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P-ISSN 1693-8666

E-ISSN 2657-1420

Scientific Journal of Pharmacy

JIF JURNAL ILMIAH FARMASI

JIF | Edisi 1 | Januari-Juli 2020 | Hal.1-95

UNIVERSITAS ISLAM INDONESIA

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JURNAL ILMIAH FARMASI
(SCIENTIFIC JOURNAL OF PHARMACY)

Penerbit
Jurusan Farmasi
Fakultas Matematika dan Ilmu Pengetahuan Alam
Universitas Islam Indonesia

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DAFTAR ISI

PENGANTAR DEWAN EDITOR

Alhamdulillah, puji syukur ke hadirat Allah Ta'ala yang telah menganugerahkan kesempatan dan kekuatan, sehingga Jurnal Ilmiah Farmasi (JIF) Vol. 16 No. 1 Tahun 2020 dapat diterbitkan. Pada edisi ini dimuat enam artikel pada kelompok Farmasi Sains dan dua artikel dari kelompok klinis. Artikel yang disajikan pada kelompok Farmasi Klinis mengulas tentang topik efektivitas terapi pada pasien di rumah sakit. Sedangkan artikel pada kelompok Farmasi Sains diantaranya mengetengahkan topik formulasi sediaan obat dari bahan alam.

Besar harapan kami semua artikel yang disajikan dalam edisi ini dapat memberikan manfaat dan menambah wawasan pembaca mengenai perkembangan penelitian dan wacana di bidang farmasi dan kesehatan. Saran dan kritik membangun dari pembaca kami harapkan. Begitu pula, kami mengundang pembaca untuk berpartisipasi mengirimkan artikel untuk dimuat dalam jurnal ini. Bagi pembaca yang berminat, dapat mencermati aturan pengiriman artikel yang sudah ditetapkan dan segera mengirimkannya ke alamat redaksi.

Akhirnya, kami ucapkan selamat membaca dan selamat mencermati, dan tak lupa kami mohon maaf apabila terdapat kesalahan dan kelalaian dalam penerbitan edisi ini.

Yogyakarta, Juli 2020

Dewan Editor

Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-89
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Antidiabetic evaluation of A*rtocarpus odoratissimus* (Moraceae) fruit

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Abstract

Background: Diabetes mellitus causes 4.2 million of deaths worldwide and 79% adults with diabetes are living in low- and middle-income countries. This research providing an alternative therapy through the prevention of postprandial hyperglycemia may help diabetic patients and provide a new utilization model of fruit peel. *Artocarpus odoratissimus*, commonly known as marang, is an edible fruit found in the southern part of the Philippines. Most of the weight of the fruit is discarded and treated as waste.

Objectives: This study aimed to utilize the by-products of marang fruit as a promising pharmaceutical agent by determining the phytochemicals present and *in vitro* antidiabetic activity of the different parts of the fruit.

Methods: Phytochemical screening of phenolics and flavonoids was done through thin layer chromatography. Ten concentrations (2-1000 µg/mL) of the extracts from the peel, pulp, and seeds were evaluated for the *in vitro* antidiabetic assay using alpha-glucosidase enzyme. Mean percent inhibition was calculated, and data was analyzed using ANOVA. The IC_{50} estimates were calculated using the program GraphPad Prism version 8.

Results: Extracts from the fruit parts of *A. odoratissimus* contained phenols and flavonoids and were active inhibitors of alpha-glucosidase enzyme. The fruit peel extract of marang was the most potent (IC_{50} = 48.19 µg/mL) compared to the seed extract, pulp extract, and the standard drug acarbose (p value = 0.035).

Conclusion: The fruit waste, the peel and seeds, has an intense activity against alpha-glucosidase enzyme because of their phenols and flavonoid contents.

Keywords: alpha-glucosidase, *Artocarpus,* diabetes, phenolics, fruit peel

1. Introduction

Diabetes mellitus (DM) is a metabolic condition of the endocrine system where there is an absolute absence or deficiency in insulin secretion or both. The disease now affects more than 100 million people worldwide and is predicted to reach 366 million by the year 2030. It is expected that one in every 10 people will be affected by diabetes in the next ten years. In the present data, China is considered to have the highest incidence of DM, affecting 94.8 million of its population and is closely followed by India and the United States of America (WHO, 2016). Antidiabetic drugs such as acarbose, a known alpha-glucosidase inhibitor, cause gastrointestinal disturbances. Thus, research is continuously being pursued to provide an alternative treatment to DM.

Plant-derived compounds for the management of diabetes have been used in folklore and traditional healing. The Philippines, being a tropical country, is rich in flora and fauna, which could promise a potential source of therapeutic agents. In spite of this, bioactive compounds must be thoroughly investigated to identify their specific mechanism of action concerning diabetes mellitus (Parveen, et al., 2018).

The *Artocarpus* covers about 50 species of deciduous fruit-bearing trees. The name *Artocarpus* is originated from two Greek words, "*artos"* and *"karpus,"* which directly translate to breadfruit (Akinloye, et al., 2015). The genus is known as a source of edible fruits and is widely used as traditional medicines. The genus is a scientific interest since the members contain medicinally important secondary metabolites that possess pharmacological activities. The extracts from the aerial and underground plant parts are used in traditional medicines for the treatment of diabetes, diarrhea, malaria, tapeworm infections, and other ailments (Bapat & Jagtap, 2010).

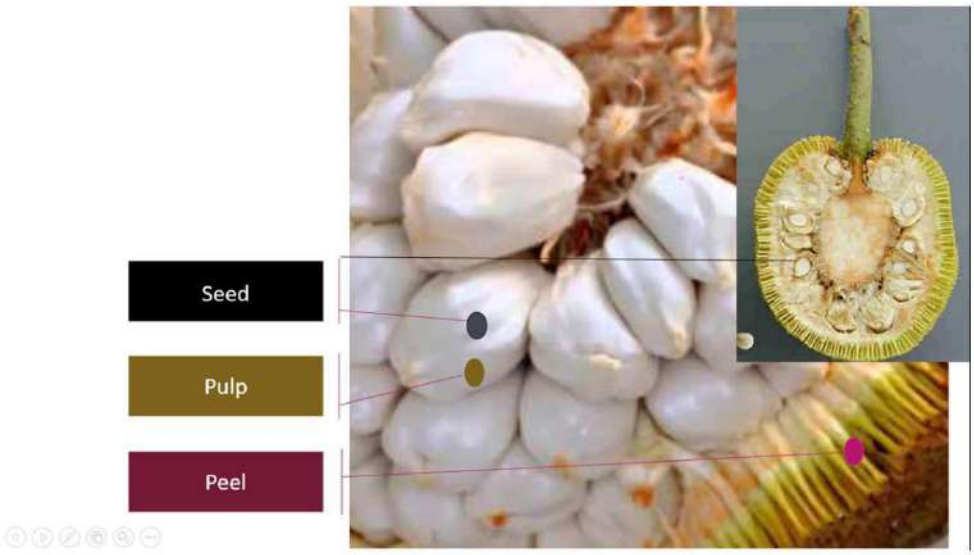

Figure 1. Fruit parts of *Artocarpus odoratissimus*

Artocarpus odoratissimus, locally known in the Philippines as marang, is a fruit native to the Mindanao islands. Locals and tourists commonly consume it because of its tasty and soft flavored pulp. Recent studies proved that the fruit parts displayed superior antioxidant properties (Bakar, et al., 2010). The fruits are sub-globose, measuring 20 cm in diameter, green-yellow and densely covered with stiff, hairy processes measured 1 cm long, borne at the end of long flexible branches, with a mass of seeds embedded in pulp (Godofredo U. Stuart Jr., 2017). The fruit is classified as a syncarp, a type of aggregate fruit that is multiple and made of fleshy fruits (Spjut & Thieret, 1989). The flesh is white, juicy, with a characteristic sweet odor, and edible. The fruits are covered in a stiff and hairy exocarp that represents 50% of the total weight

of the fruit, while the seeds represent 10% of the weight. From this, 60% of the total weight of the fruit is not utilized and considered to be a significant waste product (Bakar, et al., 2009).

To provide a new use to *Artocarpus odoratissimus* by-products and to contribute to the development of a new pharmaceutical product in the future, this research aimed to compare and investigate the presence of secondary metabolites in the different fruit parts of *A. odoratissimus*, namely the pulp, peel, and seed. The antidiabetic potential of the fruit parts was evaluated using the *in-vitro* assay by α-glucosidase enzyme inhibition.

2. Methods

2.1. Plant extraction

The fruits of *Artocarpus odoratissimus* were harvested from a farm located at Brgy. Magsaysay, Marilog District, Davao City, Philippines. It was then brought and authenticated in the Herbarium of the Research Center for Natural and Applied Sciences, University of Santo Thomas, España, Manila, Philippines. Fifty (50) kilograms of matured fruit were separated into pulp, peel, and seeds and washed with distilled water. Fruit parts were dried using a fruit drier in a maintained temperature of 42°C until devoid of watery components. Fruit parts were ground using a homogenizer. Powdered fruit parts were percolated using separate percolator containing 95% ethanol (1:10 w/v ratio) as an extracting solvent. Resulting percolates were concentrated *in vacuo*, and crude extracts were stored at -20°C until further use. The color, odor, and nature of the extracts were examined organoleptically. The percentage yield was computed using the formula:

$$\%\ Yield = \frac{weight\ of\ extract}{weight\ of\ dried\ material} \text{ x } 100 \qquad (1)$$

2.2 Phytochemical screening through Thin Layer Chromatography

The crude ethanolic extracts of *A. odoratissimus* fruit parts were used as a sample for the phytochemical screening through Thin Layer Chromatography (TLC). Approximately 5 mg of the extracts was dissolved in 1 mL dimethyl sulfoxide (DMSO). Samples were applied in commercially available Merck TLC Silica gel 60 F_{254} aluminum sheet plates measuring 20 x 65 cm.

The spotted plates were placed in the equilibrated chamber containing the solvent system to develop the chromatogram. The chromatograms were visualized by inspecting under the ultraviolet (UV) light, short-wavelength (240 nm), and long-wavelength (365 nm) UV before spraying with the reagent for the desired constituents. The spray reagents, antimony (III) chloride, vanillin-sulfuric acid, and potassium ferricyanide-ferric chloride ($K_4Fe(CN)_6$), were

utilized to screen the phenolics and flavonoid phytochemicals present in the *A. odoratissimus* fruit extracts.

2.3 Alpha Glucosidase Assay

The ability of the extracts to inhibit alpha-glucosidase enzyme was evaluated using the method from Chen et al. in 2019, with minor modifications. Quantification was done colorimetrically by monitoring the glucose released from sucrose (Bnouham et al., 2018). Concentrations (2 µg/mL – 1,000 µg/mL) of each crude extract and acarbose were prepared as samples. A concentration of 50 mM phosphate buffer system (PBS), maintained at a pH of 6.8, was used as diluent for the Para-nitrophenyl- β-D-glucuronide (p-NPG) and alpha-glucosidase enzyme.

p-NPG was used as a chromogenic substrate for the alpha-glucosidase enzyme. The p-NPG solution was prepared in 10 mM concentration to screen the most potent fruit part extract. The enzyme hydrolyzed the p-NPG and yielded the chromogenic product ρ-nitrophenol, which was yellow and measured spectrophotometrically at 405 nm at the ultraviolet to the visible range.

The crude extracts of *A. odoratissimus* fruit peel, pulp, and seed were prepared in 10 concentrations using PBS as diluent: 2, 4, 6, 8, 16, 31, 63, 125, 250, 500, and 1000 µg/mL. The same concentrations were prepared for the positive control, Acarbose. A concentration of 0.017 units/mL of alpha-glucosidase enzyme was prepared in cold PBS. All mixtures were freshly prepared for the experiment.

In a 96-well plate, 120 µL of the sample was mixed with 20 µL of α-glucosidase enzyme solution and incubated at 37°C for 15 minutes. After incubation, 20 µL of 10 mM pNPG was added to each well to catalyze the reaction mixture. The plate was then placed in an incubator at 37°C for another 15 minutes. The reaction was stopped by placing 80 µL of 0.2 M sodium carbonate into each well. The mixture was measured spectrophotometrically using the SkanIt software set at 405 nm. Percent inhibition of α-glucosidase was calculated as follows:

$$\%\ Inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of Control} x\ 100 \quad (2)$$

All the sample, blank (PBS), and positive control (acarbose) concentrations were performed in triplicates. The mean and its standard error (SEM) were used to summarize the data from the experiment. One-factor analysis of variance (ANOVA) was used to determine the effect of different concentrations on the percent inhibition of the extracts. The IC_{50} of the extracts was estimated using four-parameter logistic regression models. Also, the IC_{50} values were computed to determine which of the extracts had the most potent activity. All statistical

test were performed in SPSS version 20.0 and GraphPad Prism version 7.0. P-values less than 0.05 indicated significant differences.

3. Results and discussion

3.1. Plant extraction

The extraction done by percolation with 95% ethanol resulted in the extracts of pulp, peel, and seeds. The weight of the pulp extract obtained was 595.2 g, with a yield of 27.8%. The pulp extract is yellow, has an oily consistency and a sweet smell, resembling that of the fruit. The weight of the peel extract was 740.8 g with a yield of 46.95% and can be described as a green, syrupy consistency, and having a characteristic sweet smell. The seeds yielded a yellow, syrupy extract weighing 321.53 g (23.61%) and devoid of odor.

3.2 Phytochemical screening through Thin Layer Chromatography

Extracts of *A. odoratissimus* were screened for the presence of phenolics and flavonoids. The pulp extract chromatogram was developed using the solvent system chloroform-acetic acid-methanol (4:1:2), while the seed and peel extracts were developed using DCM-Methanol (19:2) solvent system. All developed chromatograms were sprayed with different spray reagents. Antimony (III) chloride was used to screen flavonoids and steroids. A positive result for antimony (III) chloride was in the presence of intense yellow to orange visible zones that were also fluorescent under long ultraviolet light. The pulp, peel, and seed yielded 2, 3, and 4 spots for antimony (III) chloride, respectively. Vanillin-sulfuric acid confirmed the presence of higher alcohols, phenols, steroids, and essential oils. The positive result for this spray reagent was the appearance of blue-violet colored spots. Four spots were noted for the pulp and peel, while the seed extract afforded 2 spots. The last solvent system was potassium ferricyanide – ferric chloride ($K_4Fe(CN)_6$). The display of blue spots denoted positive results. The pulp, peel, and seed extracts identified 3 positive spots for $K_4Fe(CN)_6$ spray reagent. From these results, all extracts of the fruit *A. odoratissimus* were positive for the presence of phenolics and flavonoids.

Table 1. Phytochemical screening of *Artocarpus odoratissimus* fruit parts

Extract	Solvent System	Spray Reagents	Number of Spots Identified Positive
Pulp	4 Chloroform: 1 Acetic Acid: 2 Methanol	Antimony Chloride	2
		Vanillin-Sulfuric acid	4
		$K_4Fe(CN)_6$	3
Peel	19 DCM: 2 Methanol	Antimony Chloride	3
		Vanillin-Sulfuric acid	4
		$K_4Fe(CN)_6$	3
Seed	19 DCM: 2 Methanol	Antimony Chloride	4
		Vanillin-Sulfuric acid	2
		$K_4Fe(CN)_6$	3

Phenolic acids, such as ferulic and *p*-coumaric acids, are known potent antioxidants and anticancer activities against colon cancer. Ferulic acid was detected in the seed of *A. odoratissimus* (444.40 ± 23.13 µg/g) while none was detected in the flesh (Alkhalidy, et al., 2015). Diosmin, on the other hand, is a flavonoid that could be detected only from the fruit by-product and is used pharmaceutically as an active ingredient for hemorrhoidal preparations. Diosmin was found in the seeds of *A. odoratissimus* with 288.90 ± 70.88 µg/g quantity (Bakar, et al., 2015). Artocarpin is a flavonoid previously isolated in *A. odoratissimus* that can be used in cosmetic products due to its activity on the inhibition of tyrosinase and melanogenesis (Chan, et al., 2018). The phytochemicals which may contribute to the antidiabetic activity are the phenols and flavonoids. The pharmacological activity can be contributed to the reactive phenol moiety, which can scavenge free radicals (Bakar, et al., 2009). Scavenging the free radicals that affect several pathological pathways contributing to hyperglycemia is the target of phytochemicals (Parveen, et al., 2018)

3.3 Alpha glucosidase assay

The highest percent inhibition was seen in the 1,000 µg/mL concentration among all samples. The seed extract attained the highest inhibition of alpha-glucosidase enzyme (98.25±0.16%), followed by the pulp extract (96.32 ± 0.08%), then the peel extract (95.91 ± 0.08%), and lastly, acarbose (76.07 ± 1.64%). The results are promising because all extracts exhibit a comparable anti-alpha glucosidase activity to the positive control acarbose (*p>0.05).*

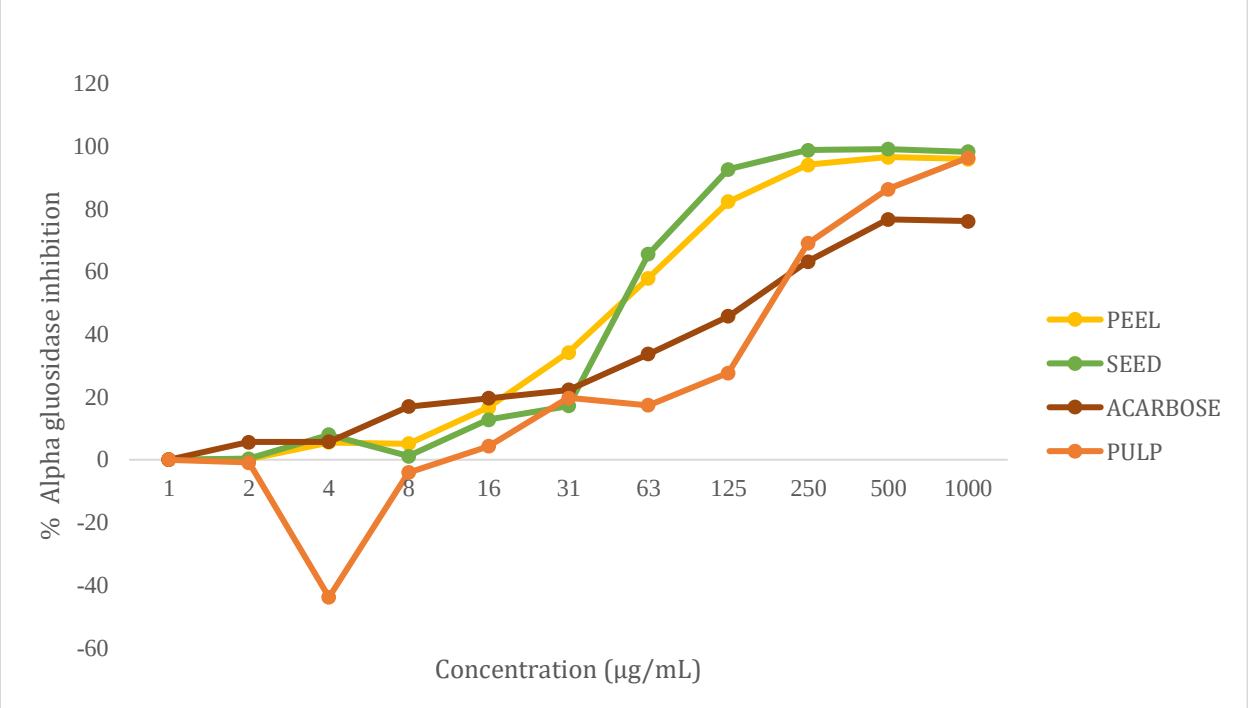

Figure 2. Inhibition activity on α-glucosidase enzyme from *A. odoratissimus* fruit extracts compared to acarbose in different concentrations. Results are reported as mean ± SEM (*n=3; p*>0.05*)* inhibition of the enzyme.

The mean was used to compare the percent inhibition of α-glucosidase and the extracts of *A. odoratissimus* fruit parts. The results reveal that the pulp, peel, and seed extract effects are comparable with the effect of the standard drug, Acarbose. Therefore, the peel and seeds of *A. odoratissimus* that are often considered as food waste can be a promising source of effectively natural alpha-glucosidase inhibitors.

The standard error of the mean was used to compare the concentrations of the extracts and the inhibitions. There is a significant interaction effect [F=3.190, p<0.05] between the extract and the concentrations, indicating that the activity of the extracts is dose-dependent. The concentration of 1000 µg/mL exhibits the highest inhibitory activity against α-glucosidase enzyme.

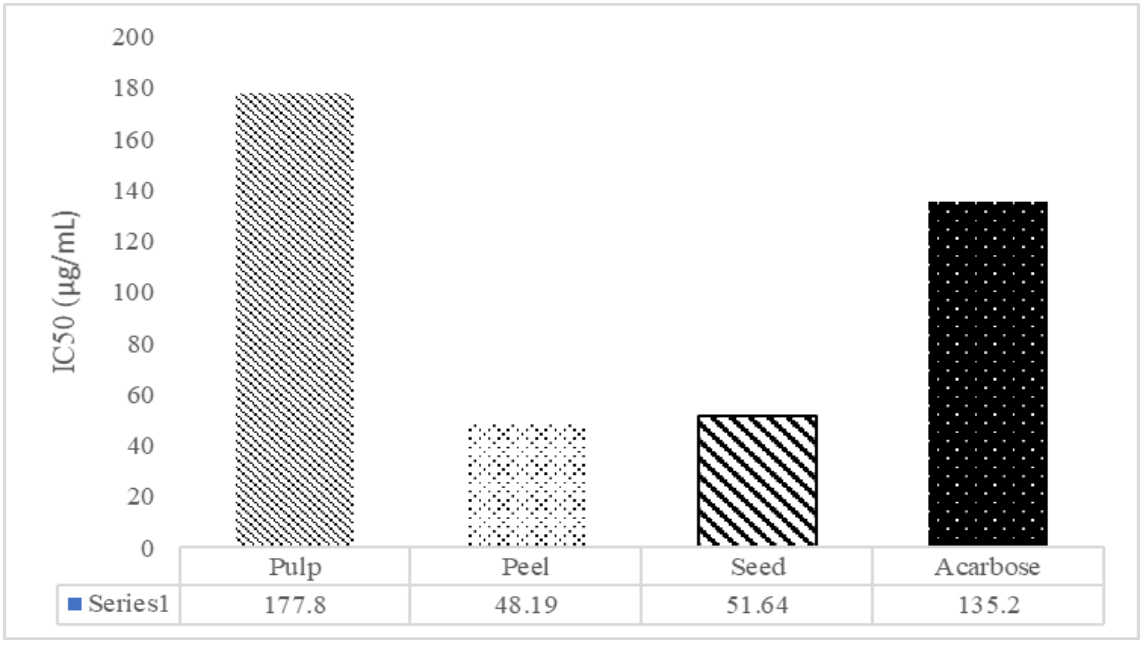

Figure 3. IC_{50} (µg/mL) estimates of extracts from the three fruit parts of *A. odoratissimus* and acarbose. Each value represents the mean (*n=3).*

The estimation of IC_{50} showed that the peel extract was the most effective with a value of 48.19 µg/mL. The extracts from the seed, pulp and acarbose yielded IC_{50} values of 51.64 µg/mL, 177.8 µg/mL, 135.2 µg/mL, respectively. Alpha glucosidase enzyme digests carbohydrates and increases the postprandial glucose level among patients suffering from diabetes mellitus. The inhibition of alpha glucosidase enzyme is an *in vitro* model to reduce the risk of developing diabetes (Parani & Poovitha, 2016). The present study is able to establish the *in vitro* antidiabetic activity from the peel of *A. odoratissimus.* Further research is required to develop a novel drug from fruit peel of *A. odoratissimus.*

The presence of phenolics and flavonoids from the fruit peel of *A. odoratissimus* is a challenge for the discovery and development of antidiabetic molecules. Isolation

of pharmaceutically active compounds against diabetes mellitus should be considered for future studies (Firdous, 2014).

4. Conclusion

The pulp, peel, and seed of *A. odoratissimus* displayed a notable inhibitory activity against alpha-glucosidase enzymes *in vitro*. The highest activity was observed in the seed extract. The pharmacological activity of the fruit parts of *A. odoratissimus* is attributed to the phenolic and flavonoid content of the fruit parts. This study has proven that the peel, which forms about 60% of the *A. odoratissimus* weight, normally underutilized and discarded as a waste product, can be a potential source of antidiabetic agent.

Acknowledgment

The authors acknowledge the National Research Council of the Philippines (NRCP) under the Department of Science and Technology (DOST) and the Philippine Council for Health Research & Development (PCHRD) for funding this research.

