax* spp. (2.22-23.04 mg g^{-1}), *S. androgynus* (11.00 mg g^{-1}), and *P. alpine* (1.12 mg g^{-1}). Sterols that are usually thought to only exist in animals as sex hormones have been found in plants as phytosterols. Apart from being a compound that plays a role in reproductive activity (steroids), Rahmawati (2008) stated that phytosterols also play a role in inhibiting the activity of Gram-negative bacteria. Furthermore, phytosterols are a group of steroid alcohols with a chemical structure similar to cholesterol, but obtained from plants and serving as fish endocrine homeostatic modulator. Previous studies have shown that stigmasterol has antioxidative, thyroid hormones inhibition, antiperoxidative, and hypoglycemic activities. Several studies have also shown that β-sitosterol has anti-inflammatory, immune-boosting, anticancer, and pro-apoptotic properties (Lee et al 2018). Phytochemicals can enhance fish non-specific immunity, specific immunity, and disease resistance. The synergism between compounds can be explained by thoroughly studying the mechanism of action of each compound (Chakraborty et al 2013).

M. malabathricum had the highest saponin values (114.60 mg g^{-1}), followed by *S. androgynus* (20.07 mg g^{-1}), SPEE of *B. umbraculum* (17.10 mg g^{-1}), *P. alpine* (6.00-10.00 mg g^{-1}), and *Panax* spp. (3.09 mg g^{-1}). Saponin value obtained from the SSPE phytochemical quantitative test was 17.10 mg g^{-1}. This value was greater than in the cinnamon leaf powder extract (0.01825%) (Susanti et al 2021). Chen et al (1998) stated that several saponins from ginseng *Panax notoginseng* have a positive effect on the *in vitro* survival of human sperm cells, which can increase sperm motility and development. Saponins are triterpene glycosides or surface-active sterols that are present in a wide range of plants. (Chakraborty et al 2013). Saponins have been reported to affect the pituitary hormone releases such as LH, considered to regulate all aspects of teleost reproduction, particularly final oocyte maturation and ovulation (Makkar et al 2007). Saponins found in *B. umbraculum* extract contribute to antioxidant activity (Aminudin 2021). Saponins are steroidal glycosides or triterpenoids play an important role in human and animal nutrition. The ginseng diet significantly increased growth, diet utilization efficiency, and haematological variables in fingerlings of *Oreochromis niloticus* and adult tilapia. Saponin triterpenoid glycosides are found in ginsenosides (or panaxosides). Plant medicinal properties are typically dependent on the presence of several phytoconstituents such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins, and polyphenols, all of which are bioactive bases responsible for antimicrobial activity (Chakraborty et al 2013). Table 3 presents that *S. androgynus* has the highest flavonoids (148.94 mg QE 100 g^{-1}), followed by *Panax* spp. (59.74-137.30 mg QE 100 g^{-1}), *M. malabathricum* (21.63-60.29 mg QE 100 g^{-1}), *P. alpine* (3.91-5.04 mg QE 100 g^{-1}), and SPEE of *B. umbraculum* (2.50 mg QE 100 g^{-1}). Flavonoids have been reported to have antioxidant and anti-inflammatory properties (Nono et al 2016). Moreover, flavonoids have been contributed to prevent lipid peroxidation, inhibit free radical chain reaction, and protect cell membrane from oxidative stress. Thus, Adhikari et al (2018) mentioned that flavonoids have medicinal properties, including the ability to reduce oxidative stress and improve reproductive performance. Flavonoids have been shown to be powerful antioxidants capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals, as well as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic, and vasodilatory properties (Chakraborty et al 2013).

The highest total phenols were found in SPEE *B. umbraculum* (531.87 mg GAE 100 g^{-1}), followed by *M. malabathricum* (2.47-431.69 mg GAE 100 g^{-1}), *S. androgynus* (276.86 mg GAE 100 g^{-1}), *Panax* spp. (10.46-95.98 mg GAE 100 g^{-1}), and *P. alpine* (3.17-4.32 mg GAE 100 g^{-1}). The total phenols concentration obtained from SPEE was 531.87 mg GAE 100 g^{-1}, much higher than by the traditional extraction of South Pacific palm with water solvent, at 147±1.24 mg GAE gDW^{-1} (Aminudin et al 2020). This difference can also be seen from the total phenol value in the ethanolic extract (50%) of South Pacific palm of *B. petersianum*, which produced 205 mg GAE gDW^{-1}, while the total phenol yield from methanolic extract (50%) was only of 87.00±0.044 mg GAE gDW^{-1} (Kumar et al 2017). Different total phenols can be caused by the use of sample types, extraction method, and different solvent types (Aminudin 2021). Consumption of bioactive compounds such as polyphenols in food or drink can boost antioxidant capacity in the body and reduce oxidative stress in reproductive organs. Dietary antioxidants can supplement endogenous antioxidants and help to limit the free radicals action (Aminudin 2020; Ly et al 2015).

