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KARAKTERISASI SENYAWA ANTIOKSIDAN DARI EKSTRAK BAMBU LAUT SULAWESI (*Isis hippuris*) DENGAN GC-MS

Characterization of antioxidant compounds from Celebes Sea bamboo extracts (Isis hippuris) using GC-MS

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ABSTRACT

Sea bamboo (Isis hippuris) is one of the octocorallia classes that has unique biological activity and is beneficial for pharmaceutical development. Evaluation of bioactive compounds from sea bamboo collected from Sulawesi (Celebes) waters has been previously reported but lacks information. Gas chromatography and mass spectrometry (GC-MS) are one of the instruments used to evaluate bioactive compounds. This study aims to evaluate bioactive compounds from extracts of sea bamboo from Sulawesi by GC-MS, analyze their antioxidant properties using the DPPH method, and predict their activity computationally using PASS. Samples of sea bamboo were collected from Kabonga Besar Coast, Palu Bay, Central Sulawesi, Indonesia. Samples were extracted by maceration method using methanol: dichloromethane solvent (1:1). GC-MS evaluation identified six alkaloid-derived compounds, namely tetramethyldiaminoethane, dimethylisopropanolamine, 2,4-dimethylpyrrole, 1-(carboxymethyl)pyridinium chloride, pyridine, 3,4-dimethyl-, and N-nitroso-2-methylthiazolidine. The Celebes sea bamboo extracts have potent antioxidant activity. Tetramethyldiaminoethane and dimethylisopropanolamine compounds are predicted to have antioxidant abilities closely related to their role as NADPH-oxidase inhibitors.

***Keywords**: alkaloids, antioxidant, Celebes, GC-MS, Isis hipurris*

ABSTRAK

Bambu laut (*Isis hippuris*) merupakan salah satu kelas octocorallia yang memiliki aktivitas biologis yang unik dan bermanfaat untuk pengembangan farmasi. Evaluasi senyawa bioaktif dari bambu laut yang dikoleksi dari perairan Sulawesi (Celebes) telah dilaporkan sebelumnya namun masih minim informasi. Kromatografi gas dan spektrometri massa (GC-MS) merupakan salah satu instrumen yang digunakan untuk mengevaluasi senyawa bioaktif. Penelitian ini bertujuan untuk mengevaluasi senyawa bioaktif dari ekstrak bambu laut dari Sulawesi dengan GC-MS, menganalisis sifat antioksidannya dengan menggunakan metode DPPH, dan memprediksi aktivitasnya secara komputasi dengan menggunakan PASS. Sampel bambu laut dikumpulkan dari Pantai Kabonga Besar, Teluk Palu, Sulawesi Tengah, Indonesia. Sampel diekstraksi dengan metode maserasi menggunakan pelarut metanol: diklorometana (1:1). Evaluasi GC-MS mengidentifikasi enam senyawa turunan alkaloid, yaitu tetrametildiaminoetana, dimetilisopropanolamina, 2,4-dimetilpirrol, 1-(karboksimetil) piridinium klorida, piridin, 3,4-dimetil, dan N-nitroso-2-metiltiazolidin. Ekstrak bambu laut Sulawesi memiliki

aktivitas antioksidan yang kuat. Senyawa tetrametildiaminoetana dan dimetilisopropanolamina diprediksi memiliki kemampuan antioksidan yang berkaitan erat dengan perannya sebagai penghambat NADPH-oksidase.

Kata kunci: alkaloid, antioksidan, Sulawesi, GC-MS, *Isis hippuris*

INTRODUCTION

Celebes (Sulawesi) waters hold many marine biota resources, especially the octocorallia class (Nurhayati *et al*., 2020; Putra *et al*., 2022). Sulawesi waters are included in the Wallacea region, an area with unique biodiversity and different from the waters of Western and Eastern Indonesia (Fisher *et al*., 2020). Marine biota resources from the octocorallia class are organisms that produce a unique diversity of chemical molecules (Tanod *et al*., 2019), which are not produced by terrestrial organisms and are reported to have biological activities that are beneficial for pharmaceutical development (Rowley, 2018).