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Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-95
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Green synthesis and antibacterial potential of artemisia vulgaris extract in silver nanoparticles against wound bacteria

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Abstract

Background: *Artemisia vulgaris* (*A. vulgaris),* a well-known Chinese traditional herb, is reported to have antibacterial properties, making it a potential agent for wound healing. In our project, we have developed *A. vulgaris* in silver nanoparticles to enhance its effect. This study investigated the antibacterial effects of the synthesised AgNP on common wound bacteria.

Objectives: The objectives of this study were to synthesise *A. vulgaris* in silver nanoparticles and to investigate the anti-bacterial effect on wound bacteria.

Methods: The AgNP was synthesised by the green synthesis method and characterisation tests were carried out to confirm the presence of AgNP in the formulation. The disc diffusion test, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests were carried out to investigate the antibacterial effects of AgNP on common wound bacteria. The AgNP was also tested on probiotics using the disc diffusion test to investigate its effect on probiotics.

Results: The characterisation tests have confirmed the presence of AgNP in the formulation. The AgNP containing all plant concentrations were able to inhibit the growth of all bacteria tested but it required a higher concentration to inhibit the gram positive bacteria. The AgNP had less inhibitory effects on probiotics compared to antibiotics and silver nitrate alone. However, statistical analysis showed that the antibacterial effect of the treatment was statistically insignificant.

Conclusion: The AgNP demonstrated anti-bacterial effects on both gram positive and gram negative wound bacteria, but the effect of the treatment was not statistically significant.

Keywords: *Artemisia vulgaris*, silver nanoparticles, antibacterial, wound bacteria

1. Introduction

Wound infection affects cuts, burns, surgical wounds, and diabetic wounds when pathogens invade through the wound opening, resulting in poor wound healing, gangrene, disability, sepsis, and death (Efron & Barbul, 2001). Normal skin flora may become pathogens that invade wound openings when given the right circumstances. Some of these bacteria have

developed resistance towards the standard antibiotic treatment (Stapleton & Taylor 2007). Hence, the development of new antibacterial agents is important to overcome such resistance.

Artemisia vulgaris, a Chinese traditional herb, is one of the plants which has a potential antibacterial effect. Commonly known as mugwort, it is often used as a traditional remedy or culinary herbs. It is much favoured by the Chinese because it is claimed to have numerous health benefits. It has produced positive results in previous antimicrobial and antioxidant studies (Temraz & El-Tantaway, 2008; Manindra, M. et al, 2016) It is also traditionally used to treat various types of health ailments, such as bleeding, menstrual problems, and skin problems (Fetrow & Avila, 2004).

Herbal agents such as *A. vulgaris* face challenges in terms of drug delivery and bioavailability due to their poor stability and rapid elimination. The extract of *A. vulgaris* can be incorporated into a novel delivery system such as silver nanoparticles to enhance its antibacterial effects while providing a safer alternative for the treatment of wound infection. Silver nanoparticles have been used as a carrier for many herbal agents and shown to have synergistic effects (Aparna, et al, 2015; Orsuwan, et al, 2017). Therefore, in this study, silver nanoparticles containing *A. vulgaris* extracts (AgNP) were tested on common wound bacteria to determine its effectiveness as an antibacterial agent.

2. Methods

2.1. Chemicals and reagents

Silver nitrate was obtained from ACROS Organics™ and used at a concentration of 0.1M to formulate silver nanoparticles. Mueller-Hinton Media was procured from Sigma-Aldrich.

2.2. Preparation of plant extracts

The leaves of *A. vulgaris* were collected from a private garden in Kota Kinabalu, Sabah. The herbarium specimen was then sent to the Sabah Forestry Department to be validated by the Forest Research Centre. Around 500g of leaves collected were dried and ground into coarse powder form. The coarse leaf powder was boiled in 100mL of distilled water at 80^{o}C for 3 hours to prepare the aqueous plant extracts in 5%, 10% and 15% w/v concentrations. The extracts were then filtered with common filter paper. (Ahmed, et al, 2016)

2.3. Green synthesis of silver nanoparticles

To synthesise the plant extract into silver nanoparticles, the filtered extracts were mixed with 50mM silver nitrate solution at a ratio of 1:9 v/v (Erjaee, et al, 2017). The 50mM silver nitrate solution was prepared earlier by diluting 0.1N Acros Organics™ Silver nitrate. The mixture of plant extract and silver nitrate was incubated at a room temperature under the stirring condition for 18 hours. The solution was then centrifuged at 13000rpm for 20 minutes

to separate the nanoparticles from the solution. The nanoparticles obtained were washed with distilled water to remove any unwanted materials.

2.3. Characterisation of silver nanoparticles

The UV–visible absorption of the silver nanoparticles was determined in quartz cuvette using the Perkin Elmer spectrometer. The wavelength range was taken from 300 to 800nm. FTIR spectroscopy was obtained using the ATR method and conducted at a room temperature under dry air. The wave range was set to 4000-400 cm^{-1} (Uznanski, et al, 2017). The particle size of silver nanoparticles was analysed using the Malvern Zetasizer Nano Instrument to determine the particle size distribution and surface charge. A high-resolution transmission electron microscope (Hitachi HT 7700) was employed to analyse the surface morphology and size of silver nanoparticles.

2.4. Investigation of antibacterial effects of AgNP on wound bacteria

The antibacterial tests were performed on common wound bacteria, which included *K. pneumonia*, *P. aeruginosa*, *E. coli*, *B. cereus*, *S. aureus,* and two strains of MRSA.

2.4.1. Disc diffusion test

Muller Hinton Agar (MHA) medium was prepared and the bacterial culture of 0.5 McFarland standard was spread thoroughly on the agar plates. The silver nanoparticles containing 5%, 10% and 15% w/v of plant extract were made into a solution and added into the wells made on the agar plates. Antibiotic discs were used as the positive control whereas 50mM silver nitrate was used as the negative control. The plates were incubated overnight at 37°C. The diameter of inhibition zone was indicative of the inhibitory effect of silver nanoparticles on the growth of the bacteria.

2.4.2. Minimum Inhibitory Concentration (MIC) test

The culture medium, bacterial suspension, and formulation samples with plant extract concentration ranging from 0.125mg/mL to 4mg/mL were added into a 96-well plate and then incubated overnight at 37°C. Dyes were added into each well to analyse the results. The MIC is the lowest concentration where bacterial growth is inhibited by 50%.

2.4.3. Minimum Bactericidal Concentration (MBC) test

Using the samples from MIC test, 5 µl of sample was taken from each well and added onto the agar plate. The plates were then incubated at 37°C overnight. The lowest concentration with no bacterial growth observed on the plates was considered the MBC.

2.5. Investigation of antibacterial effects of AgNP on probiotics

The disc diffusion test was carried out on probiotics, namely *L. casei*, *L. rhamnosus*, *L. arabinosus*, and *L. acidophilus*. The procedure was the same as that carried out on wound bacteria.

2.6. Statistical analysis

The Optima Data Analysis software version 2 was used to analyse the data obtained from the antibacterial tests of AgNP. ANOVA test was used to compare variables between groups. Statistical significance was set to <0.05 for all tests.

3. Results and Discussion

3.1. Characterization of silver nanoparticles

In accordance to previous studies, a reduction reaction of silver nitrate to silver takes place when plant extracts are added (Sathishkumar, et al, 2009; Rasheed, et al, 2017). The reaction can also be confirmed by the UV spectrum, where a broad absorption peak can be seen with λmax at 427nm, indicating the presence of silver nanoparticles (Figure 1). The plant extract itself is capable of reducing silver nitrate without the use of synthetic chemical reagents.

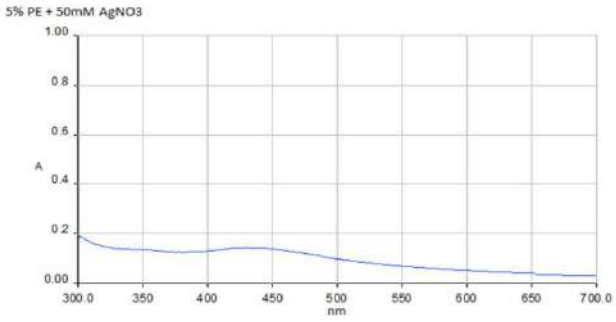

Figure 1: The UV spectra obtained which indicate the presence of silver nanoparticles

From the FTIR spectrum, significant absorption peaks of around 1100cm and 1300cm can be observed (Figure 2) The bands at 1100cm and 1300cm indicate the stretching of alkyl amine and alkyl ketone respectively. These functional groups present in *A. vulgaris* extracts were responsible for such reactions.

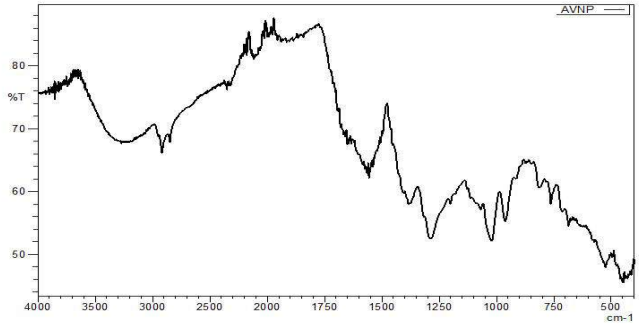

Figure 2: The FTIR spectra showing the functional groups responsible for the reduction reaction to produce silver nanoparticles

The data obtained using zetasizer show that the z-average for the nanoparticles containing 5%, 10%, and 15% plant extracts were 123nm, 240nm, and 237nm, respectively. The particle size estimated by zetasizer seemed to be larger than the usual nanoparticle size. Although the particle size was estimated to be around 200nm, the morphological analysis using TEM showed that most particle sizes fell within 50nm. The zeta potential ranged +20-30mV for all concentrations, indicating that the formulation was stable (Table 1).

Table 1: Particle size and zeta potential of silver nanoparticles containing plant extract

Concentration of plant extract in AgNP	Mean particle size (mm)	Zeta Potential (mV)
50 mg/mL	152.3	+ 24.6
100 mg/mL	224.5	+ 29.0
150 mg/mL	264.4	+ 30.1

The morphological analysis using TEM shows that the nanoparticles displayed a globular shape with a size ranging from 20 to 50nm (Figure 3). The aggregation of silver nanoparticles can be observed, which explains the larger size estimation by zetasizer. It is common that silver nanoparticles aggregate to be in a stable form (Prathna, 2011).

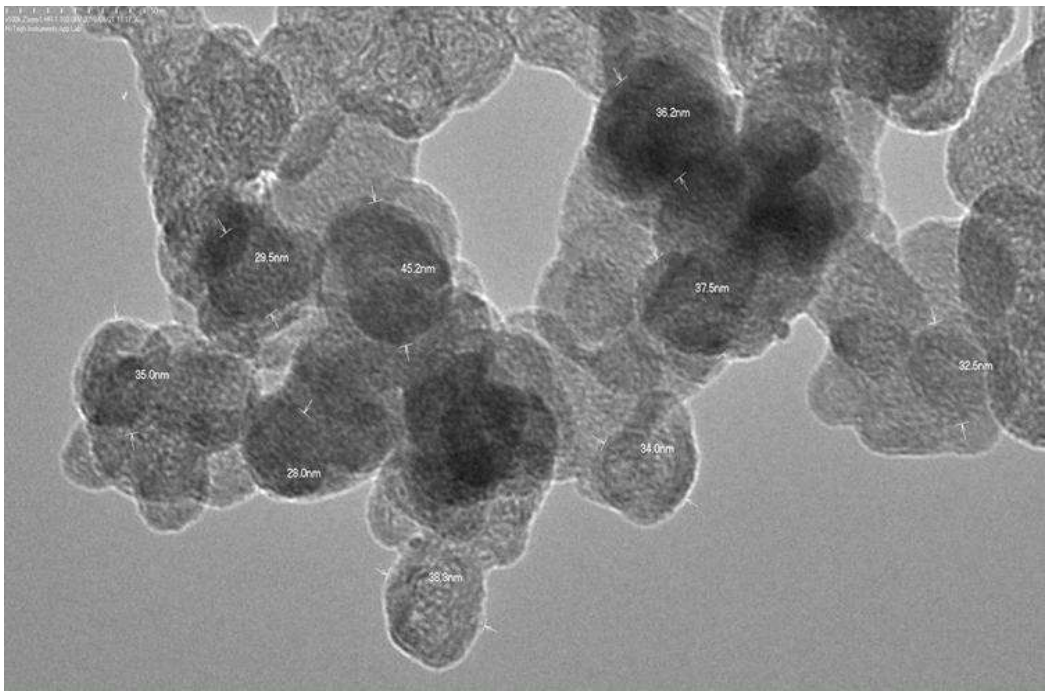

Figure 3: TEM image at 50nm magnification showing the silver nanoparticles with the size ranging between 20 and 50nm

3.2. Investigation of antibacterial effects of AgNP on wound bacteria

3.2.1. Disc diffusion plate test

AgNP with all concentrations of plant extract was able to inhibit the growth of gram positive and gram negative bacteria, although the antibacterial effect was not significant.

(Figure 4). The combination was better than both 50mM silver nitrate alone and plant extract alone. However, the AgNP was still not as effective as the positive control, the antibiotic discs. The bacterial growth inhibition was not affected by the concentration of plant extracts.

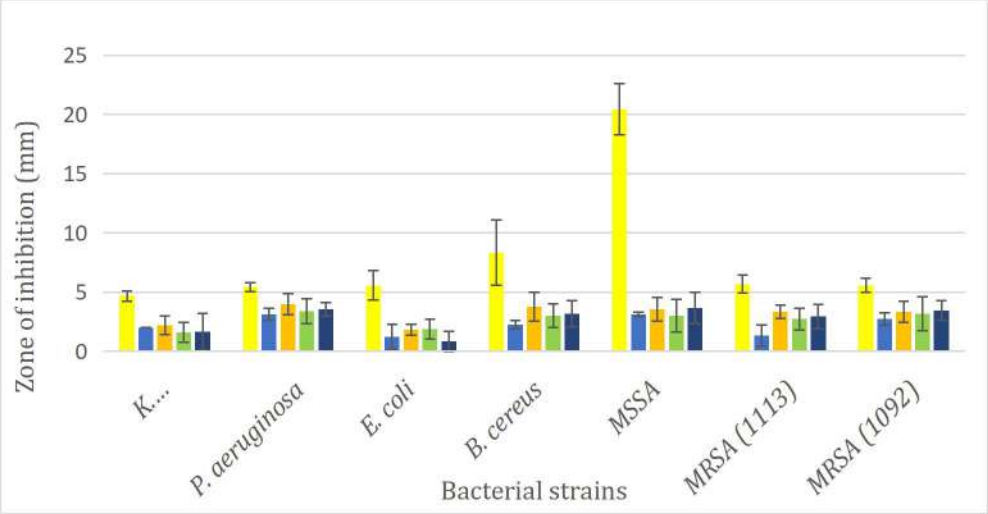

Figure 4: The inhibition of bacterial growth by disc diffusion plate test

The results indicated that the silver nanoparticles and A. vulgaris extracts can enhance each other's effect to inhibit the growth of bacteria. According to previous studies, the antibacterial effect of silver nanoparticles is mainly attributed to its small size and electrostatic attraction. (Rasheed, et al, 2017; Prathna, 2011; Nam, et al, 2015). The small size of silver nanoparticles allows it to easily penetrate the bacterial membrane. The small size of nanoparticles provides a high-surface-to-volume ratio, allowing them to have an increased contact area on the bacterial surface so that a greater amount of silver ions can exert the bactericidal effects towards the bacteria (Nam, et al, 2015; Bondarenko, et al, 2013).

The positively-charged nanoparticles and negatively-charged cell surface of gram negative bacteria cause an electrostatic attraction, which eases the diffusion of nanoparticles into bacterial cells (Pazos-Ortiz, E., et al, 2017). The permeation of nanoparticles into bacteria may result in the disruption of protein synthesis, alteration of bacterial structure, and cell death. The plant extract of A. vulgaris has a minimal bacterial effect compared to silver nitrate alone. The methanolic extract of A. vulgaris is slightly better than the aqueous extract. Terpene compounds found in *A. vulgaris* may contribute to its antibacterial effect (Zengin & Baysal, 2014)

3.2.2. Minimum Inhibitory Concentration (MIC)

From the MIC values, the silver nanoparticles containing *A. vulgaris* extract were effective in inhibiting both the gram positive and gram negative bacteria (Figure 5). The gram negative bacteria, *E. coli* and *K. pneumonia,* had the lowest MIC value, 0.25 mg/mL. Both methicillin susceptible and resistant strains of S. aureus had the highest MIC value of 1.00 mg/mL.

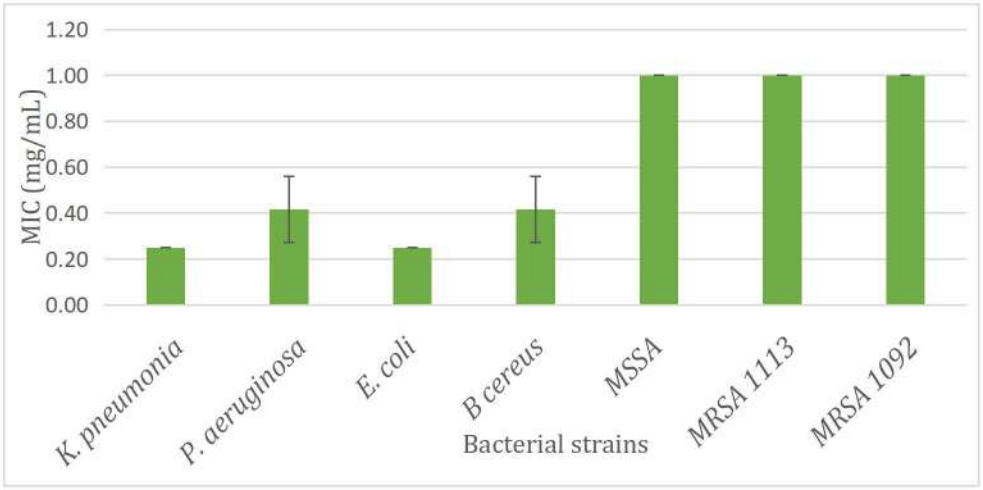

Figure 5: The minimum concentration required to inhibit bacterial growth by 50%

Although silver nanoparticles containing A. vulgaris extract were effective in inhibiting the growth of gram positive and gram negative bacteria, the results suggested that the inhibition towards gram negative bacteria was more prominent than that on gram positive ones. From the MIC values, it can be observed that a higher concentration of the formulation was required to inhibit the growth of gram positive bacteria compared to gram negative bacteria. The thick cell wall of gram positive bacteria contains a higher amount of peptidoglycan which causes the silver ions to adhere on the cell wall, resulting in a poorer antibacterial effect (Dakal, et al, 2016). The cell membrane of gram negative bacteria possesses lipopolysaccharides which are negatively charged. This promotes the adhesion of silver nanoparticles, causing the bacteria to be more susceptible to the antibacterial effect (Dakal, et al, 2016). The mechanism of A. vulgaris extract between gram negative and gram positive bacteria, however, is still not fully understood.

3.2.3. Minimum Bactericidal Concentration (MBC)

From the MBC values, it is shown that 4mg/mL of silver nanoparticles containing A. vulgaris extract, which was the highest concentration, was unable to kill the bacterial population of all the strains tested.

Table 2: Results of the MBC test

Bacteria	Minimum Bactericidal Concentration (mg/mL)
K. pneumonia	~4
P. aeruginosa	>4
E. coli	>4
B cereus	>4
MSSA	>4

3.3. Investigation of antibacterial effects of AgNP on probiotics

AgNP demonstrated lower antibacterial effects towards probiotics when compared to standard antibiotics (Figure 6). It is also noted that plant extract alone had less inhibition towards the growth of probiotics compared to AgNP. This suggests that the plant extracts exhibit a protective effect towards good bacteria so that the cells can be protected from the damaging effects of silver nanoparticles.

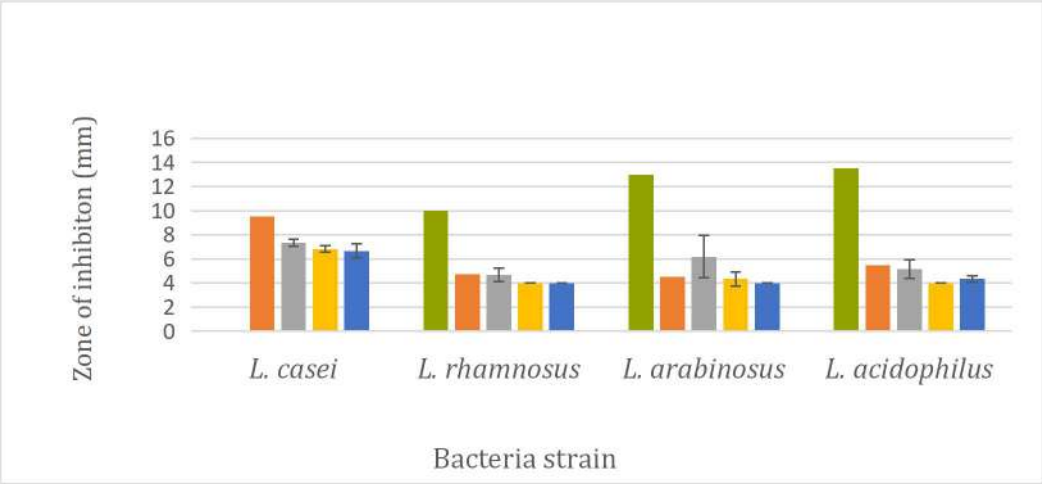

Figure 6: The inhibition of probiotics growth by disc diffusion plate test

A study has shown that a diet containing *A. vulgaris* was linked to an increase in intestinal bifidobacteria (Lee, et al, 1995). In a case report, *A. vulgaris* has been shown to speed up the wound healing process of an anaconda snake, indicating skin protective effects. In previous studies, *A. vulgaris* has shown cell protective effects, including hepatoprotective effects and less cytotoxic effects towards normal cells when compared to cancer cells (Gilani, et al, 2005; Saleh, et al, 2014)

4. Conclusion

A. vulgaris extracts and silver nanoparticles enhance each other's effect to inhibit the growth of bacteria, although not significantly. The plant extracts also exhibit a protective effect, protecting the probiotics from the damaging effects of treatment. Hence, silver nanoparticles containing *A. vulgaris* extract are a potentially safer alternative to the standard antibacterial treatment.

Acknowledgment

The research was carried out in collaboration with Queensland University of Technology, Australia, and supported by a grant from the International Medical University (IMU), Malaysia (Project ID: BP I-01/2018(39)).

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Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-89
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Physicochemical characterization of *Sargassum polcystum* C. Agardh and its activity against dinitrofluorobenzene-induced allergic contact dermatitis in mice

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Abstract

Background: *Sargassum polycystum* C. Agardh is brown seaweed abundant in the Philippines. Recent studies showed that it has an anti-inflammatory property. However, its efficacy against allergic contact dermatitis (ACD) has not yet been studied and there are no established data regarding its physicochemical properties yet.

Objectives: The objectives of this study were to evaluate the topical efficacy of *S. polycystum* crude polysaccharide (Spcp) using dinitrofluorobenzene (DNFB)-induced ACD murine model and to conduct physicochemical characterization on Spcp.

Methods: ACD was induced by sensitizing the BALB/c mice through topical application of 0.5% DNFB on the shaved ventral skin. Spcp (25%, 12.5%, 6.25% w/w) and standard drug (Betamethasone 0.10%) were topically applied on the right ear of the mice for seven days after sensitization and right after the challenge on the eighth day. Seven days after sensitization, the right ear was challenged with 0.2% DNFB. Ear thickness was measured at baseline and 24-hrs post-challenge using a dial thickness gauge. Physicochemical characterization was also performed.

Results: The results showed that topical application of Spcp inhibited the swelling produced during 24-hrs post-challenge. The analysis revealed that the 25% Spcp exhibited a statistically significant effect and was comparable with the inhibitory effect of the standard drug, betamethasone ($p<0.05$). The physicochemical characterization showed that Spcp contains a notable amount of carbohydrates, sulfate, and protein.

Conclusion: In conclusion, our results suggest that topically applied Spcp can be an effective natural product to treat allergic contact dermatitis. However, further investigations are required to understand the mechanisms involved.

Keywords: allergic contact dermatitis, physicochemical, polysaccharide

1. Introduction

Allergic contact dermatitis (ACD) is a common occupational disease and environmental health issue with a tremendous socio-economic impact. ACD is among the top 5 skin diseases in terms of lost productivity. Studies found that ACD is responsible for 20-30% of all occupational diseases and 50-60% of occupational contact dermatitis (American Academy of Dermatology, 2017).