In Table 3, *P. alpine* has the highest tannin concentration (158.83 mg TAE g^{-1}), followed by *S. androgynus* (28.51 mg TAE g^{-1}), SPEE *B. umbraculum* (15.37 mg g^{-1}), and *M. malabathricum* (0.0068 mg g^{-1}). Tannins are water-soluble polyphenols that are widely present in plant tissues. Tannins are reported to increase body growth, induce feed intake, improve body metabolism, increase protein digestibility, and act as antioxidants, due to the nature of tannins which are resistant to fungi (Delimont et al 2017). Tannins and saponins contain multiple antioxidant agents (vitamin C and E) which can maintain the semen macroscopic quality (Aminudin et al 2020). Natural products under scrutiny today are equally important anti-infective agents, and tannin structure has antifungal, antiviral, and antibacterial activity. It has been demonstrated that active tannins and antibiotics have a synergistic relationship (Kurhekar 2017).

Phytochemical screening with ethanolic extract was different from traditional extraction methods, based on Aminudin et al (2020), as the latteruse a single polar solvent, i.e. water. The screening results only obtained flavonoids, tannins, and saponins. This condition may occur as other compounds, namely semi- to non-polar compounds, are still bound in the South Pacific palm matrix (Aminudin 2021).

As substrates for biochemical reactions, cofactors and inhibitors of enzymatic reactions, absorbants/sequestrants that bind and remove unwanted constituents in the gut, and compounds that increase the absorption and/or stability of essential nutrients, phytochemicals can confer health benefits. Therefore, it may be argued that phytochemical effect can promote fish growth.

GC-MS characterization. One of six compounds found based on GC-MS analysis, namely 1,2-benzenediol or pyrocatechol, had an activity in the presence of catechol-O-methyltransferase enzymes in African catfish brains, therefore both catechol estrogen and dopamine could be methylated by this enzyme. This suggests that catechol estrogens can influence the methylation (inactivation) of dopamine. Incubation of the forebrain homogenate with dopamine and catechol estrone or catechol estradiol confirmed that these catechol estrogens can inhibit the dopamine methylation. Thereby, the presence of 1,2-benzenediol compounds can help activate dopamine for reproductive process. The primary metabolite produced by the successive actions of monoamine oxidase and catechol-O-methyltransferase on the dopamine (DA) is called homovanillic acid (HVA). Homovanillic acid is used as a reagent to detect oxidative enzymes and is associated with dopamine levels in brain. Dopamine can be metabolized via O-methylated dopamine metabolite enzyme (MAO) to dihydroxyphenylacetic acid (DOPAC) or via COMT to homovanillat acid and 3-methoxytyramine (3MT). In fish, dopamine metabolite levels and their relative importance in the assessment of dopamine catabolism are controversial. In goldfish *Carassius auratus*, the primary dopamine metabolite appears to be DOPAC, in both brain and pituitary gland, while the 3MT and HVA levels are less than 4% of the DOPAC concentrations or are even undetectable (Hernandes-Rauda et al 1996).

9,12,15 Octadecatrienoic acid (a poly-unsaturated fatty acid - PUFA) is a precursor to several hormone-like compounds, including prostaglandins, prostacyclin, thromboxane, and leukotrienes. These compounds assist in controlling blood pressure, heart rate, immune function, nervous system stimulation, muscle contractions, and wound healing. Another function is that serves as a solvent for vitamins A and E. In several studies, hexadecanoic acid and octadecanoic acid have been demonstrated to play antibacterial, antifungal, antioxidant, and anti-inflammatory roles in biological systems (Kartina et al 2019).

One of the essential fatty acids (FAs) is linoleic acid. Because the body cannot synthesize essential fatty acids, they are an important part of the diet. Essential fatty acids cannot be produced by the body and must be received from food. Linoleic acid is an arachidonic acid precursor that can be converted into a wide range of biologically active molecules. As a result, humans must obtain linoleic acid from their diet (Awaludin et al 2020). The research shows that fatty acids affect infection outcome in vivo, which benefits zebrafish by lowering mortality and virus titers. Fatty acid-induced antiviral protection appears to be related to autophagy inhibition and independent of other immune processes such as neutrophil proliferation or type I interferon (IFN) activity. Palmitic acid, when used at low concentrations as an immunostimulant, has shown great promise in preventing spring viremia of carp virus (SVCV) infection; thus, these FAs can help prevent mortality and morbidity caused by viral agents in farmed fish. Nonetheless, the potentially harmful effect of suppressing autophagy in organisms must be considered.