An organism that belongs to the octocorallia class that produce unique biological activities is sea bamboo (*Isis hippuris*). Sea bamboo is a tribe of Gorgonacea, a group of soft corals found in tropical waters (Rowley, 2018). Gorgonian *I. hippuris* is reported to contain many bioactives classified into polyoxygenated steroids, hydrocarbons, phenols, alkaloids, and fatty acids (Sheu *et al*., 2003; Chao *et al*., 2005). Sea bamboo extracts are known to exhibit potential biological activities such as antioxidant (Sayuti *et al*., 2016), anticancer (Trianto *et al*., 2018), antiviral (Chen *et al*., 2011), and cytotoxic (Liang *et al*., 2010). Previous studies reported the potential of sea bamboo from Indonesia, to date, have only come from Flores, NTT (Trianto *et al*., 2004a; Trianto *et al*., 2004b) and Biak, Papua (Sayuti *et al*., 2016; Sayuti *et al*., 2017).

Samples of sea bamboo (*I. hippuris*) collected from Biak, Papua, have previously been evaluated for bioactive compounds by using GC-MS instrument. Gas Chromatography-Mass Spectrometry (GC-MS) is a convenient instrument to determine the quantitative amount of bioactive compounds in an organism extract (Sami, 2022). GC-MS analysis has advantages, including identifying various bioactive compounds, including organic acids, phenolic compounds, alkaloids, phytosterols, esters, and ketones (El-Naggar *et al*., 2023). In addition, GC-MS can also quantify the amount of each bioactive compound identified in an natural extract (Gomathi *et al*., 2015). Therefore, GC-MS analysis can serve as a preliminary screening of the chemical composition of an natural extract, which can help determine the potential therapeutic benefits of an natural extract.

The previous study reported 53 compounds in the ethanol fraction, 40 in the ethyl acetate fraction, and 50 in the n-hexane fraction from Papua sea bamboo extracts by using the GC-MS instrument. The most dominant compounds in the ethanol fraction were 2-butoxyethanol (43.68%), methyl-D-mannopyranoside (20.41%) in the n-hexane fraction, and 2-myristynoyl-glycinamide (19.51%) in the ethyl acetate fraction (Sayuti *et al.*, 2017).

Unlike Papua sea bamboo, there are lack of studies to evaluate Celebes sea bamboo (*I. hippuris*) bioactive compounds by using GC-MS. The compound of an organism can be influenced by the habitat in which it lives. A species will interact with its environment and affect the strategies it uses to produce natural compounds used for survival (Malmstrom, 2010). Previous study was limited just

reporting the group compounds extracted from Celebes sea bamboo such as alkaloids (11.61 ± 0.24 mg CE/g), phenols (18.92 ± 0.24 mg GAE/g), flavonoids (21.24 ± 0.28 mg QE/g), and steroids (36.94 ± 1.39 mg ChE/g) (Muliadin *et al*., 2022).

This research will identify bioactive compounds from sea bamboo extracts from Celebes waters using a GC-MS instrument. In addition, compounds identified by GC-MS will be predicted for activity computationally with PASS. PASS (Prediction of Activity Spectra for Substances) is a computerized tool that predicts the biological activity profile of a drug-like substance based on its structural formula (Filimonov *et al*., 2014). PASS can predict the biological activity profile of a compound, thus saving time and resources compared to traditional compound testing methods in the laboratory (Basha *et al*., 2018). Therefore, this study aims to evaluate the bioactive compounds of sea bamboo extracts from Celebes using a GC-MS instrument and predict its activity computationally with PASS.

RESEARCH METHOD

Sampling and Extraction of Sea Bamboo Extracts

Sea bamboo samples were collected from Kabonga Besar Coast, Central Sulawesi, Indonesia, in December 2016, at coordinates 0°42'32.8" South latitude and 119°39'56.5" East longitude. After being retrieved from the water, sea bamboo samples were washed with seawater. After that, the fresh samples were cut into small pieces and put into bottles for extraction with maceration method (1:2 w/v) using methanol: dichloromethane (1:1 v/v) (MERCK, Germany). Then, the extract was filtered and evaporated with an N-1100 Rotary Vacuum Evaporator (EYELA, USA) (Muliadin *et al*., 2022).