Allergic contact dermatitis is a skin inflammatory disorder caused by T-cell mediated delayed-type hypersensitivity, which negatively affects the patient's quality of life (Salonga et al., 2014). ACD results from exposure to an allergen to which the patient has already been sensitized. There are more than 85,000 chemicals in the world environment today and almost all of these substances can be an allergen (Cashman et al., 2012). In the clinical setting, topical corticosteroids are the most common treatment for ACD, but prolonged use is prohibited

because of its known systemic adverse effects due to cumulative skin absorption (Mo et al., 2013). Therefore, there is a great need for the development of alternative treatments for ACD.

Sargassum polycystum C. Agardh, locally known as *lusay-lusay* or *boto-boto*, is a brown seaweed endemic in the Philippines. Recent studies revealed that it exhibits several pharmacological activities such as antibacterial activity (Palanisamy et al., 2019), antioxidant, anticancer activity (Palanisamy et al., 2017), and anti-HIV activity (Thuy et al., 2014). However, despite extensive studies of its pharmacological activities, none of these have focused on its topical application which offers several advantages over oral intake.

Prior to the formulation of an active compound into a dosage form, supporting scientific knowledge such as the fundamental physical and chemical properties of the active compound should be obtained. A comprehensive understanding of these properties allows the science-based development of the formulations. These data can be obtained by conducting physicochemical characterization (Verma & Mishra, 2016). This study, therefore, aimed to evaluate the topical efficacy of *S. polycystum* crude polysaccharide against DNFB-induced ACD in mice and to conduct physicochemical characterization.

2. Methods

2.1. Extraction of Spcp

Fresh *S. polycystum* seaweed was collected from Elfarco Seaweed Farm in Calatagan, Batangas, Philippines. Samples were submitted to the University of the Philippines Marine Science Institute and identified and authenticated with the accession number MSI27992. The seaweed was washed thoroughly with distilled water, air-dried, and ground into a powder.

Spcp was extracted following the hot water extraction method by Shofia et al. (2018) with some modifications. The powdered seaweed was boiled at 100°C in distilled water for 3 hours and filtered with cheesecloth. The supernatant was collected after centrifugation at 4000 rpm for 5 minutes. The crude polysaccharide was precipitated by the addition of an equal volume of absolute ethanol and was kept overnight at 4°C. The precipitated polysaccharide was collected by centrifugation at 4000 rpm for 5 minutes and was lyophilized to powder.

2.2. Animals

Healthy female BALB/c mice (6 weeks old, 20-25 grams), purchased from the Research and Biotechnology Division of St. Luke's Medical Center, Quezon City, were used in the study. The mice were housed in standard plastic cages in a temperature- and humid-controlled environment (22-27°C, 60% RH) with food and water available *ad libitum*. Mice were acclimatized for five days before the experimentation. All experiments were carried out following the guidelines for the care and use of experimental animals approved by the University of Santo Tomas Institutional Animal Care and Use Committee.

2.3. 2,4-dinitrofluorobenzene-induced allergic contact dermatitis

Allergic contact dermatitis was induced in BALB/c mice according to a published method done by Saint-Mezard et al. (2004), Salonga et al. (2014), and Yu et al. (2017). The mice were sensitized by painting their shaven abdominal skin (2x2 cm^2) with 100 µL of 0.5% dinitrofluorobenzene (DNFB, Sigma-Aldrich®) in a 4:1 acetone-olive oil solution. Seven days later, the inner and outer surface of the right ear were challenged with 20 µL of 0.2% DNFB in a 4:1 acetone-olive oil solution. The ear thickness was measured using a dial thickness gauge before challenge and 24-hrs after challenge. The ear swelling was calculated as the increase in ear thickness and percentage of ear swelling.

2.4. Drug treatment

Spcp was directly added to petrolatum, which served as the vehicle. They were mixed thoroughly in a sterile mortar. Topical treatment started right after the mice were sensitized once daily for eight days with 25%, 12.5%, 6.25%, or vehicle (petrolatum). Mice were divided into seven groups with six mice per group: normal control group, negative control group, vehicle control group, positive control group, and three Spcp groups. All groups had DNFB induction except the normal control group. The negative control group did not receive any treatment, the positive control group was treated with the standard drug, betamethasone (0.10%), the vehicle control group was treated with the petrolatum alone, and the Spcp groups were treated with ointment base containing Spcp in different concentrations (25%, 12.5%, 6.25%).

2.5. Physicochemical characterization

2.5.1 Organoleptic analysis

The general appearance, color, odor, and texture of the whole algae and the crude drug were described. The color was examined under diffused daylight or an artificial light source with wavelengths similar to those of daylight.

2.5.2 Physical Characterization

Particle size. The determination of particle size distribution of the crude drug was done through the sieving method. A 100-g crude drug was manually tapped in a stack of sieves (no. 12, 20, 40, 60) and a receiver for 30 minutes. The retained sample in each sieve was weighed.

Total ash. About 2 to 4 grams of *S. polycystum* crude drug was accurately weighed and placed in a tared crucible and incinerated at a temperature of 675 ± 25°C using a furnace until being free from carbon. After cooling in a desiccator, the weight of the ash was recorded. The percentage of total ash was calculated using the formula:

$$\%\,Total\ ash = \frac{weight\ of\ total\ ash}{weight\ of\ sample} x100 \quad (1)$$

Acid-insoluble ash. The ash obtained from the total ash determination was boiled with 25 mL of 3N HCl for five minutes. The insoluble matter was collected on a tared ashless filter paper and washed with hot water. After igniting the insoluble matter, the weight was recorded. The percentage of acid-insoluble ash was calculated using the formula:

$$\%\, Acid\ insoluble\ ash = \frac{weight\ of\ acid\ insoluble\ ash}{weight\ of\ sample} x100 \quad (2)$$

Water-soluble ash. The ash obtained from total ash determination was boiled with 25 mL of water for five minutes. The insoluble matter was collected in a sintered glass crucible and washed with water. It was ignited for 15 minutes at 450°C. Percent of water-soluble ash was computed with reference to the weight of the sample taken in the total ash determination.

$$\%\, Water\ soluble\ ash = \frac{weight\ of\ total\ ash - weight\ of\ insoluble\ matter}{weight\ of\ sample} x100 \quad (3)$$

Moisture content. The moisture analyzer was set at an analysis temperature of 105°C. An aluminum foil weighing dish with a quartz pad was placed on the balance pan. The dish and pan were allowed to dry completely to constant weight and then weighed. Ten grams of the crude drug was transferred to the quartz pad in the weighing dish. The analysis was taken as soon as the instrument balance showed a table weight. The instrument automatically shut off once the sample was dried to a constant weight. Percent moisture content was computed using the formula:

$$\%\, Moisture = \frac{weight_{driedsample+dish} - weight_{dish}}{weight\ of\ sample} x100$$

pH determination. The pH of the crude polysaccharide was determined by immersion of the electrode of a pH meter at 25°C in a 1% aqueous solution of Spcp.

Solubility test. A supersaturated solution of the crude polysaccharide was prepared by continuously dissolving an amount of the crude polysaccharide in 5 mL of the solvent until it no longer dissolved. For this purpose, the following solvents were used: distilled water, 95% ethanol, 0.1N HCl, 0.1N NaOH, and 0.9% NaCl. Solutions were agitated using an Orbital Shaker for 24 hours with the bath maintained at a temperature of 37 ± 2°C. Solutions were filtered using a Whatman filter paper (No. 1), and 3 mL aliquot portion of each filtrate was transferred to tared evaporating dishes. Filtrates were evaporated to dryness for 3 hours using an oven maintained at 100°C to 105°C. The residue obtained after heating was cooled in a desiccator for 30 minutes and weights were determined. The evaporating dishes with residue were similarly heated for another hour, cooled in the desiccator, and weighed. This procedure was repeated until two consecutive weighing stages did not differ more than 0.5 mg/g.

2.5.3 Chemical analysis

Carbohydrate content. The polysaccharide content was determined using the phenol-sulfuric acid method described by Jose and Kurup (2016). In a 96-well microplate, 20 µL of the sample (1 mg/mL) was pipetted in a well followed by the addition of 100 µL of concentrated sulfuric acid and 20 µL of 5% phenol solution. The mixture was incubated for 10 minutes at a room temperature, and absorbance was read at 490 nm. Fucose served as the standard.

Sulfate content. The sulfate content was determined through the barium chloride-gelatin turbidity method using potassium sulfate as the standard. In a microplate, 20 µL of the polysaccharide solution (1 mg/mL) was pipetted into the well followed by the addition of 190 µL of 0.5 M hydrochloric acid and 50 µL BaCl-gelatin solution. The mixture was incubated for 20 minutes at a room temperature, and absorbance was read at 360 nm (Jose & Kurup, 2016).

Protein content. Protein content was measured using the Bradford assay described by Jose & Kurup (2016). Three microliters of polysaccharide solution (1 mg/mL) was pipetted into a 96-well microplate. To each well, 150 µL of the Bradford reagent was added. The mixture was incubated for 5 minutes at a room temperature, and the absorbance was read at 595 nm. Bovine serum albumin served as the standard.

Uronic acid content. The uronic acid content of the crude polysaccharide was determined by the carbazole method using galacturonic acid as the standard. The crude polysaccharide (1 mg/mL) was heated in a boiling water bath for 10 minutes with 0.025 M sodium carbonate. Then 0.1% carbazole in methanol solution was added and boiled continuously for 15 minutes. The absorbance was read at 540 nm (Jose & Kurup, 2016).

2.6. Statistical analysis

Results were expressed as mean ± SEM of three independent measurements. Statistical analysis was conducted by ANOVA and Tukey's tests. P values of less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Extraction of the crude polysaccharide

Sargassum polycystum (Fig. 1a) was air-dried and ground into a powder to extract crude polysaccharide using the hot water extraction method. The extract was lyophilized to powder (Figure 1) to prevent further degradation. A total yield of 4.17% of crude polysaccharide was extracted from the dried material. The yield of crude polysaccharide was comparable to previous studies (Nagappan et al., 2017; Palanisamy et al., 2018). The lyophilized sample appeared as brownish powder with a distinct saltwater odor.

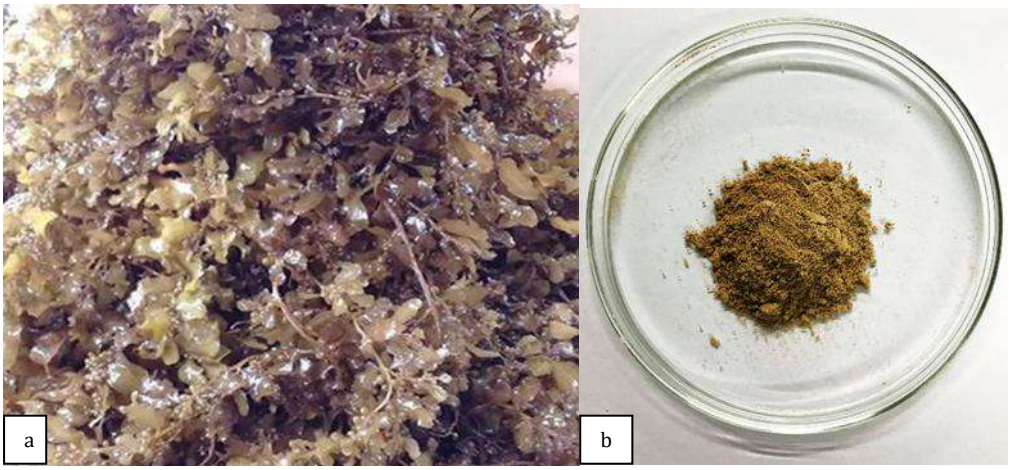

Figure 1. a. Fresh *Sargassum polycystum;* b. *Sargassum polycystum* crude polysaccharide lyophilized powder

3.2. Inhibition of DNFB-induced allergic contact dermatitis

Allergic contact dermatitis, a common clinical skin disease, is a delayed-type hypersensitivity. It is a T-cell mediated inflammatory reaction that occurs at the site of contact with the allergen; it is commonly characterized by redness, papules, and vesicles, which later develops scaling and dry skin. The most common allergens are metals, cosmetic and skincare products, fragrances, and topical antibiotics (Saint-Mezard et al., 2004).

The mouse ear swelling test seeks to identify potential contact allergens based on challenge-induced increases in ear thickness in sensitized animals. Spcp (25%, 12.5%, 6.25%) was topically applied once daily for eight days on mice to evaluate the effect of Spcp on ACD. The establishment of the DNFB-induced allergic contact dermatitis murine model and the dosage regimen are illustrated in Figure 2. Based on the average ear swelling 24-hrs post-challenge (Figure 3) and percent increase in ear thickness 24-hrs post-challenge (Figure 4), topical application of Spcp could inhibit DNFB-induced allergic contact dermatitis. The analysis reveals that all the concentrations were statistically able to decrease the ear swelling compared to the untreated group. Also, the effect of 25% Spcp was comparable to the inhibitory effect of the standard drug, betamethasone. The above mentioned results imply that the topical application of Spcp could significantly suppress the inflammatory responses in DNFB-induced allergic contact dermatitis.

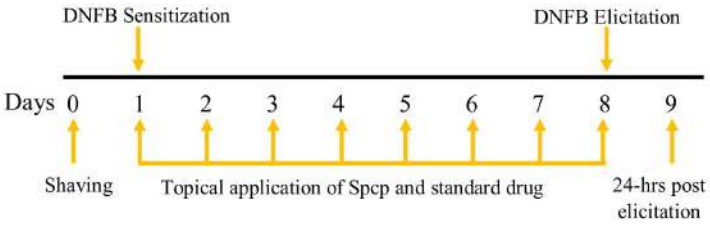

Figure 2. DNFB-induced allergic contact dermatitis murine model and dosing regimen

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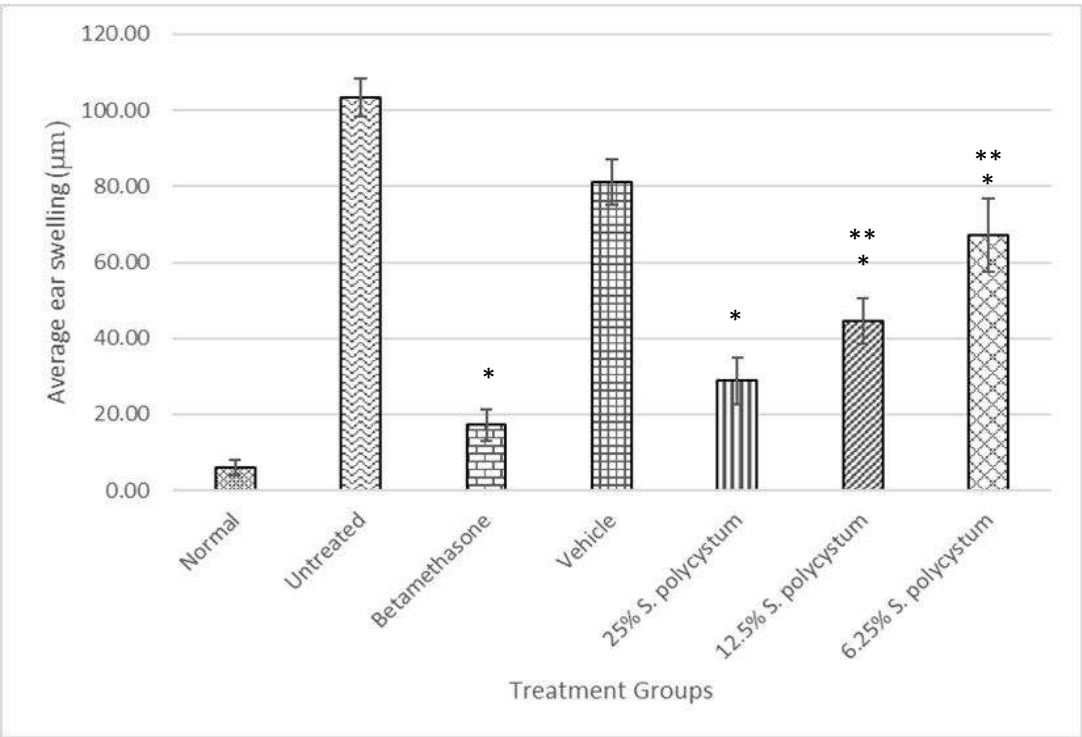

Figure 3. Average ear swelling 24-hrs post-challenge
Values are expressed as mean ± SEM of 6 mice.
*Denotes a significant difference against untreated group.
**Denotes a significant difference against the group treated with the standard drug, betamethasone. ($p<0.05$) by ANOVA and Tukey's tests.

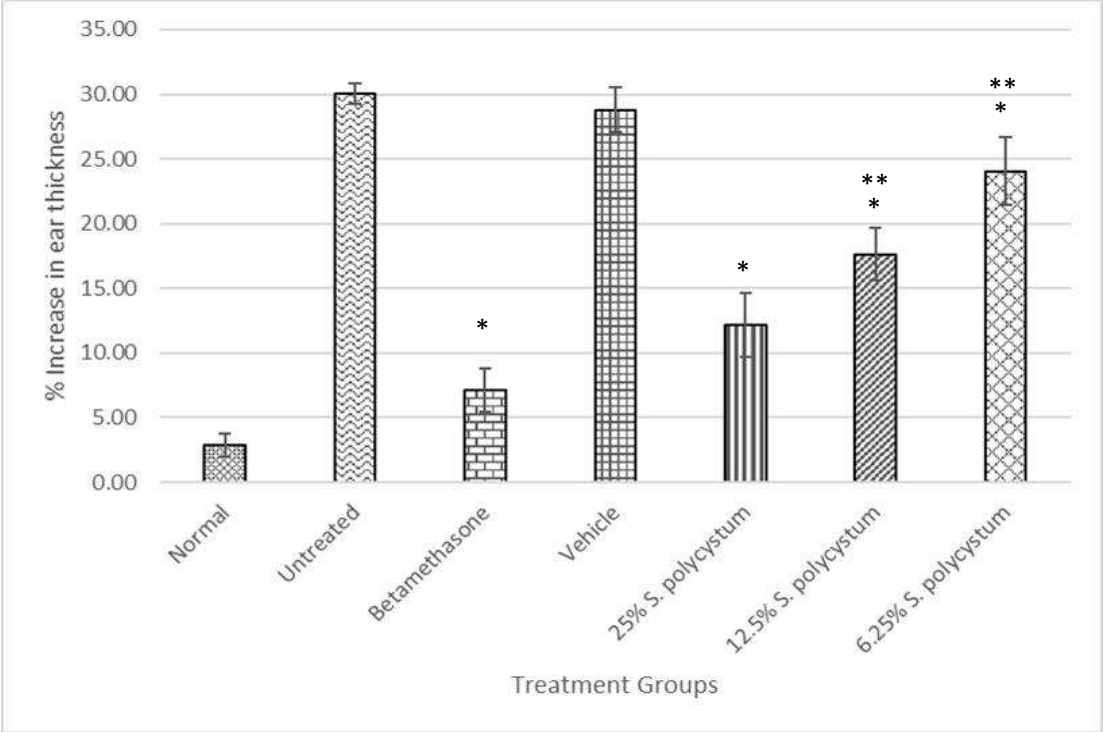

Figure 4. Percent increase in ear thickness 24-hrs post-challenge
Values are expressed as mean ± SEM of 6 mice.
*Denotes a significant difference against untreated group.
**Denotes a significant difference against the group treated with the standard drug, betamethasone. ($p<0.05$) by ANOVA and Tukey's tests.

3.3.Physicochemical characterization

To guarantee the reproducible quality of herbal medicines, proper characterization of the starting material is essential. One determinant towards ensuring the quality of starting material is establishing numerical values of standards for a comparison. In this study, the researchers aimed to characterize the physicochemical properties of Spcp which are essential to determine the possible bioactive constituent responsible for the inhibitory activity against ACD. Also, the physicochemical parameters are essential to determine the suitability of the sample to be developed into a formulated dosage form.

3.4. Organoleptic analysis

The primary step in ensuring a quality herbal plant is authentication. In the present study, the researchers aimed to provide data that will be of great importance in establishing standard parameters that may be used in the authentication and standardization of *S. polycystum.*

The thallus of *S. polycystum* is yellowish-brown, up to 90 cm tall, and holdfast a plate-like alga up to about 7 mm in diameter. The stem is brownish and finely villose, short, and usually about 10 mm long. The primary branch is highly compressed at the distal end of the stem, terete, and lumpy with elevated cryptostomata. Leaves on the main branches of vegetative materials are generally larger, broadly lanceolate, base asymmetrical, margin finely but irregularly serrate-dentate, and midrib distinct almost to apex of leaves. Cryptostomata are numerous,

distinct and elevated, and scattered on leaves and vesicles. Vesicles are numerous, very small about 1.5-2.5 mm long and 1.0-2.0 mm wide, and mainly spherical-ovate to slightly elliptical (Trono, 1992).

The histologic features of fresh *S. polycystum* stipe (Figure 5) and blade (Figure 6) were also observed. The cells in the stipe are differentiated into epidermis that contains chromatophores which are responsible for photosynthesis, cortex which is made up of parenchymatous cells and serves as the storage tissue, and medulla. On the other hand, the cells in the blade are differentiated into epidermis and mesophyll tissue. Mesophyll tissue is made up of parenchymatous cells where pigments are located.

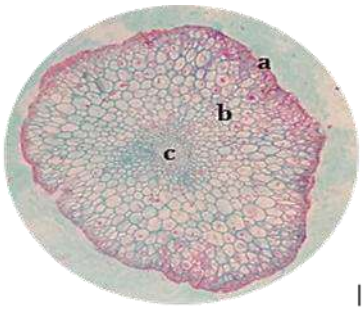

Figure 5. Histologic features of *S.polycystum* stipe blade (a)epidermis; (b) cortex; (c) medulla

Figure 6. Histologic features of *S. polycystum* (a) epidermis; (b) mesophyll tissue

Physical characteristics. Physical characterization of an herbal plant is a basic protocol for the standardization of herbal medicines. Table 1 shows the physical characteristics of Spcp. The total ash value is essential in the identification and authentication of the sample and to examine adulterants from the original species of biological importance. Establishing the pH and solubility of the sample provides useful data about the nature of the sample. These collective physicochemical properties are essential to determine the suitability of the sample to be developed into a formulated dosage form.

Table 1. Physical characteristics of Spcp

Parameters	**Value**
Particle size	425 microns – 820 microns
Total ash content	23.76%
Acid-insoluble ash	0.46%
Water-soluble ash	11.78%
Moisture content	12.90%
pH	7.40
Solubility	
Distilled Water	Slightly soluble
0.9% NaCl	Sparingly soluble
Ethanol	Very slightly soluble
HCl	Sparingly soluble
NaOH	Sparingly soluble

Chemical composition. The chemical analysis indicates that Spcp (Table 2) is composed primarily of carbohydrates (33.60%) and exhibits high sulfate content (23.66%), a notable amount of protein (7.46%), and a small amount of uronic acid (1.50%). Sulfated polysaccharide present in seaweed is composed of carbohydrate backbone with ester sulfate substitution in the sugar residue. Recent investigations showed that the biological activities of marine algae might be attributed to its sulfated polysaccharide content (Guerra Dore et al., 2013; Raghavendran et al., 2005).

Table 2. Chemical composition of Spcp

Parameters	Percentage (%)*
Carbohydrate	33.603 ± 0.371%
Sulfate	23.656 ± 0.124%
Protein	7.456 ± 0.348%
Uronic acid	1.501 ± 0.011%

*Values are expressed as mean (N=9) ± SEM.

Several studies have been done to evaluate the anti-inflammatory activity of *Sargassum* crude sulfated polysaccharide. Neelakandaan et al. (2016), evaluated *S. wightii* sulfated polysaccharide for its *in vivo* anti-inflammatory effect using carrageenan-induced rat paw edema. Results showed that *S. wightii* sulfated polysaccharide significantly reduced the paw edema in a dose-dependent manner. In a study conducted by Fernando et al., (2018), crude sulfated polysaccharide from *S. polycystum* showed a strong anti-inflammatory activity when tested against lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Decreased production of NO, PGE_2, TNF-α, IL-1β and IL-6 was also observed. In a following study, Fernando et al., (2018) found that *S. polycystum* crude polysaccharide was rich in fucoidan with high sulfate content of 27.53 ± 0.55%. The observed activity of Spcp from *S. polycystum* is attributed to its high sulfate content which is found comparable to and in good agreement with the sulfate content of other *Sargassum* species. For instance, *S. vulgare* and *S. tenerrimum* were reported to contain 22.6% and 22.14%, respectively (Guerra Dore et al., 2013; Mohan et al., 2019). Both types of seaweed were reported to have a strong antioxidant activity. Sanjeewa et al (2017) reported that the sulfated polysaccharide from *S. horneri* had the same IR spectra as a commercial fucoidan. Fucoidan is a dominant sulfated polysaccharide found in brown seaweed (Saraswati, 2019). Fucoidan from marine macroalgae has been studied for its anti-allergic activity. Yang (2012) and Tian et al. (2019) reported that fucoidan from brown seaweed is as effective as dexamethasone in improving atopic dermatitis symptoms.