Fatty acids are essential nutrients for the healthy growth of fish because they affect also the immune functions. Several studies have shown that palmitic acid (PA) can modulate infection outcome in vivo, resulting in reduced mortality and virus titers in zebrafish. Quinic acid (QA) is the active ingredient in cat's claw (*Uncaria tomentosa*), and it has been shown to be active in enhancing DNA repair and immunity in model systems, as well as having neuroprotective effects and a rejuvenating potential. Furthermore, according to Zanello et al (2015), the use of quinic acid provides anti-dengue virus activity in vitro, on human cell lines, through a mechanism that inhibits viral replication. As a result, this fact opens up the possibility of using quinic acid to prevent and treat fish health.

DPPH analysis. Low IC_{50} value of SPEE indicates high antioxidative activity. Antioxidants are free radical inhibitors. The impact of free radicals can damage cells due to oxidation, resulting in free radicals and antioxidants imbalance that can cause cell metabolism disturbance. Biological process in the body involve respiration and produce intermediate molecules called Reactive Oxygen Species (ROS). ROS molecules are oxygen-derivate radicals (OH ions, superoxide, nitric oxide, and peroxyl) and non-radical oxygen-derivate molecules (ozone, singlet oxygen, lipid peroxide, and hydrogen peroxide). These ROS condition can come from the outside (extrinsic), such as ultraviolet light, chemical compounds, and air pollution or from inside (intrinsic) such as cellular metabolism

(Aitken 2016). Excess ROS in the body can cause accumulative damage to protein, lipid, and DNA components, which cause oxidative stress.

Oxidative stress is defined as oxidant and antioxidant molecule imbalance that persists in aging and various other diseases (Dudonné et al 2009). Oxidative stress reflects an imbalance between ROS or free radicals and the body's natural defense agents called antioxidant enzymes (SOD, catalase, and superoxidase). ROS and antioxidant enzymes are important component systems in several reproductive processes, including ovarian follicular development, ovulation, fertilization, embryonal development, and implantation (Aminudin et al 2020).

BSLT analysis. Extract toxicity test with BLST method is one of many ways that can be applied to determine the potential of bioactive compounds in medicinal plant extract (Primahana et al 2015). Natural material can be determined first its safety condition by performing toxicity test. The toxicity test results showed that high SPEE concentration impacted on the mortality rate of Nauplii *Artemia* sp. Putri et al (2012) explained that high mortality rate of Nauplii *Artemia salina* was caused by higher extract concentrations, which can be marked as a starting point to determine the long-term toxic dose in animals. Toxicity level of a compound is determined when the extract can kill as many as 50% of the test animals (Awaludin et al 2020). However, the toxicity test presented in this study indicates that the toxicity level of SPEE has a low impact on Nauplii *Artemia* sp. mortality level, thus SPEE usage is in highly safe concentrations.

The *B. umbraculum* has the potential to be used as a phytopharmaceutical ingredient to replace synthetic ingredients that can be used to improve the reproductive performance, nutrition, and health of aquatic animals, especially fish. This is supported by the availability of *B. umbraculum*, which can be obtained in large quantities in the Kebar District, as well as by the ease of its production from organic and non-synthetic active ingredients, at lower prices and based of environmentally friendly raw materials.

The presence of several phytochemical compounds, such as alkaloids, steroids, triterpenoids, saponins, flavonoids, total phenols, tannins (Table 1 and Table 2), 1,2-benzenediol (pyrocatechol), homovanillic acid (HVA), 9, 12,15-octadecatrienoic acid, linoleic acid, palmitic acid and quinic acid (Table 3) in the SPEE explains a high antioxidant effect for very low toxicity values, indicating the potential to improve the reproductive performance, nutrition, and the health of aquatic animals, especially fish.

Conclusions. This study successfully identified and characterized several bioactive compounds from the South Pacific palm *B. umbraculum* ethanolic extract, namely alkaloids, steroids, triterpenoids, tannins, saponins, flavonoids, total phenol and tannins, that have a potential for improving the aspect of reproductive, nutritional, and fish health. The 1,2-benzenediol (pyrocatechol); homovanillic acid (HVA), 9,12,15-octadecatrienoic acid, linoleic acid, palmitic acid and quinic acid also have the potential to increase and improve the reproductive, nutritional and health aspects of fish, as bioactive components. Moreover, the antioxidant activity of the *B. umbraculum* ethanolic extract (SPEE) at IC_{50} was very high, with a value of 51.84±2.20 μg mL^{-1}, and supported by an LC_{50} value of 903.964 mg L^{-1}. With a lower toxicity effect, the SPEE's ethanolic extract is very safe to use to improve the reproductive, nutritional and health aspects of fish.

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