Evaluation of Compound with GC-MS

Gas chromatography and mass spectrometry were performed to evaluate the compounds from sea bamboo extracts (Tanod *et al*., 2019). The extract of the sample redissolved with 1 mL methanol (5 µg/mL). Then, the extract was injected into gas chromatography (Hewlett-Packard-HP 6890 with a mass spectrometer, Agilent Technologies Inc., USA). The column used was dimethylpolysiloxane 5% diphenyl (Elite 5MS), with a length of 30 m and a diameter of 250 µm. Helium gas was used as carrier gas. Samples were injected with a separation ratio of 50:1 v/v. The temperature was initially kept at 150°C, then increased to 240°C at 10°C/min, and maintained for 22 minutes. The scanning range was 50-550 amu.

Antioxidant activity assay of sea bamboo extracts

The antioxidant activity of the sea bamboo extract was determined by the DPPH scavenging method (Dewanto *et al*., 2018), which was compared with Vitamin E. A 20 mg of extract sample was added to 100 mL ethanol (200 mg/L), then diluted to produce a 20, 40, 60, 80, and 100 mg/L solution. The same stage was also carried out for the Vitamin E as control. Then, a 2 mL of each sample concentration was added with 2 mL of 50 µM DPPH solution. After that, it was homogenized and left for 30 minutes in a dark room at room temperature. The absorbance value was measured at a wavelength of 517 nm. The percentage of sample inhibition was using equation :

$$Inhibition\ (\%) = \frac{(Blank\ Absorbance - Sample\ Absorbance)}{Blank\ Absorbance} \times 100\%$$

Determination of IC_{50} (Inhibitory Concentration) was determined by plotting the percent of inhibiton on the y-axis and and sample concentration on the x-axis to obtain a linear regression equation (y=a+bx).

Prediction the biological activity of sea bamboo extracts

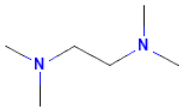
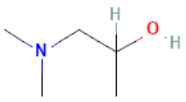
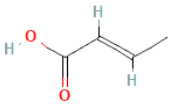
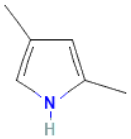
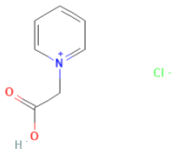
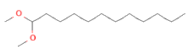
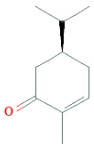
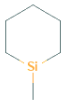
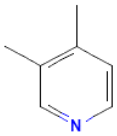
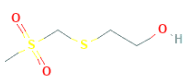
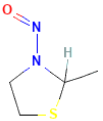
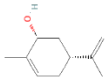
The prediction of the biological activity of each compound begins with the selection of protein targets based on the search results for literature studies related to the mechanism of action of the compounds detected from GC-MS (Riyadi *et al*., 2020). After that, based on the results of the literature study, the biological activity prediction of the sea bamboo extract was carried out with PASS prediction (https://www.way2drug.com/PassOnline/index.php) (Filimonov *et al*., 2014). After that, compounds with a probability to be active values > 0.7 were characterized as drug candidates based on the guidelines of Congreve *et al*. (2003).

RESULTS AND DISCUSSIONS

Sea bamboo extracts were identified by the GC-MS method. The results of the GC-MS evaluation of Celebes sea bamboo extracts are presented in Table 1. Table 1 shows that 14 compounds out of 15 peaks were identified by GC-MS (Figure 1). The six compounds have N atoms and are alkaloid-derived compounds. Generally, an alkaloid contains at least one nitrogen atom in an amine-type structure (Britannica, 2023). Nitrogen atoms in alkaloids are also found bound to a cyclic system (Gutiérrez-Grijalva *et al.*, 2020). Nitrogen atoms in alkaloid-derived compounds act as antioxidant substances by scavenging free radicals (Neganova *et al.*, 2012; Gan *et al*., 2017; Chen *et al*., 2020). Alkaloid components are reported to enhance the performance of phenolic compounds to provide more potent antioxidant effects in extracts from natural materials (Nantongo *et al*., 2018).