4. Conclusion

In conclusion, the crude polysaccharide from *S. polycystum* can be an effective natural product to treat allergic contact dermatitis. However, further investigations are required to understand the mechanisms involved. Also, the physicochemical properties of *Sargassum polycystum* crude polysaccharide revealed through this study can be a basis for future research in developing a formulation of dosage form.

Acknowledgment

This work was supported by financial grants from the National Research Council of the Philippines (NRCP) with the project code: O-016 and the Department of Science and Technology – Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP).

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Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-89
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Evaluation of the hepatoprotective effect of methanolic extract of *Caulerpa lentillifera* against acetaminophen-induced liver toxicity in juvenile zebrafish (*Danio rerio*)

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Abstract

Background: Liver injury is a common reason for drugs to be withdrawn from the market. Treatment options for common liver disease are limited, and therapy with modern medicines may lack effectiveness. *Caulerpa lentillifera* may have strong antioxidant systems that protect the plant from oxidative damage caused by the environment.

Objectives: The main objective of this study was to evaluate the hepatoprotective effect of the methanolic extract of *C. lentillifera* against acetaminophen-induced liver toxicity in juvenile zebrafish (*Danio rerio*).

Methods: Juvenile zebrafish (aged 1–3 months) were exposed to 10 μM and 25 μM acetaminophen (*N*-acetyl-p-aminophenol; APAP) to induce liver damage. *C. lentillifera* methanolic extracts (10 μg/L, 20 μg/L and 30 μg/L), were concomitantly added to individual tanks containing 10 μM or 25 μM APAP. The positive control group was treated with *N*-acetylcysteine/NAC (10 μM) and silymarin (10 μg/L, 20 μg/L and 30 μg/L). Hematoxylin and Eosin (H&E) staining revealed the extent of liver injury through the presence of hepatic necrosis, vacuolization, leukocyte infiltration, and ballooning. The antioxidant mechanism of hepatoprotective activity was assessed by a DPPH free radical scavenging assay.

Results: *C. lentillifera* extracts reduced the mortality of juvenile zebrafish when simultaneously exposed to 10 μM and 25 μM APAP. Upon histopathological examination of the liver tissue of juvenile zebrafish, the group treated with the 10 μM APAP together with the highest concentration (30 μg/L) of *C. lentillifera* extract showed minimal liver injury compared to the groups exposed to 25 μM APAP. However, the DPPH free radical scavenging assay performed using 24–36 mg/mL *C. lentillifera* extracts showed a minimal effect on the free radical scavenging activity.

Conclusion: The histopathological analysis of the liver showed that *C. lentillifera* extract prevented the progression of liver damage caused by APAP. The results of DPPH free radical scavenging assay indicated that the hepatoprotective activity of *C. lentillifera* extract might have other antioxidant mechanisms aside from free radical scavenging. In order to effectively assess the improvement in the survival rate of juvenile zebrafish, longer exposure in the treatments is recommended.

Keywords: *C. lentillifera*; juvenile zebrafish; hepatoprotective; drug-induced liver injury (DILI)

1. Introduction

Investigatory drugs are usually withdrawn in drug development and preclinical studies as well as after drug approval and marketing because of their ability to induce hepatotoxicity. Drug-induced liver injury results when the liver is unable to detoxify free radicals, such as reactive oxygen species (ROS), or other toxic metabolites from drug substances. This type of liver injury is a growing medical, scientific, and public health problem (Suk & Kim, 2012). Treatment choices for common liver injury are limited, and therapy with modern medicines may lack effectiveness. *N*-

acetylcysteine (NAC) is widely accepted in the prevention of hepatic injury due to acetaminophen overdose (Heard, 2008). A known hepatoprotective compound, silymarin from *Silybum marianum*, has an ability to inhibit the free radicals that are produced from the metabolism of toxic drug substances, including acetaminophen (Vargas-Mendoza et al., 2014).

Currently, there is a growing interest in the study of the antioxidant properties of marine species, such as algae, because of their inherent capability to withstand oxidative damage in the aquatic environment. *Caulerpa lentillifera*, known locally as *latô*, is commonly eaten as salad in the Philippines, and may have strong antioxidant systems that protect it from oxidative damage. Phenolic antioxidants found in *C. lentillifera* may become a possible agent used for the prevention of hepatotoxicity (Nguyen et al., 2011). Rodents are traditionally used in toxicological studies of the liver, but recently, small fish such as zebrafish (*Danio rerio*) have been used as an animal model as they present advantages, such as short generation time, high fertility, and low operational cost in terms of housing space and daily maintenance. In many liver toxicological studies, zebrafish larvae are utilized because they are optically clear and their internal organs can be directly observed without the need for dissection. Thus, real-time, simultaneous monitoring of livers in zebrafish larvae is easily achieved. Zebrafish therefore become an increasingly more valuable animal model than rodents in certain vertebrate toxicological studies (Asaoka et al., 2013). The main objective of this study was to determine the effect of *C. lentillifera* methanolic extract in reducing acetaminophen-induced liver toxicity in juvenile zebrafish (*Danio rerio*).

2. Methods

2.1. Collection and preparation of C. lentillifera extract

All seaweed specimens were collected from Barangay Talaba I in the City of Bacoor, Province of Cavite during the month of October 2018. They were immediately washed with tap water, dried, placed in wide-mouthed plastic containers covered with ice, and transported. A sample was authenticated at the Bureau of Fisheries and Aquatic Resources (BFAR) in Diliman, Quezon City. Each *C. lentillifera* specimen was washed *in situ* with distilled water, lyophilized at 70^0C for 7 days, and pulverized using a household blender. Methanolic extract was then prepared by maceration of the lyophilized and pulverized seaweed at 50^0C with sonication for 1 hour. This was then subjected to rotary evaporation to remove the solvent methanol at 40^0C and 70 rpm. The extract was dissolved in appropriate solvents for the bioassay and DPPH antioxidant assay.

2.2. Collection, acclimatization, and treatment of zebrafish

A total of 720 juvenile zebrafish (1 to 3 months old) was used in the study. Standard housing and treatment protocols were followed. The zebrafish were maintained in aerated water in the laboratory at 28 ± 2^0C in a 14 hr/10 hr light/dark cycle photoperiod and fed twice a day with fish food (sinking pellets) for 2 weeks. All zebrafish used in this study were healthy and free of any signs of disease.

After the acclimatization period, fish were randomly assigned into 24 experimental tanks, with a density of 10 zebrafish per 2 L. All treatments were done in triplicate and conducted for a total of 72 hours with twice daily feeding and regular fish tank maintenance. Stock solutions of treatments (APAP, NAC, silymarin, and *C. lentillifera*) were directly added into the fish tank water to make specified concentrations. In order to induce liver damage, the zebrafish were exposed to 10 µM and 25 µM APAP. *C. lentillifera* methanolic extracts (10 µg/L, 20 µg/L and 30 µg/L), were concomitantly added to individual tanks containing 10 µM or 25 µM APAP. Similar experiments were conducted for NAC (10 µM) and silymarin (10 µg/L, 20 µg/L and 30 µg/L) replacing *C. lentillifera* extracts. The groups were observed every 12 and 24 hrs for fish movement and mortality for 3 days. Live zebrafish were sacrificed through hypothermic shock in ice water for the histological examination.

2.3. Histological analysis

Whole body histological sections (7-µm sections) showing the liver were taken from the tail region behind the anus as prescribed by a histopathologist and using a standard protocol. Briefly, the fish was stored in Dietrich's fixative (28.5% ethanol, 1% formalin, 0.2% acetic acid) at a room temperature for several days and processed by a tissue processor (containing 70%, 80%, and 95% ethanol gradient) for dehydration. Ethanol was removed by immersing the cassettes in 100% xylene for 1 hr. The tissue was embedded in paraffin wax for 2 hrs at 56^0C and allowed to solidify. Sectioning was performed using Leica microtome. Tissue sections were stained using the hematoxylin and eosin (H & E) staining method by Ellis and Yin (2017).

2.4. DPPH free radical scavenging assay

The free radical scavenging activity of the *C. lentillifera* extracts were analyzed according to the method described by Müller et al. (2011) with the modifications from Osuna-Ruiz et al. (2016). DPPH free radical scavenging assay was also conducted to determine if the extract has the ability to scavenge free radicals as a hepatoprotective mechanism. *C. lentillifera* extracts (24 mg/mL, 27 mg/mL, 30 mg/mL, 33 mg/mL or 36 mg/mL) and L-ascorbic acid as the standard were separately incubated with 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl radical) in 1:1 hexane:methanol for 30

min in the dark and at a room temperature. The absorbance of the mixtures was determined at 540 nm using a UV/Vis spectrophotometer (Hitachi U-2910). When DPPH free radical scavengers reacted with the purple-colored DPPH, it was converted into its reduced form, which was yellow in color. This resulted in a decrease in absorbance at 540 nm. The percentage inhibition was determined using the formula:

$$\%\ Inhibition = \frac{A_{negative\ control} - \left(A_{sample} - A_{blank}\right)}{A_{negative\ control}} \times 100\% \quad (1)$$

where:

$A_{negative\ control}$ = mean absorbance of DPPH solution in methanol

A_{sample} = mean absorbance of DPPH solution with *C. lentillifera* extract (or standard, L-ascorbic acid solution)

A_{blank} = mean absorbance of *C. lentillifera* extract (or L-ascorbic acid solution)

3. Results and discussion

The number of deaths in the APAP-treated control group doubled with the increase in the concentrations of APAP from 10 μM to 25 μM. When zebrafish were exposed to the negative control (the solvent used in preparing treatments), NAC, silymarin, or *C. lentillifera* extract, no zebrafish deaths were observed at the end of 72 hours. This means that exposure to the treatment groups alone and not in combination with APAP did not adversely affect the survival rate of the zebrafish. Similar to NAC and silymarin, which are known as hepatoprotective agents, *C. lentillifera* extracts reduced the mortality of juvenile zebrafish when simultaneously exposed to 10 μM and 25 μM APAP.

The histological characteristics of the zebrafish livers were assessed using H & E staining. Liver injury was indicated when hepatic necrosis, leukocyte infiltration and hepatocyte swelling were observed in the fish sections. The latter was seen as sinusoid compression due to swollen hepatocytes (Ellis & Yin, 2017). APAP treatment showed these signs of liver injury (Figure 1). All of the observed effects of acetaminophen in zebrafish hepatocytes were consistent with previously observed effects in human liver cells. APAP-induced liver injury is known to activate neutrophils, leading to neutrophil accumulation in the hepatic vasculature. Following APAP administration, a significant number of neutrophils are recruited into the liver resulting in subsequent development of hepatocellular injury between 4 and 24 hrs after drug treatment (Xu, et al., 2014).

To ascertain that NAC, silymarin, and *C. lentillifera* extract did not adversely affect the zebrafish liver when given in the absence of liver damaging APAP, controls were set up. Zebrafish groups treated with 10 μM NAC, 10 and 20 μg/L silymarin, and 10–30 μg/L *C. lentillifera* did not show any remarkable hepatocyte changes compared with the negative control containing only 0.1% by volume DMSO, the solvent used in preparing these solutions (Figure 2).

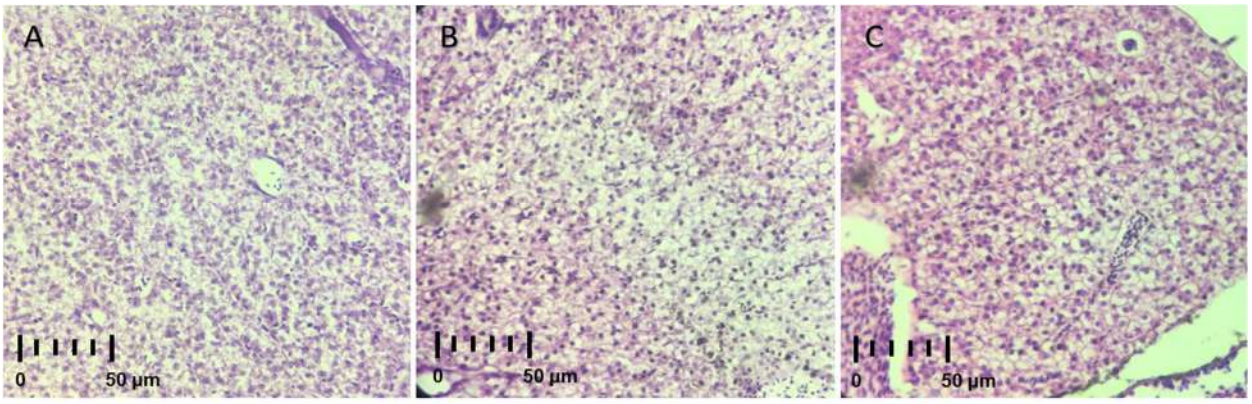

Figure 1. Exposure to APAP causing hepatic damage (400x magnification)**.** Hepatic tissue of zebrafish from the negative control group (A), after exposure to 10 µM APAP (B), and after exposure to 25 µM APAP (C)

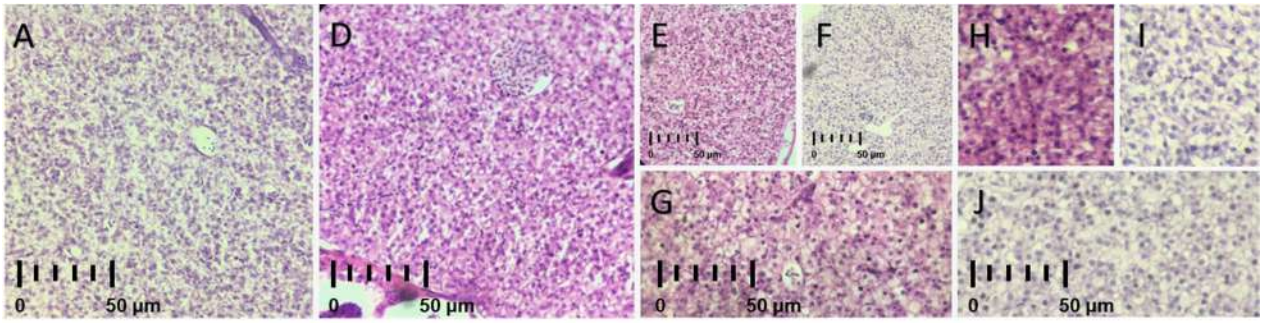

Figure 2. Treatment insignificantly affecting hepatic cells without exposure to APAP (400x magnification). Negative control (A), 10 µM NAC (D), 10 µg/L silymarin (E), 20 µg/L silymarin (F), 30 µg/L silymarin (G), 10 µg/L *C. lentillifera* extract (H), 20 µg/L *C. lentillifera* extract (I), and 30 µg/L *C. lentillifera* extract (J)

The liver histopathological features of juvenile zebrafish exposed to 10 µM APAP and concurrently treated with NAC (10 µM), silymarin (10–30 µg/L) or *C. lentillifera* extract (10–30 µg/L) showed a decrease in hepatic necrosis, leukocyte infiltration, hepatocyte vacuolization, and hepatocyte swelling in varying degrees consistent with their concentrations (Figure 3). However, hepatic tissues of zebrafish exposed to a higher concentration of APAP (25 µM) showed minimal changes on the hepatic cellular structures in the presence of the given treatments (NAC, silymarin, and *C. lentillifera* extract). These indicate that hepatic damage from exposure of zebrafish to 25 µM of APAP is irreversible with any of the known hepatoprotective agents (NAC and silymarin) and the investigational extract, *C. lentillifera*.

Treatment of zebrafish exposed to 10 µM APAP with 30 µg/L *C. lentillifera* extract showed no hepatic necrosis but minimal leukocyte infiltration and vacuolization. These were similar to those observed in 30 µg/L silymarin, possibly indicating that these plant extracts might share a similar hepatoprotective mechanism. The hepatoprotective properties of NAC and silymarin are well

established and appear to be partly related to their antioxidant activities. NAC is thought to reverse APAP-induced hepatotoxicity by replenishing glutathione, reducing the hepatotoxic metabolite of APAP, *N*-acetyl-*p*-benzoquinone imine (NAPQI), and effecting nonspecific hepatoprotective actions related to its antioxidant properties (Tardiolo, 2018). Silymarin extract contains a mixture of isomeric flavonolignans. Due to their phenolic structures, silymarin flavonoids have been reported to have antioxidant properties, which can control or inhibit free radicals produced by the hepatic metabolism of toxic substances such as APAP. In addition, the hepatoprotective activity of silymarin is shown to be caused by the maintenance of hepatocyte membrane integrity, affecting intracellular glutathione inhibition of leukotrienes and cyclooxygenase (Vargas-Mendoza, 2014). Flavonoids, which are previously reported to be present in *C. lentillifera*, may also be responsible for the observed hepatoprotective property of *C. lentillifera* (Nguyen, et al., 2011).

To determine if the hepatoprotective property of *C. lentillifera* was mediated by a free radical scavenging mechanism, the DPPH assay was performed. However, the DPPH free radical scavenging assay performed using 24–36 mg/mL *C. lentillifera* extracts showed a minimal effect on the free radical scavenging activity (Table 1). This was found to be consistent with a previous study by Nguyen, et al. (2011), indicating that the hepatoprotective activity of *C. lentillifera* extract might have other antioxidant mechanisms aside from free radical scavenging.

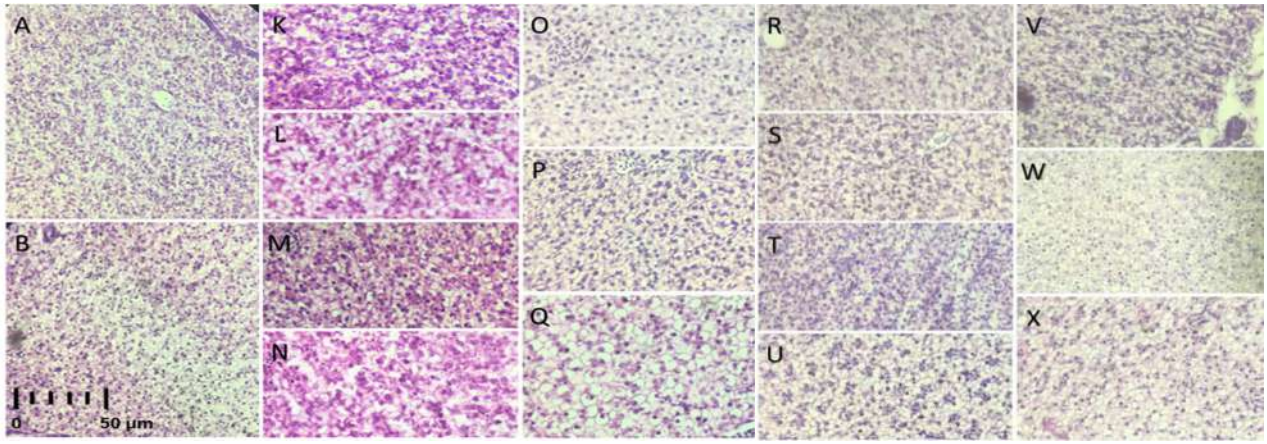

Figure 3. *C. lentillifera* reducing hepatic tissue injury after exposure of zebrafish to 10 µM APAP (K–Q) and 25 µM APAP (R–X) (400x magnification). Negative control (A), 10 µM APAP (B), with 10 µM *N*-acetylcysteine (K), with 10 µg/L silymarin (L), with 20 µg/L silymarin (M), with 30 µg/L silymarin (N), with 10 µg/L *C. lentillifera* extract (O), with 20 µg/L *C. lentillifera* extract (P), with 30 µg/L *C. lentillifera* extract (Q), with 10 µM *N*-acetylcysteine (R), with 10 µg/L silymarin (S), with 20 µg/L silymarin (T), with 30 µg/L silymarin (U), with 10 µg/L *C. lentillifera* extract (V), with 20 µg/L *C. lentillifera* extract (W), with 30 µg/L *C. lentillifera* extract (X)

Table 1. Percentage Inhibition of Free Radical Activity Using DPPH Assay

Concentration (mg/mL)	Percentage Inhibition	
	L-Ascorbic Acid Standard	*C. lentillifera* Extract
24.0	96.1	29.7
27.0	95.9	17.1
30.0	97.5	20.4
33.0	97.5	5.6
36.0	97.3	43.2

4. Conclusion

After 72 hours of exposure to 10 μM and 25 μM APAP, zebrafish showed an increased mortality rate with increasing APAP concentrations. Concurrent treatment with NAC, silymarin, and *C. lentillifera* extract for 72 hours resulted in zero deaths. *C. lentillifera* might have a potent hepatoprotective property similar to known hepatoprotective agents, NAC and silymarin. The histopathological analysis of the hepatic tissues showed that *C. lentillifera extracts* (at 10–30 μg/L) prevented the progression of hepatic damage caused by 10 μM APAP. The results of DPPH free radical scavenging assay indicated that the hepatoprotective activity of *C. lentillifera* extract might have other antioxidant mechanisms aside from free radical scavenging. In addition, the concentration of the extract might be insufficient to show its antioxidant activity.

In order to effectively assess the improvement in the survival rate of juvenile zebrafish, longer exposure in the treatments is recommended. Additional antioxidant assays may be performed on the methanolic extract of *C. lentillifera* to determine its mechanism of hepatoprotective activity.

Acknowledgment

We would like to thank the faculty and staff of the De La Salle Medical and Health Sciences Institute College of Pharmacy and College of Medical Laboratory Science for the technical support in completing this research. We would also like to express our gratitude to the organizers of the 5th Asian Young Pharmacists Group (AYPG) Leadership Summit and the Indonesian Young Pharmacists Group for accepting this research paper for oral presentation in the summit.

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Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-89
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Acute toxicity study of *Andrographis paniculata* (Burm.f) Ness herbs and *Gynura procumbens* (Merr) leaves extracts combination

Studi toksisitas akut kombinasi ekstrak daun *Andrographis paniculata* (Burm.f) dan *Gynura procumbens* (Merr)

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Abstract

Background: Development of medical plants as an alternative treatment needs support in terms of scientific evidence to increase public confidence to ensure the safety of its use. Recent research on *Andrographis paniculata* (Burm. f) Ness and *Gynura procumbens* (Lour.) Merr showed that the combination of these extracts has a potential to be developed into antihyperglycemic agent and there's no any potential toxicity for each extract.

Objective: The aim of this study was to evaluate the acute toxicity level of these two extracts combination. From this research, it is expected that information can be obtained regarding the safety of extracts to support the further development of the extract combination.

Method: The method that used in this research is based on OECD 423. Observation was intensively done to animal behavior 4 h after acute exposure and continued up to 14 days after acute exposure to evaluate whether there were animal died. After the 15 days, all the animals were sacrificed and the vital organ was isolated for histological study.

Results: The results showed that the exposure of these combination didn't caused any to toxicity symptoms and there's no animals died. Histological study on hepar showed that there's no mayor damage in the hepar even after exposure of 2000 mg/kgBW dose.

Conclusion: The combination of ethanol extract of *A. paniculata* herbs and *G. procumbens* leaves was categorized as unclassified (>2000 mg/kgBW) in term of toxicity levels based on *Globally Harmonized Classification System*.

Keywords: *Andrographis paniculata* (Burm.f) Ness, *Gynura procumbens* (Lour.) Merr, acute toxicity

Intisari

Latar belakang: Pengembangan tanaman obat sebagai alternatif pengobatan perlu dukungan dari segi *scientific evidence* untuk meningkatkan kepercayaan masyarakat dan menjamin keamanan penggunaannya. Penelitian terbaru tentang sambiloto dan sambung nyawa menunjukkan bahwa kombinasi ekstrak tersebut berpotensi untuk dikembangkan menjadi agen antihiperglikemia dan dibutuhkan pemastian keamanannya.

Tujuan: Penelitian bertujuan untuk mengevaluasi potensi ketoksikan secara akut kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa.

Metode: Metode yang digunakan pada penelitian ini mengacu pada panduan OECD 423. Pengamatan yang dilakukan termasuk pada tingkah laku hewan uji tikus betina galur Wistar berjumlah 15 ekor, secara intensif terhadap gejala toksisitas selama 4 jam awal setelah paparan sediaan uji kemudian dilanjutkan hingga 14 hari pasca paparan untuk melihat ada/tidaknya hewan uji yang mati. Pada hari ke-15, seluruh hewan uji dikorbankan dan dibedah untuk diisolasi, ditimbang organ vitalnya dan dilakukan pengamatan histologi.

Hasil: Hasil penelitian menunjukkan bahwa kombinasi ekstrak tersebut tidak menyebabkan gejala toksik terhadap hewan uji dan tidak ada satupun hewan uji yang mati. Hasil histopatologi organ

hepar menunjukkan bahwa kombinasi ekstrak ini tidak menunjukkan efek berbahaya pada organ hepar hewan uji yang telah diberi paparan akut dengan dosis 2000 mg/kgBB.
Kesimpulan: Ketoksikan kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa masuk dalam kategori *unclassified* (>2000mg/kgBB) menurut *Globally Harmonized Classification System*.
Kata kunci : *Andrographis paniculata* (Burm.f) Ness, *Gynura procumbens* (Lour.) Merr, toksisitas akut

1. Pendahuluan

Indonesia memiliki keanekaragaman hayati yang sangat beragam. Banyak tanaman yang telah dimanfaatkan oleh masyarakat dalam berbagai hal termasuk untuk pengobatan sebagai obat tradisional. Saat ini, eksplorasi khasiat tanaman herbal telah banyak dilakukan. Banyak tanaman herbal yang diteliti khasiatnya dalam bentuk ekstrak tunggal. Di sisi lain, eksplorasi kombinasi tanaman obat dapat menjadi alternatif dalam pengembangan tanaman obat untuk mendapatkan hasil atau keuntungan yang lebih baik dalam terapi penyakit. Salah satunya adalah herba sambiloto (*Andrographis paniculata* (Burm.f) Ness) dan daun sambung nyawa (*Gynura procumbens* (Lour.) Merr) yang telah dikenal terkait efek hipoglikemiknya.

Berbagai penelitian yang menggunakan herba sambiloto dan sambung nyawa secara tunggal maupun dalam bentuk kombinasi dengan ekstrak lain telah banyak dilakukan. Salah satunya adalah terkait aktivitas ekstrak tunggal sambiloto dan ekstrak tunggal sambung nyawa sebagai agen hipoglikemia (Algariri, *et al*., 2014; Reyes *et al*., 2006; Zhang & Tan, 2000a, 2000b). Ekstrak sambung nyawa juga diketahui memiliki kemampuan atau manfaat sebagai antioksidan (Puangpronpitag *et al*., 2010).

Pengembangan obat herbal harus terus dilakukan secara berkesinambungan dan jangan sampai terputus pada satu tahap hingga uji farmakologi untuk bisa dimanfaatkan secara luas. Penelitian terkait efek herba sambiloto dan daun sambung nyawa yang diberikan secara kombinasi sebagai agen hipoglikemia telah dilakukan sejak tahun 2015 untuk mendapatkan komposisi perbandingan ekstrak kombinasi yang optimal (Sari *et al*., 2015). Sehingga langkah selanjutnya adalah studi untuk mengetahui potensi toksisitas akut apabila kedua ekstrak tersebut diberikan secara kombinasi. Apabila kombinasi kedua ekstrak tidak menyebabkan toksisitas akut pada hewan uji, maka pengembangan selanjutnya adalah untuk formulasi kombinasi kedua ekstrak tersebut.

Dari hasil pengujian aktivitas farmakologi herba sambiloto yang telah dilakukan, diketahui bahwa sambiloto berkhasiat sebagai antibakteria (Sule *et al*., 2010), antidiabetes mellitus (Zhang & Tan, 2000b), dan antiinflamasi (Chao, *et al.*, 2010),. Penelitian khasiat

sambiloto sebagai agen antidiabetes telah banyak dilakukan antara lain oleh Zhang dan Tan (2000a). Sambung nyawa telah lama digunakan dalam pengobatan seperti antihiperglikemik dan antihiperlipidemia (Zhang & Tan, 2000b), antiinflamasi (Iskander *et al*., 2002; Tan *et al*., 2016), antikarsinogen (Agustina, *et al*., 2006), penurun tekanan darah (Hoe *et al*., 2007; Kaur *et al*., 2013; Kim *et al*., 2006), antiproliferasi pada *human mesangial cell* (Tan *et al*., 2016), antioksidan (Puangpronpitag *et al*., 2010; Rosidah *et al*., 2009), dan anti ulcer (Mahmood *et al*., 2010).

Khasiat hipoglikemik kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa pernah diteliti oleh (Sari *et al*., 2015) pada tahun 2015 dan menunjukkan bahwa pemberian kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa dapat menurunkan kadar glukosa darah *preprandial* dan *postprandial* pada tikus terinduksi aloksan dengan daya hipoglikemik yang lebih baik daripada ekstrak tunggalnya. Selain itu, diketahui bahwa pemberian kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa, secara kualitatif dapat memperbaiki morfologi kerusakan pulau Langerhans dan ekspresi insulin pankreas pada tikus terinduksi aloksan.

Studi toksisitas akut ekstrak sambiloto secara tunggal telah dilakukan oleh (Chandrasekaran *et al*., 2009). Pada penelitian tersebut menunjukkan bahwa pemberian ekstrak sambiloto pada dosis hingga 5000 mg/kgBB tidak menunjukkan tanda-tanda toksisitas pada hewan uji. Oleh karena itu, ekstrak sambiloto dapat dikategorikan dalam kategori aman. Toksisitas ekstrak sambung nyawa secara tunggal telah diteliti sebelumnya. (Algariri *et al*., 2014) menyebutkan bahwa hasil dari studi toksisitas akut dan subkronis yang telah dilakukan berdasarkan OECD 425 dan 407 menunjukkan nilai LD_{50} melebihi dosis 2000 mg/kg. Oleh karena itu, ekstrak sambung nyawa masuk dalam kategori aman secara biokimia dan hematologi.

Adanya potensi yang baik dari kombinasi herba sambiloto dan daun sambung nyawa untuk dikembangkan menjadi alternatif pengobatan dalam penyakit hiperglikemik, mendorong peneliti untuk menguji keamanan kombinasi tanaman tersebut. Oleh karena itu, melalui penelitian ini akan dilakukan pengujian toksisitas akut kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa untuk melihat keamanan setelah dipaparkan terhadap hewan uji dalam 24 jam. Sehingga akan dapat diperoleh informasi sifat ketoksikan akut kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa yang akan menjadi dasar pengembangan kombinasi kedua ekstrak tersebut.

2. Metodologi penelitian

2.1. Deskripsi bahan

Bahan pembuatan ekstrak adalah herba sambiloto (*Andrographis paniculata* (Burm. f) Ness) dari daerah Sidoarum Sleman, daun sambung nyawa (*Gynura procumbens* (Lour.) Merr) dari daerah Sidoarum Sleman, dan etanol 70%. Bahan lain yang digunakan selama penelitian yaitu akuades, CMC Na 0.5%, buffer formalin 10%, NaCl fisiologis, dan pewarna Hematoxillin-Eosin.

2.2. Proses pembuatan ekstrak sambiloto dan sambung nyawa

Herba sambiloto dan daun sambung nyawa yang telah dipanen kemudian dikeringkan di bawah sinar matahari. Simplisia yang telah kering kemudian diserbuk dengan *grinder*. Serbuk herba Sambiloto (1022,2 gram) dan daun Sambung nyawa (1164,6 gram) diekstraksi dengan metode maserasi secara terpisah menggunakan pelarut etanol 70% (perbandingan 1:10). Proses maserasi pertama dilakukan pada suhu kamar selama 3 hari dan dilakukan pengadukan secara berkala. Setelah diremaserasi dua kali masing-masing 3 hari, filtrat yang diperoleh dikumpulkan dan kemudian dievaporasi hingga diperoleh ekstrak kental.

2.3. Pengujian toksisitas akut

Uji toksisitas akut dilakukan berdasarkan metode OECD 423 (OECD, 2002). Sebelum perlakuan, hewan uji tikus betina galur Wistar diaklimatisasi pada kandang uji selama 7 hari dan dipantau berat badannya. Sebanyak 15 hewan uji kemudian dibagi menjadi 5 kelompok perlakuan, yang terdiri dari 1 kelompok kontrol dan 4 kelompok uji (kelompok perlakuan pelarut, kelompok perlakuan ekstrak etanol herba sambiloto tunggal, kelompok perlakuan ekstrak etanol daun sambung nyawa tunggal, kelompok perlakuan kombinasi ekstrak etanol herba sambiloto dan daun sambung nyawa dengan masing-masing jumlah hewan uji per kelompok adalah tiga ekor.

Untuk sampel uji kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa, masing-masing ekstrak ditimbang sesuai proporsi dengan perbandingan komposisi 100 mg ekstrak larut etanol herba sambiloto dan 112 mg ekstrak larut etanol daun sambung nyawa. komposisi ekstrak yang dipilih berdasarkan hasil penelitian Sari *et al* (2015) yang menyatakan bahwa perbandingan optimal kedua ekstrak sebagai agen hipoglikemik adalah

100 mg ekstrak sambiloto dan 112 mg ekstrak sambung nyawa. Ekstrak uji kombinasi ini kemudian dihomogenkan dan disuspensikan dengan CMC Na menjadi sediian uji kelompok ekstrak kombinasi sambiloto dan sambung nyawa.

Pada tahap pertama, dosis yang diberikan terhadap hewan uji (15 ekor) merupakan dosis tunggal yang diberikan secara peroral dengan dosis awal adalah 300 mg/kgBB. Pengamatan yang dilakukan meliputi pengamatan perilaku hewan uji terhadap gejala toksik selama 4 jam setelah pemberian sediaan uji, kemudian dilanjutkan selama 24 jam dan dihitung jumlah hewan uji yang mati di setiap kelompoknya, serta dilakukan penimbangan bobot hewan uji pada hari ke-0, ke-7, dan ke-14 setelah perlakuan. Pada tahap kedua, dosis paparan akut dinaikkan menjadi 2000 mg/kgBB dengan jumlah hewan uji yang sama karena pada dosis sebelumnya tidak ditemukan adanya hewan uji yang mati.

Setelah selesai masa uji, seluruh hewan uji yang masih hidup dikorbankan dan dibedah untuk diambil organ vitalnya. Organ vital yang terdiri dari hepar, usus, ginjal, dan lambung ditimbang bobotnya sedangkan untuk organ hepar dimasukkan ke dalam pot plastik berisi larutan buffer formalin 10%, untuk selanjutnya dilakukan pembuatan preparat untuk melihat gambaran histopatologis tikus pada tiap kelompok perlakuan. Pembuatan preparat dan pewarnaan hematoxylin eosin (HE) dilakukan di Laboratorium Balai Besar Veteriner Wates. Pewarna HE merupakan senyawa pewarna yang umum digunakan untuk sel dan jaringan. Hematoksilin akan mengecat inti sel menjadi berwarna biru sedangkan eosin akan mengecat sitoplasma dan matriks ekstraseluler menjadi berwarna merah. Pengamatan perubahan histologis hati tikus dilakukan dengan bantuan mikroskop cahaya pada pembesaran 10x10 dan 10x40 untuk mengidentifikasi sel-sel hepar yang mengalami degenerasi dan nekrosis kemudian dibandingkan dengan kondisi organ hepar hewan uji kelompok kontrol yang hanya diberi pelarut.

3. Hasil dan pembahasan

Penelitian ini sebelumnya telah mendapatkan ijin *ethical clearance* dari tim Etik Penelitian Fakultas Kesehatan Universitas Jenderal Achmad Yani Yogyakarta dengan Nomor Skep/0170/KEPK/VII/2019. Pada penelitian ini, diperoleh rendemen ekstrak kental sambiloto sebesar 9,7% dan rendemen ekstrak kental sambung nyawa sebesar 8,1%. Hasil rendemen ekstrak sambiloto yang diperoleh memenuhi nilai yang dipersyaratkan Farmakope

Herbal Indonesia (FHI) tahun 2010 yaitu tidak kurang dari 9,6%. Hasil rendemen ekstrak kental sambung nyawa juga memenuhi persyaratan FHI yaitu tidak kurang dari 7,2%.

Hasil uji organoleptik ekstrak sambiloto yang diperoleh adalah berwarna hijau kecoklatan, bau khas pahit, konsistensi kental dan lengket, dan rasa sangat pahit. Sedangkan hasil uji organoleptik ekstrak daun sambung nyawa adalah berwarna hijau pekat, bau khas, konsistensi kental, dan rasa pahit. Uji organoleptik bertujuan untuk memberikan pengenalan awal ekstrak secara objektif berupa bentuk, warna, bau, dan rasa. Data ini juga dapat digunakan sebagai dasar untuk menguji ekstrak secara fisis selama penyimpanan. Uji organoleptik yang dilakukan meliputi pengamatan terhadap warna, bau, rasa dan konsistensi dari masing-masing ekstrak. Hasil uji organoleptik ekstrak berupa warna yang menunjukkan hasil ekstrak kental sambiloto bewarna hijau kecokelatan adalah akibat dari terjadinya polimerisasi senyawa fenolik dalam ekstrak sambiloto. Rasa pahit pada ekstrak sambung nyawa dapat disebabkan oleh kandungan senyawa turunan seskuiterpen lakton yang terdapat pada tanaman suku Asteraceae sedangkan pada ekstrak sambiloto rasa pahit akibat adanya kandungan metabolit turunan diterpen lakton seperti andrografolid.

Dosis yang diberikan pada hewan uji dimulai dari dosis 300 mg/kg BB sesuai petunjuk OECD 423 untuk senyawa bahan alam yang belum ada informasi dosis toksiknya dengan perhitungan volume pemberian maksimal 3 mL untuk tikus berbobot 200 gram. Dosis kemudian dinaikkan menjadi 2000 mg/kg BB sesuai dengan panduan OECD 423 dengan aturan volume pemberian yang sama. Pada pengujian tahap 1 dan tahap 2, pengamatan dilakukan selama 24 jam setelah pemberian sediaan uji dengan masa pengamatan intensif adalah 4 jam setelah paparan akut. Apabila ada hewan uji yang mati sebelum 24 jam maka hewan uji tersebut segera dibedah, diambil organ vitalnya seperti hati, ginjal, usus halus, jantung, dan lambung untuk diamati secara makroskopis. Pada kasus ini tikus yang dipejani sediaan uji tidak ada yang mengalami kematian pada dosis 300 mg/kgBB dan 2000 mg/kgBB pada 24 jam pertama baik pemberian pertama maupun pengulangan pada hewan yang berbeda hingga hari ke 14 penelitian. Sehingga pada hari ke 14 hewan uji dikorbankan untuk melihat efek tertunda yang mungkin muncul. Pengamatan secara mikroskopis dilakukan pada 4 organ vitalnya yaitu hati, ginjal, usus, dan lambung.

Tidak terdapat perubahan yang signifikan pada pengamatan kualitatif berupa gejala klinis pada kulit dan bulu, membran mukosa, sistem pernapasan, sistem sirkulasi, somatomotor, mata, sistem otonom, perilaku dan koma pada seluruh kelompok perlakuan

setelah pengamatan intensif selama 4 jam dan 24 jam setelah paparan senyawa uji. Seluruh hewan uji masih bersifat normal dan tidak ada keanehan dalam perilaku. Efek menegangnya bulu hewan uji hanya terlihat sementara segera setelah pemaparan yang mungkin diakibatkan stress-nya hewan uji karena menerima dosis paparan yang tinggi.

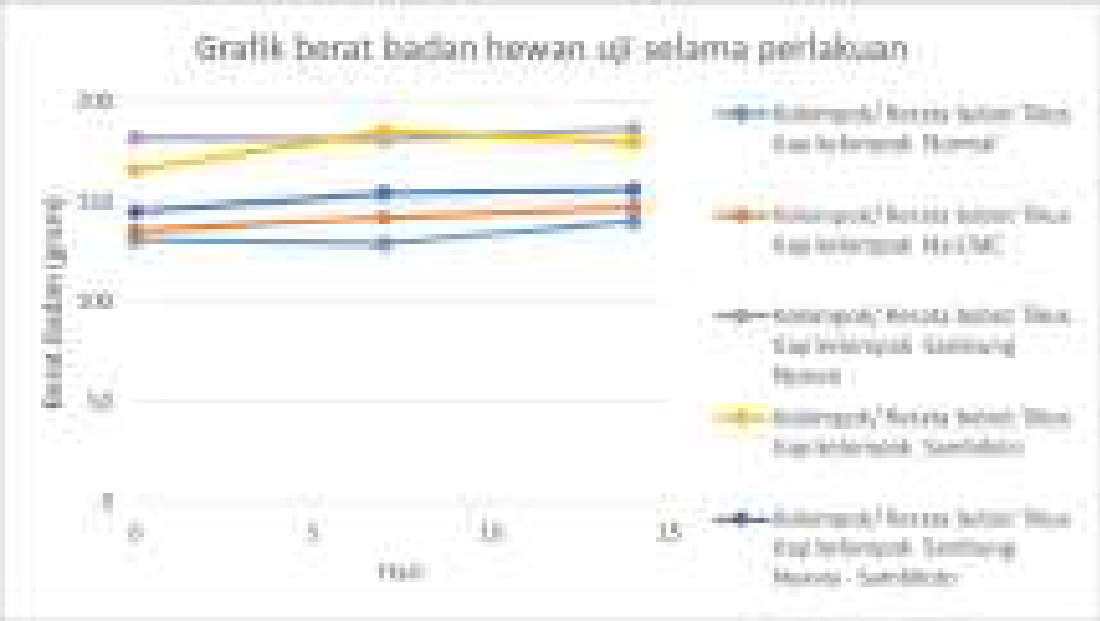
Jumlah kematian hewan uji pada masing-masing kelompok kontrol maupun kelompok dosis setelah pemberian ekstrak larut etanol herba sambiloto, daun sambung nyawa, dan kombinasi keduanya dapat dilihat pada Tabel 2. Pada tabel tersebut dapat dilihat bahwa pada tahap pertama, tidak ditemukan adanya hewan uji yang mati sehingga dilanjutkan dengan tahap ke-2 dan setelah akhir masa uji tidak ditemukan hewan uji yang mati.

Tabel 1. Perbandingan jumlah hewan uji antar kelompok perlakuan selama masa uji

Kelompok Perlakuan	**Jumlah Tikus Awal (ekor)**	**Jumlah Tikus Akhir Tahap I (ekor)**		**Jumlah Tikus Akhir Tahap II (ekor)**	
		Mati	**Hidup**	**Mati**	**Hidup**
Kontrol Normal	3	0	3	0	3
Kontrol Pelarut	3	0	3	0	3
Ekstrak Sambiloto Tunggal (100 mg/kgBB)	3	0	3	0	3
Ekstrak Sambung Nyawa Tunggal (112 mg/kgBB)	3	0	3	0	3
Ekstrak Kombinasi Sambiloto (100 mg/kgBB) - Sambung Nyawa (112 mg/kgBB)	3	0	3	0	3
Jumlah	15	0	15	0	15

Pada penelitian ketoksikan akut data perubahan berat badan hewan uji merupakan salah satu parameter yang digunakan untuk mengevaluasi kondisi kesehatan secara umum dari hewan uji. Pengamatan ini dapat digunakan untuk mempelajari kemungkinan mekanisme efek toksik akibat pemberian sediaan uji. Penimbangan berat badan hewan uji dilakukan pada hari ke-0, yaitu sebelum pemejanan sediaan uji,

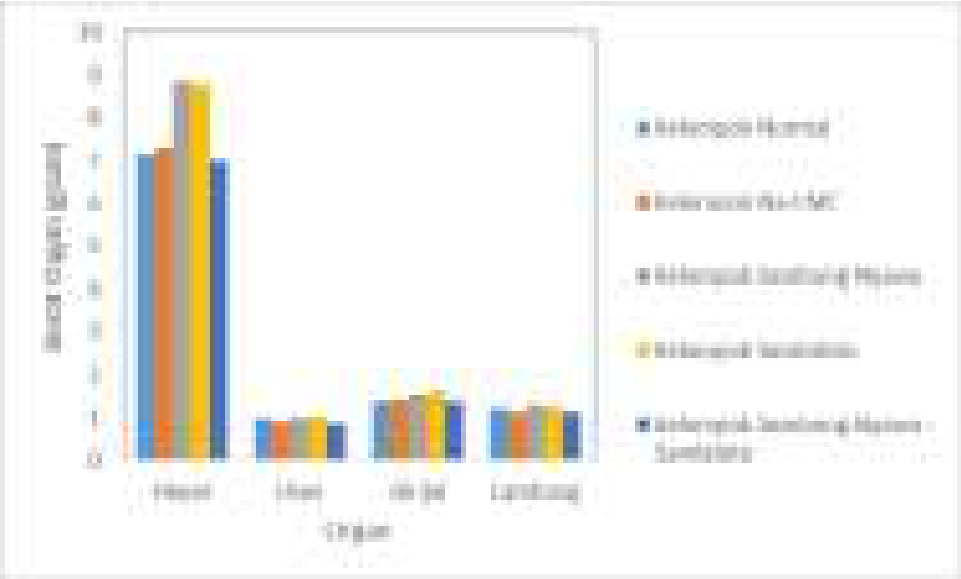
kemudian penimbangan dilakukan lagi sebelum hewan uji dikorbankan. Hewan uji dipelihara sampai 14 hari dan dilakukan penimbangan berat badan pada hari ke 0, 7, dan 14 untuk menghindari terjadinya stres. Salah satu tanda terjadinya ketoksikan adalah adanya penurunan berat badan hewan uji akibat pemaparan sampel uji. Adapun perubahan berat badan hewan uji dapat dilihat pada Gambar 1 dan terlihat jika pemberian sampel uji tidak memepengaruhi berat badan hewan uji selama perlakuan.



Gambar 1. Grafik berat badan hewan uji selama perlakuan dari hari ke-0, 7, dan 14

Pemeriksaan makroskopis dikenal juga dengan gross patologi. Pemeriksaan gross patologi menggunakan kaca tidak memperlihatkan perbedaan secara kasat mata antara organ vital hewan uji kelompok kontrol dengan perlakuan. Pengamatan makroskopis yang dilakukan terhadap organ vital hewan uji dengan cara mengamati organ hewan uji dibawah lup/kaca pembesar dilengkapi dengan penerangan yang cukup. Hasil pengamatan pada kelompok kontrol Na CMC 0,5% tidak menunjukkan adanya kerusakan secara kasat mata. Hal yang sama juga terjadi pada kelompok perlakuan dosis 300 mg/kg BB dan dosis 2000 mg/kg BB juga tidak menunjukkan adanya kerusakan pada organ hewan uji. Pengamatan makroskopis pada semua kelompok perlakuan selama 14 hari untuk melihat efek yang tertunda tidak menunjukkan adanya kerusakan. Dari hasil pengamatan makroskopis ini masih belum dapat disimpulkan pengaruh sediaan uji terhadap hewan uji karena perlu dilihat hasil pemeriksaan histopatologinya.

Sedangkan data bobot hewan uji setelah dikorbankan dapat dilihat pada Gambar 2 di bawah ini.

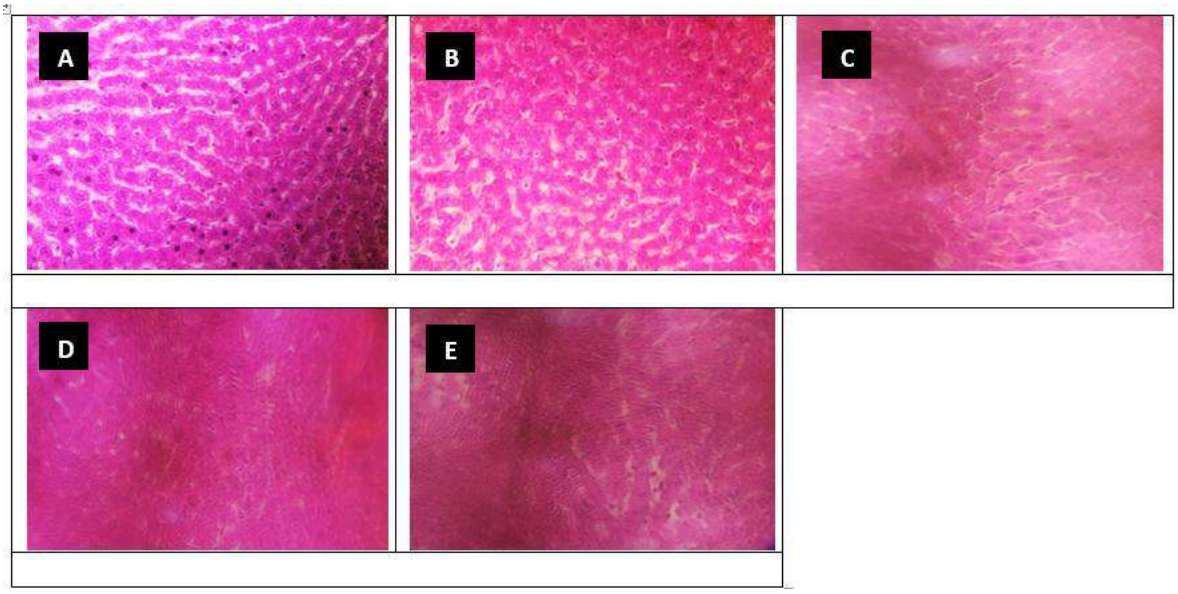


Gambar 2. Grafik bobot organ vital hewan uji yang terdiri dari hepar, usus, ginjal, dan lambung dari tiap kelompok perlakuan

Organ hepar memiliki berbagai fungsi penting dalam tubuh, beberapa diantaranya adalah menetralisir zat toksik, sintesis serum protein, pengaturan nutrisi dan menskresikan garam empedu (Dray, 2011). Berdasarkan hasil interpretasi preparat hispatologi pada organ hepar hewan uji dapat terlihat tidak adanya kerusakan sel kelompok uji kontrol normal (Gambar 3A) dan kelompok kontrol pelarut (Gambar 3B). Pada sel organ hepar, kemungkinan terjadi nekrosis yang ditandai dengan hancur atau hilangnya nukelus (inti sel). Penyebab terjadinya nekrosis adalah adanya toksin atau keracunan. Pada kelompok perlakuan akut senyawa uji ekstrak larut etanol daun sambung nyawa, diketahui bahwa pada histologi organ hepar terjadi pelebaran vena sentralis pada hepar yang kemungkinan disebabkan oleh tingginya kandungan flavonoid pada ekstrak sambung nyawa sebagai antioksidan (Gambar 3C). Tidak ada kerusakan berarti pada organ hepar kelompok perlakuan akut ekstrak larut etanol daun sambung nyawa. Pengamatan preparat organ hepar tikus yang mendapat perlakuan akut senyawa uji ekstrak larut etanol herba sambiloto tunggal dosis 2.000 mg/kg BB menunjukkan adanya pengecilan ukuran vena sentralis, terjadinya perlemakan hati, dan sinusoid yang ditandai dengan adanya bentuk inti sel yang gepeng dan gelap dengan sedikit

sitoplasma (Gambar 3D). Pada pengamatan histopatologi organ hepar hewan uji kelompok perlakuan akut kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa dosis 2000 mg/kgBB terlihat adanya vena sentralis yang berukuran normal dan adanya perlemakan sel hepar (Gambar 3E). Meskipun demikian tidak dijumpai adanya tikus yang mati selama pemberian perlakuan dan secara umum tidak ditemukan adanya kerusakan mayor pada organ hepar hewan uji.