The results of GC-MS analysis support the detection of bioactive components in previous studies, which showed the presence of alkaloids and steroids compound from Celebes sea bamboo extracts (Muliadin *et al*., 2022). The research of Qi *et al*. (2010) had previously reported hippuristerone A, an alkaloid compound from *I. hippuris*. A previous study reported that the isolation of a compound from *I. hippuris*, which was identified as a new ester compound (Tanaka *et al*., 1982). Tanaka *et al*. (2002) also reported the chemical screening of a formosan sea bamboo *I. hippuris*, which resulted in the identification of 19 distinct metabolites involved in sphingolipid metabolism, including a sulfoxide compound.

Table 1. GC-MS evaluation of Celebes sea bamboo extracts (*Isis hippuris*)

RT (min)	Area (%)	Compound(s)	Molecular Formula	Groups	2D Structure
2.630	5.39	Tetramethyl-diaminoethane	$C_6H_{16}N_2$	Amine-alkaloi-ds	
2.814	22.01	Dimethylisopro-panolamine	$C_5H_{13}NO$	Amine-alkaloi-ds	
3.445	8.14	Crotonic acid, (E)-	$C_4H_6O_2$	Carbo-xylic acid	
3.762	6.54	2,4-Dimethylpyrrole	C_6H_9N	Hetero-cyclic alkaloi-ds	
3.948	9.01	1-(Carboxymethyl)-pyridinium chloride	$C_7H_8ClNO_2$	Hetero-cyclic alkaloi-ds	
4.145	4.68	Dimethyl sulphoxide	$(CH_3)_2SO$	Sulfo-xide	
4.292	6.96	Dodecanal dimethylacetal	$C_{14}H_{30}O_2$	Ester	
4.961 5.171	4.31 5.86	Silacyclopentane	$C4H_{10}Si$	Cyclo-silane	
6.037	3.74	(+)-carvotanacetone	$C_{10}H_{16}O$	Ester	
6.451	2.36	1-methyl-1-silacyclohexane	$C_6H_{13}Si$	Cyclo-silane	
6.569	6.31	Pyridine, 3,4-dimethyl-	C_7H_9N	Hetero-cyclic alkaloi-ds	
7.336	6.29	3,5-Dithiahexanol 5,5-dioxide	$C_4H_{10}O_3S_2$	Sulfo-xide	
9.870	4.04	N-Nitroso-2-methylthiazolidine	$C_4H_8N_2OS$	Amine alkaloi-ds	
20.724	4.36	(-)-cis-Carveol	$C_{10}H_{16}O$	steroids	