Adanya senyawa flavonoid dan fenolik pada kedua ekstrak tersebut dapat bertindak sebagai antioksidan yang dapat mengurangi efek berbahaya dari senyawa toksin. Mekanisme efek potensiasi ini juga terjadi pada efek kombinasi kedua ekstrak tersebut sebagai agen hipoglikemik. (Sari *et al*., 2015) menyatakan bahwa tingginya senyawa flavonoid dan fenolik pada ekstrak sambung nyawa dapat membantu efek dari ekstrak sambiloto dalam memperbaiki sel β pulau Langerhans yang rusak akibat paparan aloksan sehingga terjadi pengurangan dosis dari ekstrak larut etanol herba sambiloto untuk menimbulkan efek hipoglikemik yang optimal.



Gambar 3. Gambaran histopatologi hepar tikus dengan pewarnaan HE. (A) tikus normal; (B) kontrol pelarut; (C) ekstrak tunggal sambung nyawa (112 mg/kgBB); (D) ekstrak tunggal sambiloto (100 mg/kgBB); (E) ekstrak kombinasi sambiloto (100 mg/kgBB) dan sambung nyawa (112 mg/kgBB) (perbesaran 400x)

Berdasarkan hasil kajian toksikologi akut dengan metode OECD 423 dan dilanjutkan dengan pemeriksaan histopatologi organ hepar hewan uji setelah paparan dosis 2000 mg/kgBB, maka dapat diketahui bahwa sampel uji kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa setelah pengujian toksisitas akut dengan metode OECD 423 tidak menunjukkan tanda toksisitas pada hewan uji. Hal ini didasarkan pada tidak adanya tanda-tanda toksisitas yang muncul setelah paparan akut sampel uji, tidak adanya hewan uji yang mati setelah paparan sampel uji, dan tidak adanya perubahan yang signifikan terhadap berat badan hewan uji yang diamati hingga 14 hari setelah paparan sampel uji.

Hasil penelitian menunjukkan bahwa pemaparan secara akut kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa pada hewan uji hingga dosis 2000 mg/kgBB tidak menunjukkan adanya kematian maupun gejala toksik pada hewan uji, sehingga kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa masuk dalam kategori *unclassified* (>2000mg/kgBB) menurut *Globally Harmonized Classification System* (GHS) (OECD, 2002). Sampel uji kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa berdasarkan pengamatan gross anatomi pada organ vital hewan uji tidak ditemukan adanya kerusakan dibandingkan dengan hewan uji normal. Hewan uji yang diberi paparan sampel uji kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa tidak menunjukkan adanya kerusakan histopatologi mayor apabila dibandingkan dengan gambaran pada hepar tikus normal.

Kesimpulan

Kombinasi ekstrak larut etanol herba sambiloto dan daun sambung nyawa tidak menunjukkan tanda toksisitas pada hewan uji setelah paparan akut sesuai metode OECD 423 dan masuk dalam kategori *unclassified* (>2000mg/kgBB) menurut *Globally Harmonized Classification System* (GHS). Oleh karena itu kombinasi kedua ekstrak ini berpotensi untuk dikembangkan menuju proses formulasi optimal sebagai produk hipoglikemik dan dapat dilanjutkan pada pengujian toksisitas sub kronis.

Ucapan terimakasih

Terima kasih kami ucapkan kepada Kemenristek DIKTI yang telah mendanai penelitian ini dan Fakultas Kesehatan Universitas Jenderal Achmad Yani Yogyakarta yang telah turut mendukung terlaksananya penelitian ini.

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Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-95
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Fungal endophytes as the source of medicinal natural product

Jamur endofit sebagai sumber obat bahan alam

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Abstract

Massive exploration of medicinal plants as a source of medicinal raw materials and high demand for traditional medicines on the market has been a threat to biodiversity and plant species. To respond to the challenge of more efficient access to chemical diversity in a sustainable way, researchers have begun to focus their research on renewable sources, under-explored, but that have the prospect as the reservoir of new structures of bioactive metabolites, namely fungal endophytes. Fungal endophytes grow within the internal tissue s of the plant, without causing pathogenic symptoms and to have succeeded in producing secondary metabolites with diverse chemical structures and pharmacological activities such as antibacterial, antifungal, insecticide, antioxidant, anti hyperlipidaemia, cytotoxic and anticancer. However, under conventional laboratory conditions, a plethora of secondary metabolites encoded in fungal endophytes were not produced presumably because the genes responsible for the secondary metabolites biosynthetic are not transcribed (remain silent). Several methods have been explored to activate these silent genes, including optimization parameters of fermentation, co-culture techniques, precursors/ plant extracts feeding, the addition of epigenetic modifiers such as *DNA methyltransferase* (DNMT) or *histone deacetylase* (HDAC) inhibitors, and genetic manipulation of biosynthetic and regulatory genes. The approaches in culture techniques are expected to bridge the debate in drug discovery and natural material production from endophytic fungi.
Keywords: fungal endophytes, pharmacological activities, activating silent gene

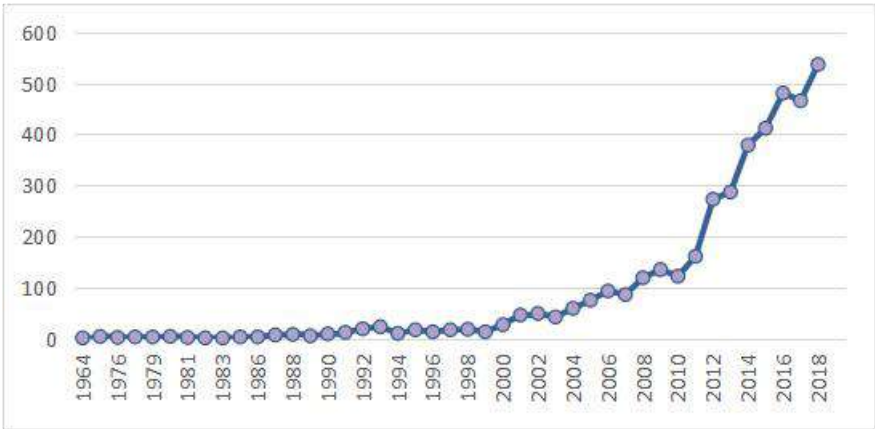
Intisari

Eksplorasi besar-besaran tanaman obat sebagai sumber bahan baku obat dan tingginya permintaan akan obat tradisional di pasaran telah menimbulkan permasalahan dalam biodiversitas dan ancaman bagi spesies tanaman. Peneliti bahan alam telah mulai memfokuskan penelitannya pada sumber terbarukan yang belum tereksplorasi namun memiliki prospek sebagai penyedia keanekaragaman struktur kimia, yaitu jamur endofit. Jamur endofit hidup di dalam jaringan tanaman tanpa menimbulkan simptom patogenik dan telah dilaporkan menghasilkan metabolit sekunder dengan struktur kimia yang beragam dengan aktivitas farmakologi yang luas seperti antibakteri, antijamur, insektisida, antioksidan, antihiperlipidemia, sitotoksik dan antikanker yang sangat potensial untuk dikembangkan dalam industri farmasi. Dalam pengembangannya, pemanfaatan jamur endofit memiliki beberapa kendala, utamanya dalam teknik kultur / fermentasi dalam rangka mengaktifkan gen penyandi biosintesis metabolit sekunder yang relatif inaktif selama kultur. Metode untuk mengaktifkan gen diam (*silent gen*e) dapat dilakukan dengan beberapa cara yaitu: optimasi parameter fermentasi, teknik ko-kultur, penambahan prekursor atau zat antara ke dalam media kultur, penambahan modifikator epigenetik seperti inhibitor *DNA methyltransferase* (DNMT) dan atau inhibitor *histone deacetylase* (HDAC), dan manipulasi genetik. Pendekatan dalam teknik kultur jamur endofit diharapkan dapat menjembatani permasalahan dalam penemuan obat dan produksi bahan alam dari jamur endofit.
Kata kunci: jamur endofit, aktivitas farmakologi, aktivasi gen diam

1. Pengantar

Bahan alam (*natural products*) merupakan kunci utama dalam pengembangan obat terutama sebagai sumber senyawa penuntun. Bahan alam menjadi sumber utama penyedia keanekaragaman struktur senyawa kimia dibandingkan pengembangan struktur melalui kimia kombinatorial (Newman & Cragg, 2016). Dalam tiga dekade terakhir eksplorasi besar-besaran untuk menemukan senyawa baru bahan baku obat dari tanaman telah dilakukan namun proses uji farmakologi yang dilakukan baru mencapai sekitar 6% dari total species tanaman yang ada (24 ribu dari 391 ribu species), dan hanya 15% diantaranya yang dilakukan uji fitokimia (Newman & Cragg, 2012).

Proses penemuan senyawa penuntun dengan metode konvensional merupakan proses yang panjang, membutuhkan biaya yang relatif mahal, dan membutuhkan banyak pelarut dan bahan kering tanaman sebagai bahan baku. Selain itu, proses tersebut dilakukan dengan teknik ekstraksi dan isolasi yang umum yang seringkali memperoleh senyawa yang pernah ditemukan sebelumnya (*re-discovery*) dengan aktivitas biologi yang tidak konsisten. Proses penemuan senyawa dari bahan alam telah mengalami perubahan perspektif dengan lebih memperhatikan biodiversitas sebagai dampak dari pemanasan global. Peneliti bahan alam telah mulai memfokuskan penelitannya pada sumber terbarukan yang belum tereksplorasi namun memiliki prospek sebagai penyedia keanekaragaman struktur kimia, yaitu jamur endofit. Jamur endofit tumbuh di dalam jaringan tanaman dan menyebabkan infeksi yang tidak terlihat dan tidak bergejala. Jamur endofit semakin dikenal sebagai produsen dalam biosintesis produk alami sejak penemuan Taxol yang berhasil disintesis oleh jamur *Taxomyces andreana* yang diisolasi dari batang *Taxus brevifolia* (Stierle *et al*., 1993; Y. Yang *et al*., 2014). Jamur ini telah terbukti sangat potensial untuk sintesis *de novo* dari berbagai metabolit bioaktif yang dapat secara langsung atau tidak langsung digunakan sebagai agen terapi terhadap berbagai penyakit (Kusari *et al*., 2012; Strobel, 2003). Riset tentang jamur endofit telah sangat meningkat jumlahnya dalam dua dekade terakhir. Berdasarkan data dari PubMed dari tahun 1964-2018, terdapat 4047 publikasi dengan kata kunci “fungal endophytes”. Banyaknya riset per tahun yang memfokuskan penelitiannya pada jamur endofit dapat dilihat pada Gambar 1 berikut.



Gambar 1. Jumlah publikasi dengan topik jamur endofit (Grafik diolah dari data yang diperoleh dari publikasi di PubMed dengan kata kunci "*fungal endophytes"* dari tahun 1964 - 2018)

2. Hutan Indonesia sebagai hotspot jamur endofit

Jamur endofit dapat ditemukan pada semua tanaman mulai dari daerah Arktik sampai daerah Tropis, dengan famili terbesar Actinomycetes, Dothideomycetes, Sordariomycetes, Pezizomycetes, Leotiomycetes dan Eurotiomycetes (Arnold, 2007; Higginbotham *et al*., 2013). Daun tanaman di daerah Tropis terkolonisasi 100% oleh jamur endofit sementara di daerah Arktik hanya 20%. Bahkan, satu tanaman di daerah Tropis bisa dikolonisasi 30-40 jenis jamur endofit (Herre *et al*., 2009). Hal ini menunjukkan potensi tanaman di daerah tropis sebagai sumber jamur endofit. Sebagai salah satu contoh adalah kawasan hutan baku Indonesia yang tumbuh di sepanjang garis pantai Indonesia sepanjang 95.000 km (23 persen dari semua ekosistem bakau di dunia)(Giri *et al*, 2011; von Rintelen *et al*., 2017) merupakan sumber dari lebih dari 200 spesies jamur endofit (M. Y. Li *et al*., 2009) dengan serangkaian bioaktivitas luar biasa seperti sitotoksik dan antiinfeksi serta aktivitas khusus sebagai penghambat protein kinase, α- glukosidase, asetilkolinesterase dan tirosinase (Debbab *et al*., 2013). Sayangnya, penelitian pemanfaatan jamur endofit masih sangat minim dan terbatas pada penggunaannya sebagai agen biokontrol dalam bidang pertanian dan agroindustri (Ministry of Environment and Forestry of Indonesia, 2014). Pemanfaatan jamur endofit untuk pengembangan obat masih sangat terbatas baik dari segi jenis penelitian dan sumber dana penelitian (Kementerian Riset Teknologi dan Pendidikan Tinggi, 2017) sehingga diperlukan langkah strategis dan integratif dalam pengembangannya.

3. Aktivitas farmakologi dari jamur endofit

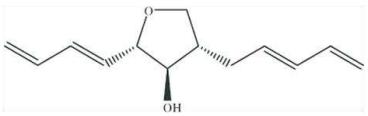
Jamur endofit telah banyak diaplikasikan dalam bidang pertanian sebagai biopestisida atau pengatur pertumbuhan tanaman (Butt TM, Jackson C, 2001; Gao *et al*., 2010; S. N. Kumar *et al*., 2014) , dalam bidang industri sebagai sumber enzim dan katalis (Corrêa *et al*., 2014; Toghueo & Boyom, 2020), dan dalam bidang teknik lingkungan digunakan untuk fitoremediasi atau pengontrol polusi (Rozpadek *et al*., 2017; C. Wang *et al*., 2014). Di dalam bidang kesehatan, jamur endofit telah banyak diteliti dan dilaporkan memiliki aktivitas farmakologi yang cukup luas. Senyawa aktif beberapa jamur endofit telah berhasil diisolasi dan menunjukkan struktur kimia yang bervariasi mulai dari benzopiran, poliketida, terpenoid, senyawa fenolik, alkaloid, peptida dan peptida siklik, serta diketopiperazin (Barakat *et al*., 2018; Mousa & Raizada, 2013). Contoh metabolit beserta jamur penghasil dan aktivitas farmakologinya dapat dilihat pada Tabel 1 dan contoh struktur metabolit sekunder dari jamur endofit dapat dilihat pada Gambar 2

Tabel 1. Metabolit dan aktivitas farmakologi jamur endofit

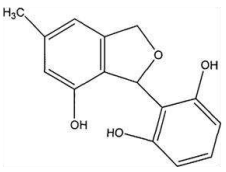
Metabolit	Golongan senyawa	Aktivitas	Endofit	Referensi
Aureonitol	Aromatik sederhana	Antibakteri	*Chaetomium globosum* dari tanaman tomat	Kurt *et a*l., 2016
Biscogniazaphilone A *Biscogniazaphilone B*	Azapilon 2-benzopiran	Antimikobakterium	*Biscogniauxia formosana* BCRC 33718 dari *Cinnamomum* sp.	Cheng *et al*., 2012
Beauvericin	Depsipeptidaa	Antibakteri terhadap MRSA dan *B. subtilis*	*F. oxysporum* dari tanaman *C. kanehirae*	Q.-X. Wang *et al*., 2011
Cochlioquinone A *Isocochlioquinone A*	Meroterpenoid	Antileishmania	*Cochliobolus sativus* dari *Vernonia polyanthes*	do Nascimento *et al*., 2015
cis-4-acetoxyoxymellein *8-deoxy-6-hydroxy-cis-4-acetoxyoxymellein*	Poliketida	Antibakteri terhadap *E. coli* dan *Bacillus megaterium,* Antijamur terhadap *Microbotryum violaceum* dan *Botrytis cinerea*	Jamur dari tanaman *Meliotus dentatus*	Hussain *et al*., 2015
Guanacastepene	Diterpenoid	Antibakteri terhadap MRSA dan *Enterococcus faecium* yang resisten *vancomycin*	Jamur dari tanaman *Daphnopsis americana*	Brady *et al*., 2001

Metabolit	Golongan senyawa	Aktivitas	Endofit	Referensi
Phomopsichalasin	Sitokalasin	Antibakteri terhadap *B. subtilis, S. aureus*, dan *Salmonella gallinarum* (patogen pada unggas) dan antijamur terhadap *Candida tropicalis*	*Phomopsis* sp. yang diisolasi dari tanaman *Salix gracilostyla*	Sunil K. Deshmukh *et al*., 2018 Sunil Kumar Deshmukh *et al*., 2014
Sordaricin	Diterpenoid	Antijamur terhadap *C. albicans*	*Xylaria* sp. yang diisolasi dari tanaman *Garcinia dulcis*	Mousa & Raizada, 2013
1α-10α-Epoxy-7α-hydroxyeremophil-11-en-12,8-β-olide	Sesquiterpenoid	Antimalaria terhadap *Plasmodium falciparum*	*Xylaria* sp. BCC 21097, yang diisolasi dari *Licuala spinosa*	Isaka *et al*., 2010
monocerin dan *11-hydroxymonocerin*	Poliketida	Antimalaria terhadap *P. falciparum*	Jamur yang diambil dari *Exserohilum rostratum*	Sappapan *et al*., 2008
Palmarumycin CP17 Palmarumycin CP18	Spirobisnaftalen	*Antileishmania*	*Edenia* sp. dari tanaman *Petrea volubilis*	Martínez-luis *et al*., 2011
Cercosporin	Poliketida	*Antileishmania*	*Mycosphaerella* sp. nov. strain F2140 dari *Psychotria horizontalis*	Moreno *et al*., 2011
Pestacin Isopestacin	Isobenzofuran	*Antioxidant*	*P. microspora* dari *T. morobensis*	Kouipou & Boyom, 2019 Strobel & Daisy, 2003
Tauranin	Sesquiterpenoid	*Sitotoksik pada* sel NCI-H460 (*non small cell lung cancer*), MCF-7 (sel kanker payudara), SF-268 (glioma), PC-3M (sel kanker prostat metastatik), dan MIA Pa Ca-2	*Phyllosticta spinarum* yang diisolasi dari tanaman *Platycladus orientalis*	Aly *et al*., 2011
Altersolanol	Antranoid	Antiangiogenesis	*Alternaria* sp. dari tanaman *Erythrina variegata*	Pompeng *et al*., 2013
Guignasulfide	Benzofenon	Sitotoksik terhadap sel kanker manusia HepG2	*Guignardia* sp. IFB-E028 yang diambil dari tanaman *Hopea hainanensis*	F. W. Wang *et al*., 2010

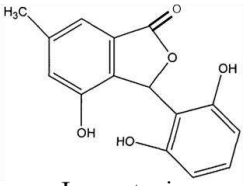
Metabolit	Golongan senyawa	Aktivitas	Endofit	Referensi
Diaporthesin C	Poliketida	Penghambatan trigliserida pada sel *steatotic L-02*	*Diaporthe* sp. JC-J7	Hu *et al*., 2018
Cycloepoxylactone	Monokarbosiklik poliketida	Antijamur, antibakteri	*Phomopsis* sp dari *Laurus azorica*	Hussain *et al*., 2009
Cryptocandin	Lipopeptida	Antijamur	*Cryptosporiopsis cf. quercina* dari *Tripterigium wilfordii*	Strobel *et al*., 1999
Deacetyl-mycoepoxydiene	Monokarbosiklik poliketida	Antikanker pada sel MCF	*Phomopsis* sp. dari tanaman bakau	Zhu *et al*., 2015
7-desmethyl fusarin C - derivates	Alkaloid pirolidin	Antibakteri terhadap *E. coli*	*Fusarium solani JK10* yang diambil dari *Chlorophora regia*	Kyekyeku *et al*., 2017
Epichlicin	Peptida siklik	Antijamur	*Ephichloe typina* dari *Phleum pretense*	Seto *et al*., 2007
Pestalofone F	Asam amino dan peptida	Sitotoksik terhadap sel HeLa dan MCF-7 cells	*Pestalotiopsis fici*	S. Kumar & Kaushik, 2012
Phomone D	Poliketida	Antikanker	*Phoma* sp. YN02-P-3	S. J. Li *et al*., 2018
Pycnophorin	Meroterpenoid	Antibakteri terhadap *S. aureus* dan *B. subtilis*	*Botryosphaeria dothidea*, diisolasi dari *Melia azedarach*	Xiao *et al*., 2014
Paclitaxel	Diterpenoid	Antikanker	*Taxomyces andreanae*	Heinig *et al*., 2013
Torreyanic acid	Alifatik-Polisikloheteroaliksiklik	Sitotoksik	*Pestalotiopsis microspora*	Kaul *et al*., 2012
10-Hydroxycamptothecin	Alkaloid kamptotesin	Antikanker	*Fusarium solani* dari *Camptotheca acuminata*	Pu *et al*., 2013



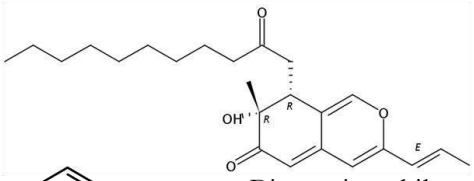
Aureonitol



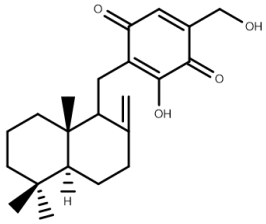
Pestacin



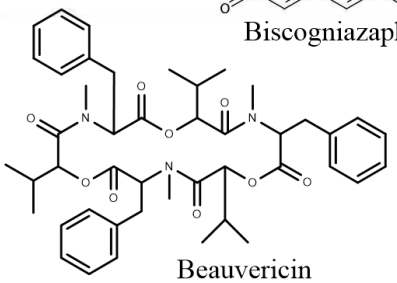
Isopestacin



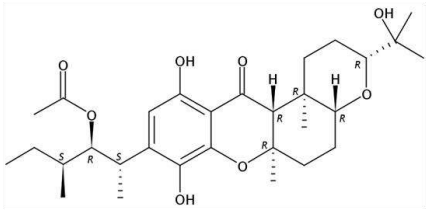
Biscogniazaphilone A



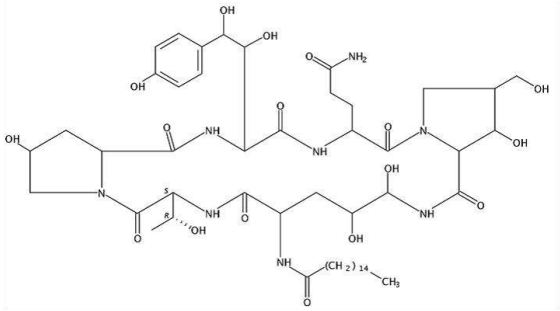
Tauranin



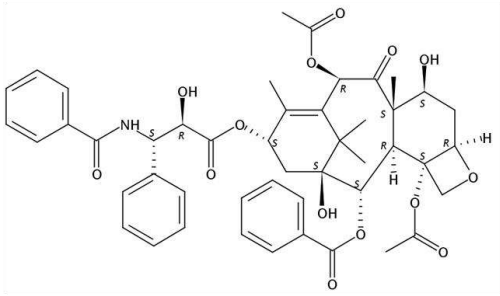
Beauvericin



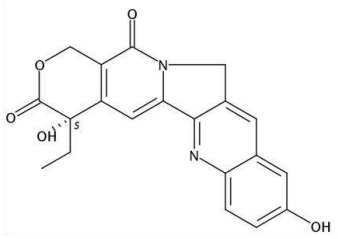
Isocochlioquinone A



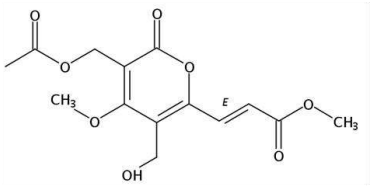
Cryptocandin



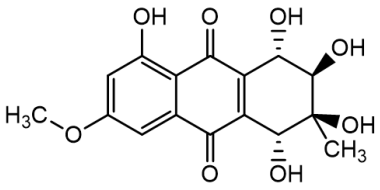
Paclitaxel



10-Hydroxycamptothecin



Phomone D



Artesolanol A

Gambar 2. Beberapa contoh metabolit sekunder dari jamur endofit

3.1. Antimikroba

Jamur endofit melindungi tanaman terhadap berbagai macam patogen seperti bakteri, jamur, dan serangga yang memungkinkan sifat antimikroba tersebut umum ditemukan pada beberapa genera jamur seperti Aspergillus, Alternaria, Colletotrichum, Fusarium, Penicillium, dan Pestalotiopsis (Casella *et al*., 2013; Gupta *et al*., 2020; Martín-Rodríguez *et al*., 2014; Selim *et al*., 2012). Beberapa jenis jamur yang potensial misalnya *Fusarium tricinctum* yang diisolasi dari *Rhododendron tomentosum*, menghasilkan senyawa antibakteri dan antijamur terhadap *Staphylococcus carnosus* dan *Candida albicans* serta *C. utilis*. Sementara itu ekstrak heksan jamur *Colletotrichum gloeosporioides* yang diisolasi dari tanaman obat *Vitex negundo* memiliki aktivitas antibakteri terhadap bakteri *S. aureus* yang resisten terhadap *methicillin*, *penicillin* dan *vancomycin* (Arivudainambi *et al*., 2011). Jamur *F. oxysporum* dari tanaman *C. kanehirae* memproduksi senyawa *beauvericin* yang memiliki aktivitas kuat terhadap MRSA dan *Bacillus subtilis* dengan nilai MIC 3.125 µg/mL (Q.-X. Wang *et al*., 2011). Jamur dari tanaman *Meliotus dentatus* memproduksi senyawa poliketida yaitu *cis-4-acetoxyoxymellein* dan *8-deoxy-6-hydroxy-cis-4-acetoxyoxymellein* yang aktif terhadap *Escherichia coli* dan *Bacillus megaterium*. Kedua poliketida tersebut juga aktif terhadap jamur *Microbotryum violaceum* dan *Botrytis cinerea* (Hussain *et al*., 2015).