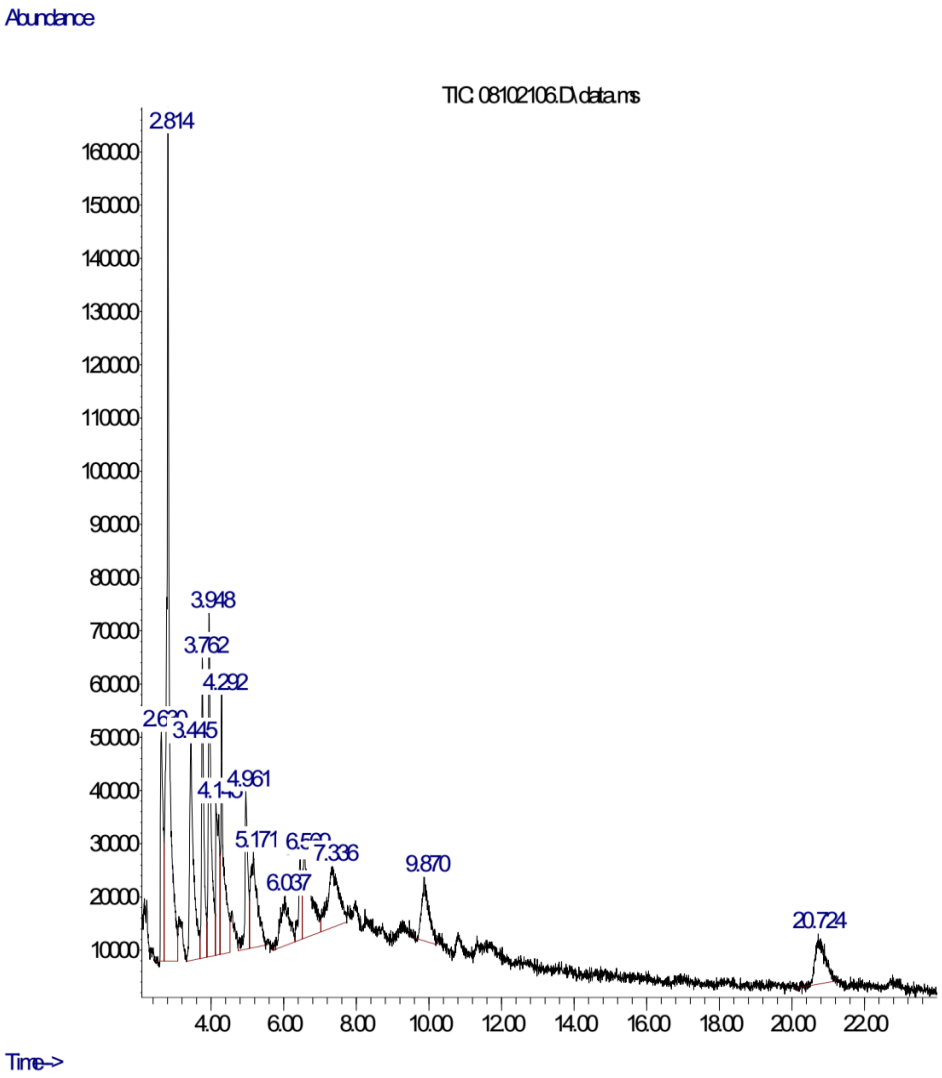


Figure 1. Chromatogram of GC-MS evaluation of Celebes sea bamboo extracts

The bioactivity of compounds analyzed by GC-MS from Celebes sea bamboo extract based on literature studies was presented in Table 2. Table 2 shows GC-MS compounds from Celebes sea bamboo extracts reported to have antioxidant and anti-inflammatory potential. Previous studies reported a positive correlation between many natural products' antioxidant and anti-inflammatory activities (Diaz *et al*., 2012). Preclinical research results show that the antioxidant properties of some natural products correlate with their anti-inflammatory properties (Allegra, 2019). Table 2 also strengthens the notion that alkaloid derivatives have strong potential as antioxidants.

Furthermore, an antioxidant assay was conducted using the DPPH scavenging method to strengthen the suspected antioxidant properties of Celebes sea bamboo extracts. The antioxidant power of Celebes marine bamboo extract was compared with Vitamin E, which is reported to be a potent antioxidant in DPPH scavenging method (Dewanto *et al*., 2021; Muliadin *et al*., 2021). Table 3 shows the IC_{50} of Celebes sea bamboo extracts compared to Vitamin E.

Table 2. The bioactivity of GC-MS compounds from Celebes sea bamboo extracts (*Isis hippuris*)

Compound(s)	Bioactivity	References
Tetramethyldiaminoethane	Anticancer, anti-microbial, antitripanosomatids, anti-inflammatory, and antileishmanial	Thurakkal *et al*. (2022)
Dimethylisopropanolamine	Reduce inflammation	Sliva *et al*. (2019)
Crotonic acid, (E)-	Antioxidant	Jasicka-Misiak *et al*. (2005)
2,4-Dimethylpyrrole	Antioxidant, Inhibitory effects on cholinesterase and α-glucosidas, anti-fungi	Sefer *et al*. (2017); Barut *et al*. (2021)
1-(Carboxymethyl)pyridinium chloride	Antimicrobial	Balasubramaniam *et al*. (2021)
Dimethyl sulphoxide	Neuroprotective antioxidants	Bulama *et al*. (2022)
Dodecanal dimethylacetal	Antimicrobial	Chanprapai *et al*. (2018)
Silacyclopentane	Antimicrobial, antiviral, anticancer and antioxidant	Sarai *et al*. (2021)
(+)-carvotanacetone	Antioxidant, antibacterial, anti-inflammatory and anticancer	Šmejkal *et al*. (2016); Dini *et al*. (2022)
1-methyl-1-silacyclohexane	Anti-inflammatory and anti-viral	Wu *et al*. (2021); Rafiq *et al*. (2022)
Pyridine, 3,4-dimethyl-	Antioxidant, antimicrobial and antiviral	Altaf *et al*. (2015)
3,5-Dithiahexanol 5,5-dioxide	Antimicrobial and anticancer	Hameed *et al*. (2018); El-Bondkly *et al*. (2022)
N-Nitroso-2 methylthiazolidine	Antioxidant	Herrmann *et al*. (2015)
(-)-cis-Carveol	Cytoprotective, antioxidant, antisecretory, and immunoregulatory mechanisms	Serafim *et al*. (2021)