Guanacastepene, suatu diterpenoid yang diproduksi oleh jamur dari tanaman *Daphnopsis americana* memiliki aktivitas antibakteri terhadap MRSA dan *Enterococcus faecium* yang resisten *vancomycin* dengan mekanisme perusakan membran bakteri (Brady *et al*., 2001). *Phomopsichalasin* dari jamur *Phomopsis* sp. yang diisolasi dari tanaman *Salix gracilostyla* memiliki aktivitas anti bakteri terhadap B*. subtilis, S. aureus*, dan *Salmonella gallinarum* (patogen pada unggas) dan antijamur terhadap *Candida tropicalis* (Sunil K. Deshmukh *et al*., 2018; Sunil Kumar Deshmukh *et al*., 2014). *Sordaricin* yang diisolasi dari *Xylaria* sp. dari tanaman *Garcinia dulcis* menunjukkan aktivitas antijamur moderat terhadap *C. albicans*. Sordarin sebelumnya terbukti menghambat sintesis protein jamur dengan mekanisme mengikat secara selektif dan menghambat faktor pemanjangan 2 (EF-2) yang mengkatalisasi translokasi ribosom selama proses translasi (Justice *et al*., 1998; Mousa & Raizada, 2013)

3.2. Antiparasit

Jamur *Xylaria* sp. BCC 21097, yang diisolasi dari *Licuala spinosa* menghasilkan senyawa *1α-10α-Epoxy-7α-hydroxyeremophil-11-en-12,8-β-olide* yang aktif terhadap *Plasmodium falciparum* dengan mekanisme terkait dengan struktur epoksid dari senyawa tersebut (Isaka *et al*., 2010). Beberapa metabolit dari jamur endofit juga menunjukkan aktivitas terhadap *P. falciparum* (K1,

multidrug-resistant strain) seperti *monocerin* dan *11-hydroxymonocerin* yang diisolasi dari *Exserohilum rostratum* dengan IC_{50} berturut-turut 0.68 dan 7.70 µM dan metabolit turunan *benzoquinone* dan *xylariaquinone* A dari *Xylaria* sp. dengan IC_{50} 1.84 dan 6.68 µM, dan P*homoxanthones* A dan B, dari jamur *Phomopsis* sp. BCC 1323 (Sappapan *et al*., 2008; Tansuwan *et al*., 2007).

Palmarumycin CP17 dan *palmarumycin CP18* yang diisolasi dari *Edenia* sp. dari tanaman *Petrea volubilis* mampu menghambat amastigot dari *Leishmania donovani*, penyebab leishmaniasis dengan EC_{50} berturut-turut 1.34 and 0.62 µM, (kontrol positif *amphoterycin* B=EC_{50} 0.09 µM) dan memiliki aktivitas toksisitas rendah terhadap sel Vero (Martínez-luis *et al*., 2011). *Cercosporin* dan metabolit analog dari *Mycosphaerella* sp. nov. strain F2140 yang diambil dari tanaman *Psychotria horizontalis* juga menunjukkan aktivitas penghambatan pada *L. donovani* (IC_{50} 0.46 dan 0.64 µM), *Tripanosoma cruzi* (IC_{50} 1.08 and 0.78 µM) dan *P. falciparum* (IC_{50} 1.03 dan 2.99 µM) (Moreno *et al*., 2011).

3.3. Antioksidan

Ekstrak dari jamur endofit juga dilaporkan memiliki aktivitas sebagai antioksidan berdasarkan uji antioksidan dengan metode DPPH seperti jamur *Aspergillus awamori* DT11 yang diisolasi dari tanaman stroberi. Senyawa golongan flavonoid dan terpenoid yang terkandung dalam ekstrak memungkinkan efek antioksidan tersebut (Hipol *et al*., 2014). Selain itu, ekstrak dari jamur *Aspergillus* sp.JPY1 dan *Phoma* sp. dari tanaman *S. oleoides* juga memiliki aktivitas antioksidan yang tinggi dan tidak menunjukkan toksisitas pada hewan uji sampai dosis 1000mg/kgBB (Dhankhar *et al*., 2012).

Huang *et al*., (2007) telah meneliti 292 jamur endofit dari 29 tanaman obat dan menemukan aktivitas antioksidan yang bervariasi dari jamur endofit. Aktivitas terbesar dimiliki oleh jamur *AcapF3* dari *Artemisia capillaris* dengan aktivitas of 526.93 µmol trolox/100 ml kultur dan jamur *TwL3* dari tanaman *T. wightianus* dengan aktivitas antioksidan (298.35 µmol/100 ml kultur. Analisis terbaru dari Gupta *et al*., (2020) menyatakan bahwa senyawa seperti *Pestacin, Isopestacin, Rutin, Corynesidones A* dan *B, Borneol, Lapachol, Coumarin, p-Tyrosol* dari jamur endofit memiliki aktivitas antioksidan yang potensial.

3.4. Antikanker

Banyak jamur endofit yang memiliki aktivitas sitotoksik kuat terhadap beberapa jenis sel kanker sehingga potensial dikembangkan sebagai senyawa antikanker. Sebagai contoh, senyawa *tauranin* dari jamur *Phyllosticta spinarum* yang diisolasi dari tanaman *Platycladus orientalis*

memiliki aktivitas sitotoksik dengan EC_{50} berturut-turut 4.3, 1.5, 1.8, 3.5, and 2.8 μM terhadap sel NCI-H460 (*non small cell lung cancer*), MCF-7 (sel kanker payudara), SF-268 (glioma), PC-3M (sel kanker prostat metastatik), dan MIA Pa Ca-2 (sel karsinoma pankreas), dibandingkan dengan *doxorubicin* sebagai kontrol positif dengan EC_{50} berturut-turut 0.01, 0.07, 0.04, dan 1.11 μM. Mekanisme dari *tauranin* adalah dengan menginduksi apoptosis pada sel kanker (Aly *et al*., 2011). Selain itu, *altersolanol* yang diproduksi oleh *Alternaria* sp. dari tanaman *Erythrina variegata* dilaporkan memiliki aktivitas antiangiogenesis (penghambatan pembentukan pembuluh darah baru pada kanker). Pada model sel endotelial vena umbelikal manusia, altersolanol mampu menghambat proliferasi, pembentukan pembuluh darah, dan migrasi dari sel endotelial (Pompeng *et al*., 2013). Sementara itu, senyawa *guignasulfide* dari jamur *Guignardia* sp. IFB-E028 yang diambil dari tanaman *Hopea hainanensis* memiliki aktivitas sitotoksik terhadap sel kanker manusia HepG2 dengan EC_{50} 5.2±0.4 μM (F. W. Wang *et al*., 2010).

3.5. Antituberkulosis

Sebanyak 1,5 juta orang meninggal karena tuberkulosis (TB) pada tahun 2018 (termasuk 251.000 orang dengan *HIV*). Di seluruh dunia, TB adalah salah satu dari 10 penyebab utama kematian dan penyebab utama dari satu agen infeksius (di atas *HIV* / *AIDS*) (World Health Organization, 2020). Jamur endofit telah dilaporkan memiliki aktivitas terhadap *Mycobacterium tuberculosis*. Sebagai contoh, senyawa *diaporthein* B dari kultur jamur *Diaporthe* sp. BCC 6140 dan *Phomoenamide* dari jamur *Phomopsis* sp. dari tanaman *Garcinia dulcis* memiliki aktivitas penghambatan pertumbuhan bakteri TB dengan metode kolorimetri menggunakan *Alamar Blue* (Dettrakul *et al*., 2003; Rukachaisirikul *et al*., 2008). Contoh lain, jamur *Chaetomium globosum* menghasilkan alkaloid *piperazine* yang aktif terhadap *Mycobacterium tuberculosis* H37Ra dengan konsentrasi hambat minimum (MIC) sebesar 169.92 mM (Martins & Carvalho, 2007) dan *3-nitropropionic acid* berhasil diisolasi dari *Phomopsis longicolla* dari tanaman *Trichilia elegans* yang aktif terhadap bakteri TB (Flores *et al*., 2013). Senyawa *Chaetoglobosin A* dan *chaetoglobosin B* dari *Asperillus fumigatus* juga aktif terhadap *Mycobacterium tuberculosis* H37Ra selain aktif pada bakteri *S. aureus* dan MRSA (Flewelling *et al*., 2015).

3.6. Antihyperlipidemia

Jamur *Diaporthe arengae* yang diisolasi dari *Terminalia arjuna* mengandung senyawa fenolik yang memiliki aktivitas anti-hiperkolesterol dengan penghambatan peroksidasi lipid secara *in vitro* dan mampu menurunkan kolesterol total dan LDL kolesterol pada hewan uji (Patil *et al*., 2017).

Selain itu, senyawa *diaporthesin C* yang diisolasi dari fermentasi *Diaporthe* sp. JC-J7 menunjukkan penghambatan trigliserida pada sel *steatotic L-02* (Hu *et al*., 2018). Hal ini merupakan bukti potensi jamur endofit untuk dapat dikembangkan sebagai sumber bahan baku obat.

4. Tantangan dalam pengembangan jamur endofit

4.1. Seleksi tanaman sebagai sumber jamur endofit

Mempertimbangkan besarnya jumlah dan biodiversitas tanaman, strategi yang tepat harus digunakan untuk mempersempit pencarian endofit yang potensial sebagai sumber senyawa obat. Beberapa hipotesis yang mengatur strategi pemilihan tanaman ini telah dikemukakan oleh Strobel dan Daisy (2003) yaitu: (i) Tanaman yang hidup di ekosistem khas, dengan kondisi biologi yang tidak biasa dan memiliki strategi unik untuk bertahan hidup, misalnya tanaman bakau (Apurillo *et al*., 2019; Calcul *et al*., 2013; Osorio *et al*., 2017); (ii) Tumbuhan yang memiliki sejarah etnobotani (digunakan oleh masyarakat secara turun-temurun) yang terkait dengan penggunaan sebagai obat (etnomedisin) misalnya *Melia azadirachta* (mimba) atau *Centella asiatica* (pegagan) dan *Piper betle* (sirih) yang penggunaanya sangat luas di masyarakat untuk pengobatan (Alam *et al*., 2015; James & Dubery, 2011; Srinivasan *et al*., 2016; Xiao *et al*., 2014); (iii) Tumbuhan yang endemik, yang memiliki umur panjang yang tidak biasa, cenderung untuk berkoloni dengan endofit dibandingkan tanaman lain, misalnya *Taxus* sp. penghasil obat kanker *paclitaxel* (Stierle *et al*., 1993); (iv) Tanaman yang tumbuh di daerah dengan keanekaragaman hayati yang besar seperti tanaman yang hidup di hutan Indonesia atau hutan Amerika Selatan (Ferreira *et al*., 2015; Sieber, 2007).

4.2. Kondisi kultur jamur endofit di laboratorium

Gen penyandi biosintesis metabolit sekunder pada jamur terletak pada segmen gen sepanjang lebih dari 10 kb dan tersusun dalam suatu kluster atau multidomain (Reen *et al*., 2015). Pada kondisi kultur laboratorium, jamur ditumbuhkan pada cawan petri secara aksenik (monokultur) sehingga "komunikasi mikrobial" yang awalnya tersedia sebagai interaksi antara jamur endofit dengan tanaman inang atau mikrobial lain yang tumbuh pada tanaman tersebut dan sebagai sinyal pengkode sintesis metabolit sekunder menjadi hilang. Dengan tidak adanya rangsangan ini mengakibatkan produksi metabolit sekunder hanya sedikit. Untuk meniru komunikasi mikrobia sehingga produksi metabolit sekunder pada isolat fungi bisa meningkat, dapat dilakukan proses penambahan ekstrak tanaman pada kultur jamur atau dengan mengkulturkan jamur endofit dengan jamur lain. Selain komunikasi mikrobial, rangsang berbeda selama kultur seperti perbedaan

pada komposisi media, pH, suhu, kondisi osmotik juga dapat mempengaruhi pertumbuhan dan jenis metabolit sekunder yang dihasilkan oleh jamur (Nützmann *et al*., 2012). Sebagian besar spesies jamur tumbuh subur dalam kondisi hangat, bergula, asam, dan aerobik. Sedangkan untuk suhu, kisaran untuk pertumbuhan jamur cukup luas, tetapi secara umum sebagian besar spesies tumbuh sangat baik sekitar 25°C. Parameter fisik lain yang mempengaruhi fisiologi jamur termasuk radiasi (cahaya atau UV dapat menimbulkan diferensiasi miselia dan sporulasi pada beberapa jamur yang menghasilkan spora di udara), aerasi, dan gaya sentrifugal (misal pada kultur kinetik) (Kavanagh, 2005). Faktor-faktor tersebut dapat dimodifikasi dengan harapan akan mempengaruhi pertumbuhan dan fisiologi jamur dan produksi metabolit.

4.3. Metode mengaktifkan jalur kriptik pada jamur endofit

Beberapa metode telah dieksplorasi untuk mengaktifkan jalur biosintetik diam yang juga disebut "jalur kriptik". Menariknya, pendekatan ini tidak hanya mengarah pada penemuan metabolit sekunder baru, tetapi juga pada akumulasi senyawa yang sudah diproduksi sebelumnya Optimalisasi parameter yang mempengaruhi produksi metabolit dari strain jamur endofit yang potensial dapat dilakukan dengan menggunakan berbagai media kultur dan kondisi kultur yang berbeda. Bode *et al*. (2002) mengenalkan istilah "*One Strain Many Compounds*" (OSMACs), untuk menggambarkan bagaimana strain jamur tunggal dapat diinduksi untuk menghasilkan banyak senyawa dengan hanya memvariasikan parameter kultur seperti mengubah pH dan mengubah komposisi nutrisi seperti mengubah kadar glukosa dan kadar asam amino (Bode *et al*., 2002). Berbagai jenis media komersial seperti PDA (*Potato dextrose agar*), atau PDB (*Potato dextrose broth*), MEA (*Malt Extract agar*), dan YMA (*Yeast malt agar*) atau media alami seperti media dari beras yang direbus dan media ekstrak buah *cherry* dengan kandungan nutrisi yang bervariasi dapat digunakan untuk melihat pertumbuhan produksi metabolit sekunder. Kondisi yang berbeda ini dapat secara dramatis mengubah profil metabolit sekunder dan bahkan menginduksi sintesis beberapa metabolit baru (Suryanarayanan *et al*., 2009). Penelitian menunjukkan bahwa ketika jamur ditanam pada media yang miskin nutrisi dibandingkan dengan media yang kaya nutrisi akan menghasilkan metabolit sekunder yang lebih banyak sebagai tanggapan atas rangsang “stress nutrisi” (Martínez-luis *et al*., 2011). Sebagai contoh, jamur endofit yang diisolasi dari tanaman di Panama yang dikulturkan pada media *Czapek Dox* (mengandung sukrosa, $NaNO_3$ dan K_2HPO_4) memberikan aktivitas anti kanker dan antiparasit yang lebih baik dibandingkan jika ditanam pada media kaya seperti MME (*Modified malt extract*) yang mengandung malt, peptone, dan dextrose atau PDB yang mengandung ekstrak kentang dan dekstrosa. Keterbatasan kandungan nitrogen dan

karbon pada media *Czapek Dox* menyebabkan stres pada jamur, dan transduksi sinyal stres ini menginduksi respons perlindungan berupa sintesis metabolit sekunder untuk memungkinkan bertahan hidup di media tersebut. Metode modifikasi media jamur merupakan metode yang relatif sederhana dan mudah dilakukan. Kelemahan pada metode ini adalah banyaknya modifikasi parameter pertumbuhan (seperti media, pH, temperatur) yang harus diamati hingga diperoleh parameter pertumbuhan optimal untuk memacu produksi metabolit sekunder sehingga akan banyak ekstrak yang diperoleh dan dianalisa.

Metode kedua adalah dengan teknik ko-kultur, yaitu teknik menumbuhkan bakteri-bakteri, jamur-jamur atau bakteri-jamur pada media yang sama untuk meniru kondisi fisiologis alami pada tanaman sehingga "komunikasi mikrobial" dapat terbentuk (Netzker *et al*., 2015). Beberapa penelitian terbaru melaporkan bahwa teknik ko-kultur dapat meningkatkan produksi metabolit sekunder dan juga menginduksi sintesis senyawa baru yang tidak dihasilkan oleh masing-masing mikrobia jika dikulturkan secara monokultur (Bertrand, Schumpp, Bohni, Bujard, *et al*., 2013; Chagas *et al*., 2013; Serrano *et al*., 2017). Sebagai contoh, ko-kultur jamur *Trametes versicolor* dan *Ganoderma applanatum* menginduksi biosintesis senyawa baru *N-(4-methoxyphenyl)formamide -2-O-β-D-xyloside,* dan *N-(4-methoxyphenyl) formamide 2-O-β-D-xylobioside* (Yao *et al*., 2016); *Trichophyton rubrum* dan *Bionectria ochroleuca* menginduksi metabolit baru *4- hydroxysulfoxy-2,2-dimethylthielavin* (Bertrand, Schumpp, Bohni, Monod, *et al*., 2013); dan ko-kultur *Alternaria tenuissima* dan *Nigrospora sphaerica* secara signifikan meningkatkan produksi poliketida termasuk senyawa antifungi *stemphyperylenol* (Chagas *et al*., 2013). Keuntungan menggunakan metode ko-kultur adalah jamur dapat dikulturkan pada media agar atau media cair dengan berbagai modifikasi parameter pertumbuhan dan dapat dilakukan pada bebagai ukuran petri dish mulai dari 5 cm sampai 15 cm, atau menggunakan sumuran ukuran 24 atau 6 (Bertrand, Azzollini, *et al*., 2014). Kultur pada petri dish langsung dapat diamati apakah ada fenomena interaksi yang menarik seperti inhibisi, atau produksi metabolit berwarna yang tidak diproduksi apabila jamur dikulturkan snediri-sendiri (monokultur). Kekurangan dari metode ini adalah diperlukan analisa metabolit sekunder yang dihasilkan oleh masing-masing jamur dan metabolit sekunder yang dihasilkan ketiga jamur dikulturkan bersama. Teknik ini membutuhkan instrumen yang bisa mendeteksi adanya perubahan (baik jumlah maupun jenis) metabolit pada mono dan ko-kultur seperti HPLC-MS(Bertrand, Bohni, *et al*., 2014).

Selanjutnya, penambahan prekursor atau zat antara dalam jalur biosintetik ke media kultur dapat juga dilakukan untuk meningkatkan metabolit sekunder yang diinginkan. Penambahan ekstrak tanaman dari tanaman inang dapat dilakukan untuk mengoptimalkan kondisi kultur karena

kesamaan kimia yang lebih dekat dengan lingkungan inang. Sebagai contoh, penambahan ekstrak tanaman *Torreya taxifolia* meningkatkan produksi Taxol dalam kultur jamur *Periconia* sp. dan penambahan asam benzoat sebagai zat antara sintesis Taxol juga mengakibatkan peningkatan 8 kali lipat dalam produksi Taxol (J. Y. Li *et al*., 1998). Metode ini membutuhkan analisa metabolit sekunder yang dapat membedakan metabolit dari ekstrak yang ditambahkan dan metabolit hasil produksi jamur. Selain itu, untuk penambahan precursor diperlukan suatu studi pendahuluan mengenai biosintesis dari senyawa target agar dapat menentukan precursor yang tepat.

Metode lain yang dapat dilakukan adalah dengan menambahkan suatu modifikator epigenetik seperti inhibitor *DNA methyltransferase* (DNMT) dan atau inhibitor *histone deacetylase* (HDAC) (Lamoth *et al*., 2015; Triastuti *et al*., 2019). Penambahan inhibitor DNMT dan HDAC meningkatkan diversitas kimiawi dengan cara menginduksi jamur untuk menghasilkan senyawa baru yang tidak diproduksi pada kultur normal (González-Menéndez *et al*., 2016; Siless *et al*., 2018; Triastuti *et al*., 2019; X. L. Yang *et al*., 2014). Sebagai contoh, penambahan inhibitor HDAC yaitu *suberanilohydroxamic acid* (SAHA) pada media kultur endofit tanaman kecubung (*Datura stramonium*) mampu menginduksi biosintesis senyawa baru, asam fusarat (Chen *et al*., 2013) dan tiga senyawa baru *cyclodepsipeptides*, *desmethylisaridin E*, *desmethylisaridin C2*, dan *isaridin F* pada jamur *Beauveria feline* (Chung *et al*., 2013). Penambahan asam valproat dan SAHA juga mampu mengubah komposisi metabolit sekunder pada kultur jamur *Botryosphaeria mamane* yang diisolasi dari tanaman *Bixa orellana* yang dikulturkan pada media cair (Triastuti *et al*., 2019). Keterbatasan metode ini adalah adanya kemungkinan reaktivitas modifikator epigenetik dengan media atau terjadinya biotransformasi modifikator epigenetik oleh jamur (Allard *et al*., 2016; Siless *et al*., 2018; Triastuti *et al*., 2019). Diperlukan suatu teknik analisa yang dapat mendeteksi proses degradasi atau biotrasnformasi tersebut seperti dengan teknik *molecular networking*.

Metode terakhir yang dapat dilakukan dan merupakan metode yang paling maju adalah dengan manipulasi genetik. Profil metabolit suatu jamur dapat diubah dengan memodifikasi gen yang mengkode protein pengatur suatu ekspresi metabolit sekunder. Sebagai contoh, penghapusan gen yang ditargetkan (*knockout gen*e) dapat digunakan untuk menghapus ekspresi dan penggantian promotor dapat digunakan untuk memodifikasi ekspresi gen yang diinginkan. Ketika promotor induksi dipilih, peneliti dapat secara reversibel mengontrol keadaan ekspresi (*on* atau *off*) dan, dalam beberapa kasus, dapat mengatur tingkat ekspresi itu sendiri (Lim *et al*., 2012). Teknik-teknik ini berlaku untuk jamur yang telah dipelajari dengan luas dan diketahui profil genetikanya seperti *Aspergillus nidulans* (Brakhage & Schroeckh, 2011). *Knockout* dari gen *easA* dan *easB* yang masing-masing mengkode NRPSs (non-ribosomal peptide synthetases) dan PKS (*polyketide synthases*)

mengarah pada penemuan *emericellamides* (Brakhage *et al*., 2009; Brakhage & Schroeckh, 2011). Langkah-langkah mengaktifkan gen kriptik dapat dipilih dengna menyesuaikan kapasitas laboratorium dan jenis jamur yang diteliti.