Table 3. IC_{50} of Celebes sea bamboo extracts (*Isis hippuris*) compared with Vitamin E

Samples	IC_{50} (µg/mL)	Compared with Previous Study
Isis hippuris	99.49 ± 0.72	IC_{50} = 469.50 – 3221.07 µg/mL (Sayuti *et al*., 2019)
Vitamin E	39.73 ± 0.67	IC_{50} = 15.87 – 40.83 µg/mL (Tanod *et al*., 2019; Muliadin *et al*., 2021)

According to Blois, there are four categories of antioxidant activity: very strong (IC_{50}<50 µg/mL), strong (IC_{50} between 50-100 µg/mL), moderate (IC_{50} ranging between 100-150 µg/mL), and weak (IC_{50} ranging between 150-200 µg/mL) (Tanod *et al*., 2019). Table 3 shows that Celebes sea bamboo extracts have strong antioxidant activity compared to Papua sea bamboo extracts. This result is probably because Papua sea bamboo has been dried before extraction. The drying process can have an impact on the antioxidant substance of a sample (Husni *et al*., 2014). This antioxidant activity assay strengthens the results of GC-MS analysis of potential compounds in Celebes sea bamboo extract and the literature study in Table 2.

Furthermore, literature studies show that the mechanism of action of alkaloid-derived compounds as antioxidants is related to the inhibition of synthesis, activation, or translocation of NADPH-oxidase subunits (Macáková *et al.*, 2019). Therefore, using PASS prediction, the sea bamboo extracts compounds possessing N atoms were predicted for their activity as NADPH-oxidase inhibitors (Figure 2).

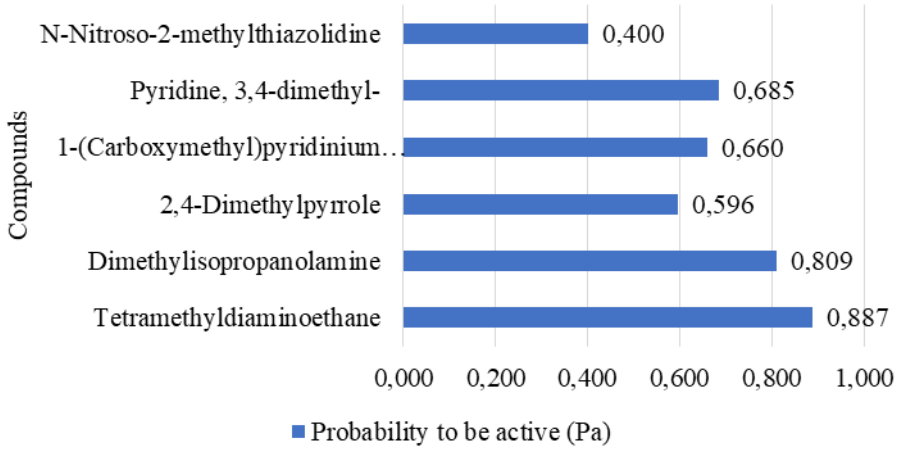


Figure 2. PASS prediction of Celebes sea bamboo extracts as NADPH-oxidase inhibitors

PASS analysis provides the probability to be active (Pa), which estimate the chances of a compound's biological potential being active (Filimonov *et al*., 2014). Pa values also determine the chance of finding biological activity in an experiment; if Pa>0.7, the chance of finding potential biological activity in the laboratory assay is high. Suppose 0.5<Pa<0.7, the chance of finding activity in the laboratory assay is moderate. If Pa<0.5, the chance of finding biological activity in an laboratory assay is very small (Basha *et al*., 2018; Aisiah *et al*., 2020).

Figure 2 shows that two out of six compounds that have N atoms have a high potential as NADPH-oxidase inhibitors, three compounds have moderate, and one compound has a very small potential as NADPH-oxidase inhibitors. NADPH-oxidase is a regulator of reactive oxygen species (ROS) propagation (Rodiño-Janeiro *et al*., 2013; Meitzler *et al*., 2014). In addition, NADPH-oxidase activation is prepared as a substrate (hydrogen peroxide) for myeloperoxidase (Hansson *et al*., 2006). Myeloperoxidase is an oxidant substance toxic to microorganisms, but if its production is excessive, it will cause tissue damage (van der Veen *et al*., 2009).

Therefore, the two compounds from Celebes sea bamboo extracts are predicted to act as antioxidants by inhibiting the spread of ROS and excessive production of myeloperoxidase enzyme, closely related to their role as NADPH peroxidase inhibitors. A previous study reported that tetramethyldiaminoethane acts as a nitrogen oxide inhibitor, which can help reduce oxidative stress and inflammation in cardiovascular disease (Sylvester *et al*., 2022). Dimethylisopropanolamine inhibited NADPH-oxidase activity in human neutrophils (Altenhöfer *et al*., 2015).

Furthermore, the two compounds with N atoms and having high potential as NADPH-oxidase inhibitors were characterized to see the potential of both compounds as drug candidates (drug-lead-like) based on the instructions of Congreve *et al*. (2003). The two compounds were also predicted for toxicity computationally to obtain LD_{50} (Banerjee *et al*., 2018). Characterization of two compounds of Celebes sea bamboo extract can be seen in Table 4.

Table 4. Drug-lead-like compounds and prediction toxicity of Celebes sea bamboo extracts

Parameters	Tetramethyldiaminoethane	Dimethylisopropanolamine
Molecular Weight < 300 Dalton (g/mol)	116.20	103.16
High Lipophilicity (expressed as Log P<3)	0.11	0.07
Hydrogen Bond Donors < 3	0	1
Hydrogen Bond Acceptors < 3	2	2
Rotatable Bonds < 3	3	2
Predicted LD_{50} (mg/kg)	268	1739
Prediction Toxicity Class	III	IV
Prediction Accuracy (%)	100	100

Table 4 shows that both compounds have a lipophilic weight of < 300 Dalton, thus having the potential to be optimized in the discovery of new drug candidates (Raymer and Bhattacharya, 2018). In addition, lipophilicity is related to the transportation process of compounds, including absorption in the gut, cellular level inner membrane permeability, protein binding, and distribution to various tissues and organs, including the brain (Van De Waterbeemd, 2006). Both compounds have lipophilicity < 3, which indicates the compounds have high solubility and low toxicity. High lipophilicity (logP > 5) often contributes to high metabolic turnover, low solubility, and poor oral absorption. In addition, highly lipophilic compounds tend to bind to hydrophobic targets other than the desired target, and therefore, there is an increased risk of promiscuity and toxicity (Hughes *et al*., 2008; Chen *et al*., 2013).

Table 4 also shows that both compounds have hydrogen bond donors and acceptors < 3, which indicates that all nine compounds can bind to target proteins in aqueous media and potentially reduce active depletion and destabilize the solid state without resulting in increased lipophilicity (Kenny, 2022). Rotatable bonds indicate that the number of rotatable bonds is a measure of molecular flexibility and is essential in determining the oral bioavailability of drugs (Khanna and Ranganathan, 2009). In general, both compounds in Table 4 fulfill the drug-lead-like rule, which indicates that both compounds tend to have chemical and physical properties that are orally bioavailable. Both compounds were also evaluated to predict their toxicity with Pro-Tox II software to determine the LD_{50} prediction. The LD_{50} prediction of both compounds is essential in future research using doses in vivo assay (Pillai *et al*., 2021).

CONCLUSION

These research findings show that are 14 compounds contained in Celebes sea bamboo extract detected by GC-MS. A total of 6 out of 14 compounds are alkaloid derivatives, namely tetramethyldiaminoethane, dimethylisopropanolamine, 2,4-dimethylpyrrole, 1-(carboxymethyl)pyridinium chloride, pyridine, 3,4-dimethyl-, and N-nitroso-2-methylthiazolidine. The Celebes sea bamboo extracts have potent antioxidant activity, using the DPPH scavenging method. The 2 out of 6 compounds detected by GC-MS from Celebes sea bamboo extract are predicted to have high antioxidant ability, closely related to their role as NADPH-oxidase inhibitors.

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