Kesimpulan

Jamur endofit dapat menjadi calon produsen sumber senyawa bioaktif dan kimiawi yang melimpah dan dapat diandalkan untuk penggunaan pada bidang kedokteran, pertanian, dan industri. Penelusuran aktivitas farmakologi jamur endofit hendaknya mengaplikasikan teknologi yang tepat dan efisien mengingat tingkat biodiversitas tanaman dan jamur yang sangat tinggi. Penelitian jamur endofit di Indonesia harus ditingkatkan mengingat Indonesia kaya akan tanaman obat yang secara tidak langsung sebagai sumber '*hotspot*' jamur endofit. Selain itu, modifikasi kultur jamur endofit dapat diterapkan untuk meningkatkan produksi metabolit sekunder dan juga untuk menginduksi penemuan senyawa baru. Ke depan, bioprospeksi jamur endofit dari tanaman obat Indonesia diharapkan dapat mengungkapkan lebih banyak potensi metabolit untuk terapi.

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Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-89
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Dizziness and nausea vomitting induced by ropinirole therapy in an elderly patient with Parkinson's disease : a case report

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Abstract

Background: Ropinirole is a non-ergoline dopamine agonist drug that is widely used in a therapy for patients diagnosed with Parkinson's disease. In long-term use, several published studies have mentioned the occurrence of side effects of ropinirole in the therapy of Parkinson's disease, but there has been no case report on the occurrence of side effects in the form of dizziness and nausea-vomiting, especially in Indonesia.

Case Presentation: This case study reported the occurrence of side effects in the form of dizziness and nausea-vomiting experienced by a 74-year-old elderly who was undergoing a treatment in a hospital in Indonesia. The patient was diagnosed with Parkinson's 8 months ago and has been given a combination therapy of levodopa-benserazide and trihexyphenidyl. During such period, no side effects occurred. The therapy was then supplemented with 2 mg ropinirole because the patient complained that his hand started shaking again. Some side effects arose after the addition of 2mg ropinirole; therefore, the side effects were thought to be associated with ropinirole. The assessment methods used were the time-series data collection followed by causality analysis using the Naranjo Scale. The analysis showed a score of 6, indicating Probable. Based on the literature review, side effects such as nausea and vomiting may occur due to the activation of dopamine D2 receptors in the Chemoreceptor Trigger Zone (CTZ) area. The CTZ area consists of several receptors, which are sensitive to the causative agent of emesis and produce information on the vomiting center that has a role in triggering the vomiting reflexes.

Conclusion: Analysis using Naranjo Scale shows a score of 6 which indicates a probable association between dizziness, nausea-vomiting and ropinirole in an elderly patient with Parkinson's Disease.

Keywords: ropinirole, Parkinson, case report, elderly, side-effects

1. Introduction

Ropinirole is a non-ergoline dopamine-agonist that is widely used as a therapy for patients with Parkinson's disease. As a therapy at the early stage of Parkinson, ropinirole can be used as a single therapy or combined with levodopa (Pahwa et al., 2004). Long-term use of ropinirole possibly causes several side effects. Some common side effects of ropinirole can be known through tertiary information sources and drug leaflets. Based on the information on the product leaflet, some side effects are commonly found, including nausea and vomiting. Apart from the information in the product leaflet, several published studies have reported some side effects caused by ropinirole, for example, Othello Syndrome (Pal et al., 2012) and psychosis (Grover & Ghosh, 2010). Although some literature has mentioned the side effects that can be caused by the long-term use of ropinirole, the number of case reports and published studies about this topic remains limited, especially in Indonesia.

2. Methods

We reported a case of ropinirole side effects and described the pharmacological background of these side effects. The assessment methods used were time-series data collection followed by causality analysis using the Naranjo Adverse Drug Probability Scale.

3. Case Report

Mr. BK, a 74-year-old man, had been diagnosed with Parkinson's Disease since October 2018. At that time, he went to the doctor because he had had troublesome symptoms such as tremor and walking disorders for six months. After the doctor did some examination, he was diagnosed with Parkinson's Disease and given a therapy of 2 mg trihexyphenidyl and a combination of 100 mg levodopa and 25 mg benserazide. Both medications were taken 3 times a day. The treatment gave him improved conditions, evidenced by the doctor's assessment 2 months after that. Throughout the treatment, the patient also used a number of heart medications, such as 5 mg isosorbide dinitrate which was taken twice a day every morning and night, and 300 mg irbesartan taken every morning. The patient said that he had used the drug for several years without experiencing significant problems.

However, 6 months later (around June 2019), the patient came back to the doctor and complained that his right hand was shaking again. Therefore, the doctor added 2 mg ropinirole which was advised to be taken once a day at night. Two weeks after using 2 mg of ropinirole therapy, the patient came back to the hospital and told the pharmacist that he had dizziness also nausea and vomiting after using the additional medicine.

To overcome those symptoms, he stopped using ropinirole for several days and the symptoms did not occur anymore. After the symptoms disappeared, the patient continued using the medication and the symptoms reappeared. Finally, the patient divided ropinirole tablet into 2 pieces and only drank half of it at night without any consultations with the doctor. The effects of dizziness and nausea-vomiting had been reduced but still felt by the patient after taking only half of the tablet of ropinirole. The pharmacist conducted an assessment of the patient's complaints and analyzed the possibility of drug side effects.

After investigating the side effects of the drug using the Naranjo Adverse Drug Probability Scale, a score of 6 was obtained, which indicated Probable. Therefore, in July 2019, the doctor decided to stop the treatment involving 2 mg ropinirole because it was suspected that the patient suffered from the side effects caused by the use of Ropinirole.

4. Discussion

Since being approved as an initial therapy and adjunctive therapy for Parkinson's by the United States' Food and Drug Administration (FDA) in 1997 and Badan Pengawas Obat dan

Makanan (BPOM) in 2012, ropinirole has been widely used as a treatment for Parkinson's. Ropinirole is a non-ergoline dopamine agonist with a preferential affinity for D2-like receptors (D2, 3, 4). It has the highest affinity with D3 receptors which are concentrated in the limbic areas of the brain and may account for some of the neuropsychiatric effects. Lesser, but still significant, affinity is seen at D2 receptor in the striatum, accounting for the prominent benefits on the motor symptoms of Parkinson's Disease (Shill & Stacy, 2009).

Several published journals and drug leaflets have mentioned that one of the common side effects that often occur in the long-term use of ropinirole are nausea and vomiting (Pahwa et al., 2004). A systematic review conducted in the United States stated that the use of ropinirole is associated with high nausea (HR 5,924 [4,410–7,959], $p < 0.001$) and vomiting (HR 4,628 [3,035–7,057], $p < 0,0001$). The publication also mentioned that of all the side effects that have been reported, nausea and vomiting were experienced by more than 50% of patients (Kurin et al., 2018).

In the case of this 74-year-old man, the effects of dizziness and nausea and vomiting were felt after using 2 mg ropinirole for about 2 weeks. Analysis of the occurrence of side effects of the drug was carried out by the pharmacist using the Naranjo Adverse Drug Probability Scale. This showed a score of 6, indicating Probable (Table 1 and 2). Based on the literature review, these side effects can occur due to the stimulation of dopamine D2 receptors in the Chemoreceptor Trigger Zone (CTZ) area.

Table 1. Naranjo adverse drug reaction probability scale

No.	Question	Yes	No	Don't Know	Score
1	Are there previous conclusive reports on this reaction?	+1	0	0	1
2	Did the adverse event appear after the suspected drug was administered?	+2	-1	0	2
3	Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	+1	0	0	1
4	Did the adverse reaction reappear when the drug was re-administered?	+2	-1	0	2
5	Are there alternative causes (other than the drug) that could solely have caused the reaction?	-1	+2	0	-1
6	Did the reaction reappear when a placebo was given?	-1	0	0	0
7	Was the drug detected in the blood (or other fluids) in a concentration known to be toxic?	+1	0	0	0

No.	Question	Yes	No	Don't Know	Score
8	Was the reaction more severe when the dose was increased, or less severe when the dose was decreased?	+1	0	0	1
9	Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0	0
10	Was the adverse event confirmed by objective evidence?	+1	0	0	0
	Total Score				**6**

Table 2. Interpretation of scores

Score	Result
Total Score >9	**Definite.** The reaction (1) followed a reasonable temporal sequence after a drug or in which a toxic drug level had been established in body fluids or tissues, (2) followed a recognized response to the suspected drug, and (3) was confirmed by improvement on withdrawing the drug and reappeared on reexposure
Total Score 5 to 8	**Probable**. The reaction (1) followed a reasonable temporal sequence after a drug, (2) followed a recognized response to the suspected drug, (3) was confirmed by withdrawal but not by exposure to the drug, and (4) could not be reasonably explained by the known characteristics of the patient's clinical state.
Total Score 1 to 4	**Possible.** The reaction (1) followed a temporal sequence after a drug, (2) possibly followed a recognized pattern to the suspected drug, and (3) could be explained by characteristics of the patient's disease.
Total Score ≤0	**Doubtful.** The reaction was likely related to factors other than a drug.

The CTZ area consists of several receptors that are sensitive to the causative agent of emesis and produce information on the vomiting center that has a role in the occurrence of vomiting reflexes. Some receptors on CTZ that are identified to cause nausea and vomiting in patients include opioids mu, kappa, dopamine-type 2 (D2), neurokinin-1 (NK-1), and serotonin-type 3 (5-HT3) (MacDougall & Sharma, 2019). As for the side effects of dizziness, it is thought to be due to an imbalance of neurotransmitters in vestibular neuroepithelium (Lee & Jones, 2017). However, the mechanism of these side effects has not been widely published.

There are various factors other than ropinirole that possibly cause side effects in this patient (as stated in Naranjo Scale point 5), including other medicines, food, or patient's condition. As stated in the paragraph above, the patient also took other medicines, such as trihexyphenidyl, levodopa-benserazide, irbesartan, and isosorbide dinitrate. Those medicines might contribute to the occurrence of side effects in the patient.

Meanwhile, based on the literature review, another factor that contributes to the occurrence of these side effects in the elderly patient is a change in the patient's pharmacokinetic profile. Ropinirole is inactivated by metabolism in the liver. The principal metabolic enzyme is the cytochrome P450 (CYP) isoenzyme CYP1A2 (Kaye & Nicholls, 2000). Ropinirole's metabolites are mainly excreted in the urine. Based on previous findings, oral clearance of ropinirole is reduced by approximately 15% in elderly patients (65 years or above) compared to younger patients (Jost & Angersbach, 2005). This fact is also supported by the lipophilic properties of ropinirole. The volumes of distribution of ropinirole increase with age. The main effect of the increased volume of distribution is a prolongation of half-life (Mangoni & Jackson, 2004). Both of these reasons are likely to cause an increase in the concentration of drug which can trigger an increase in drug action and also the side effects. Unfortunately, in this case, the patient did not perform any laboratory tests, so that the other parameters such as AST (aspartate aminotransferase)/ALT (alanine aminotransferase) or creatinine clearance cannot be determined.

The side effects that can be caused by the use of ropinirole depend on the individual sensitivity of the patient. For example, if a patient experiences nausea and vomiting on high-dose ropinirole (4 mg), the dose can be reduced to 2 mg. However, if it occurs in a small dose (2 mg), it can be recommended to stop the treatment and switch to alternative drugs with different target actions. Currently, there is only one brand name which is available in Indonesia, namely ReQuip 2 mg and 4 mg available in a 24-hour prolonged release. Therefore, in the case of this patient, if the side effects occur in low dose use, it is not justified to divide the tablet into 2 pieces since the drug is formulated for controlled release. Splitting controlled-release tablets can damage the film layer of the tablet resulting in a disruption of the method of controlled release of the drug. Therefore, in this patient, drug withdrawal is the appropriate choice while monitoring the effectiveness and side effects of the drug that may occur.

There are several limitations in this study. Some points in the Naranjo Adverse Drug Probability Scale cannot be performed by the researchers, including the administration of placebo to the patient and analysis of blood levels of ropinirole. Both of these are important to support the results of the analysis of drug side effects. Therefore, further research is required to ensure that the side effects occur due to the use of ropinirole.

5. Conclusion

Analysis using the Naranjo Adverse Drug Reaction Probability Scale shows that ropinirole possibly causes dizziness and nausea-vomiting in the elderly patient with Parkinson's Disease. The use of ropinirole in Parkinson's patients needs to get more attention from professionals, especially pharmacists. Monitoring should be carried out not only related to the effectiveness of

the drug but also to the possibility of side effects. Individual dosage adjustments should be applied according to the sensitivity of each individual, especially in elderly patients who have different physiological profiles. Also, pharmacists are expected to be able to provide more education about the possible side effects of the drug to patients and the steps that must be taken when experiencing these side effects. Therefore, the patient's awareness can be increased to support the effectiveness and safety of the therapy.

Acknowledgment

We would like to express our gratitude to the National Hospital, Surabaya for supporting us during this project.

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Jurnal Ilmiah Farmasi 16(1) Januari-Juli 2020, 1-95
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Analysis of the level of knowledge of mothers about self-medication to children in Cangkringan District, Yogyakarta

Analisis tingkat pengetahuan para ibu tentang swamedikasi pada anak di Kecamatan Cangkringan Yogyakarta

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Abstract

Background: Self-medication refers to an endeavor that is mostly frequently done by society in coping with any symptoms of disease prior to have an aid from medical practitioner. In this case, knowledge about medication and any disease complaints will bring about the impact on the medication use. Insufficiency of mother in understanding about drug and the way of using it in self-medication is potential to be a factor of medication error both for the mothers themselves and for their family. Knowledge required to properly do self-medication is by identifying the active substances, indication, contraindication, dosage and side effect of the medication**.**

Objective: This research is designed to observed the description of the implementation of self-medication, the description of knowledge level of mothers about self-medication and factors determining the knowledge level of mothers.

Method: In addition, this research used questionnaires written in accordance with the Guidelines of Free Medicine Use and Limited Free Medicine. Categorization of the knowledge level of mothers is based on the final score of the questionnaires.

Results: The result then showed that the knowledge level of the mothers about the general knowledge of medicine was at 61% for those categorized into good knowledge and 39% for those categorized into medium-level knowledge. Meanwhile, in terms of knowledge level of mother about complaint and diseases treatable using self-medication was at 90% for those categorized into good knowledge and 10% for those categorized at medium-level knowledge.

Conclusion: The factors determining the knowledge level of mothers included age, educational level and income. On the other hand, the factor that mostly determined the knowledge level of mother was educational level.

Keywords: self-medication, knowledge level, Yogyakarta

Intisari

Latar belakang: Swamedikasi adalah upaya yang paling banyak dilakukan masyarakat untuk mengatasi gejala penyakit sebelum mencari pertolongan dari tenaga kesehatan Pengetahuan tentang obat dan keluhan penyakit berdampak pada penggunaan obat. Keterbatasan pengetahuan para ibu akan obat dan cara penggunaannya dalam swamedikasi dapat menjadi sumber terjadinya kesalahan pengobatan (medication error) pada diri sendiri dan anggota keluarganya. Pengetahuan yang dibutuhkan untuk melakukan swamedikasi dengan benar adalah mengetahui bahan aktif, indikasi, kontraindikasi, dosis, dan efek samping pengobatan.

Tujuan: Tujuan penelitian untuk mengetahui gambaran pelaksanaan swamedikasi,mengetahui gambaran tingkat pengetahuan para ibu tentang swamedikasi dan mengetahui faktor- faktor yang mempengaruhi tingkat pengetahuan para ibu**.**

Metode: Penelitian menggunakan kuesioner yang disusun berdasarakan Pedoman Penggunaan Obat Bebas dan Obat Bebas Tebatas. Pembagian golongan tingkat pengetahuan para ibu berdasarkan skor

akhir kuesioner.
Hasil: Tingkat pengetahuan para ibu tentang informasi umum obat, sebanyak 61% ibu tergolong pengetahuan baik dan 39% ibu tergolong pengetahuan sedang. Tingkat pengetahuan para ibu tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi, sebanyak 90% ibu tergolong pengetahuan baik dan 10% ibu tergolong pengetahuan sedang.
Kesimpulan: Faktor yang mempengaruhi tingkat pengetahuan ibu antara lain usia, tingkat pendidikan dan tingkat penghasilan. Sedangkan faktor yang paling mempengaruhi tingkat pengetahuan para ibu adalah tingkat pendidikan.

Kata kunci : Swamedikasi, tingkat pengetahuan, para ibu, Cangkringan

1. Pendahuluan

Pengobatan sendiri, atau yang disebut dengan swamedikasi, merupakan upaya yang paling banyak dilakukan masyarakat untuk mengatasi gejala penyakit sebelum mencari pertolongan dari tenaga kesehatan (Kemenkes, 2008). Hasil Susenas pada tahun 2009 juga mencatat bahwa 66% orang sakit di Indonesia melakukan swamedikasi untuk mengatasi penyakitnya (Kartajaya, 2011). Dari data *World Health Organization* (1998), di banyak negara sampai 80% orang yang sakit mencoba untuk melakukan pengobatan sendiri oleh penderita. Sedangkan data di Indonesia menunjukkan bahwa sekitar 60% masyarakat melakukan swamedikasi dengan obat modern sebagai tindakan pertama bila sakit (Kemenkes, 2009).

Kesehatan didefinisikan sebagai keadaan sejahtera dari badan, jiwa, dan sosial yang memungkinkan setiap orang hidup produktif baik secara sosial dan ekonomi (Kemenkes, 2009). Dalam upaya pemeliharaan kesehatan, pengobatan sendiri merupakan upaya pertama dan yang terbanyak dilakukan masyarakat untuk mengatasi keluhan kesehatannya sehingga peranannya tidak dapat diabaikan begitu saja. Keterbatasan pengetahuan masyarakat tentang obat dan penggunaannya merupakan penyebab terjadinya kesalahan pengobatan dalam swamedikasi (Suryawati, 1997). Keterbatasan tersebut dapat menyebabkan rentannya masyarakat terhadap informasi komersial obat, sehingga memungkinkan terjadinya pengobatan yang tidak rasional jika tidak diimbangi dengan pemberian informasi yang benar (Kemenkes, 2013). Untuk itu penelitian ini akan mtenganalisis tingkat pengetahuan yang dimiliki para ibu tentang swamedikasi pada anak.

Ibu dapat diasumsikan sebagai “dokter keluarga” yang bertanggung jawab terhadap kesehatan anak-anaknya. Saat ini, dengan makin berkembangnya teknologi sebagai sumber informasi khususnya iklan tentang produk kesehatan (obat) menjadikan para ibu mudah mendapat pengetahuan tentang obat bebas. Penulis mendapati tidak sedikit para ibu membeli sendiri obat bebas di Apotek tanpa resep dokter untuk mengatasi gejala penyakit pada anaknya. Dengan begitu, sangat penting untuk mengetahui tingkat pengetahuan para ibu tentang swamedikasi untuk

mencegah terjadinya *self medication error*. Keterbatasan pengetahuan tentang pilihan penggunaan obat dan pemilihan dosis obat pada anak dapat berdampak terjadinya *medication error.* Dalam keluarga, ibu adalah sosok yang bertanggung jawab terhadap kesehatan anak-anaknya. Informasi yang benar, dapat mendukung keberhasilan swamedikasi yang dilakukan para ibu pada anak.

Kecamatan Cangkringan merupakan salah satu kecamatan yang berdampak langsung ketika erupsi gunung Merapi. Praktek swamedikasi di daerah bencana umumnya meningkat (Purwanti, *et al.*, 2004). Selain daerah yang rawan bencana, kecamatan Cangkringan juga merupakan kecamatan yang terletak di daerah pedesaan. Bagi masyarakat di daerah terpencil, swamedikasi akan menghemat banyak waktu yang diperlukan untuk ke kota mengunjungi dokter (Purwanti, 2008). Tingkat pengetahuan yang baik akan berdampak pada keberhasilan terapi dan menurunkan kesalahan pengobatan yang banyak terjadi pada praktek swamedikasi. Oleh karena itu, penting mengetahui tingkat pengetahuan yang dimiliki para ibu tentang swamedikasi yang tinggal di Kecamatan Cangkringan.

2. Metodologi penelitian

2.1. Populasi dan sampel

Populasi ibu yang memiliki anak ≤ 12 sebesar 406 orang. Dengan menggunakan rumus Slovin (Sastroasmoro, 2008). Jumlah sampel minimum sebesar 202 orang, dan ditambah 30 orang untuk mengukur validitas dan reabilitas kuesioner. Teknik sampling dilakukan dengan metode non probabilitas.

2.2. Tempat dan waktu penelitian

Penelitian dilakukan pada bulan Mei sampai Juni 2014 dari rumah ke rumah di Kecamatan Cangkringan, Sleman Yogyakarta.

2.3. Kriteria inklusi dan eksklusi

Kriteria inklusi dalam penelitian ini adalah para ibu yang memiliki anak ≤ 12 tahun dan para ibu yang melakukan swamedikasi menggunakan obat bebas dan obat bebas terbatas untuk anaknya. Kriteria eksklusi pada penelitian ini adalah para ibu yang tidak bersedia bekerja sama dalam penelitian.

2.4. Alat dan bahan

Alat yang digunakan dalam penelitian ini adalah kuesioner yang dibuat sendiri berdasarkan Pedoman Penggunaan Obat Bebas dan Obat Bebas Terbatas yang dikeluarkan oleh Ditjen Bina Kefarmasian Dan Alat Kesehatan Departemen Kesehatan 2006.

2.5. Pengolahan data dan analisis data

a. Penilaian kuesioner

Data yang telah didapatkan kemudian dilakukan pemeriksaan atas kelengkapan pengisian kuesioner, kejelasan makna jawaban dan perbaikan isian kuesioner tersebut. Uji validitas digunakan untuk mengukur sah atau valid tidaknya suatu kuesioner. Kuesioner dikatakan valid jika pertanyaan pada kuesioner tersebut mampu mengungkapkan sesuatu yang akan diukur oleh kuesioner tersebut. Uji Reabilitas adalah indeks yang menunjukkan sejauh mana suatu alat pengukuran dapat dipercaya atau dapat diandalkan. Jika nilai *Cronbach's Alpha* lebih besar dari 0,600, maka kuesioner dapat dinyatakan realibel (Gaspersz, 1991).

b. Interpretasi data

Gambaran pelaksanaan swamedikasi didapat dari tabulasi jawaaban dari kuesioner yang dibagikan.

Gambaran Pengetahuan :

1. Tingkat pengetahuan tentang swamedikasi
    a) Baik, apabila skor responden ≥80
    b) Sedang, apabila skor responden 60-79
    c) Buruk, apabila skor responden < 60
2. Tingkat kejadian swamedikasi (dalam 3 bulan). Dibagi dalam 4 kategori kejadian :
    a. Sangat sering, bila tingkat kejadian > 75% atau > 30 kali dalam 3 bulan
    b. Sering, bila tingkat kejadian 50-75% atau 15- 30 kali dalam 3 bulan
    c. Jarang, bila tingkat kejadian 20-49% atau 6-14 kali dalam 3 bulan
    d. Sangat jarang, bila tingkat kejadian <20% atau kurang dari 6 kali dalam 3 bulan.

3. Faktor yang mempengaruhi tingkat pengetahuan para ibu. Beberapa faktor yang mempengarui tingkat pengetahuan seseorang di dapat dari kajian literature. Diantaranya umur, tingkat pendidikan, dan tingkat penghasilan. Data dianalisa dengan analisa univariat dan regresi linear menggunakan *software* SPSS versi 17 dan hasil disajikan dalam bentuk tabel frekuensi dan persentase.

3. Hasil dan pembahasan

3.1. Penyusunan kuesioner

Kuesioner yang dibuat terdiri dari tiga bagian, bagian pertama memuat gambaran pelaksanaan swamedikasi, bagian kedua tentang informasi umum obat dan bagian ketiga tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi. Masing- masing bagian memuat sepuluh butir soal. Kuesioner bagian pertama terdiri dari sepuluh butir soal dengan lima pilihan jawaban. Soal pada kuesioner bagian pertama tidak dijadikan sebagai alat untuk mengukur tingkat pengetahuan para ibu melainkan dijadikan sebagai informasi mengenai gambaran pelaksanaan swamedikasi yang dilakukan para ibu di Kecamatan Cangkringan. Sedangkan kuesioner bagian kedua dan ketiga masing-masing terdiri dari sepuluh butir soal dengan dua pilihan jawaban. Kuesioner bagian kedua dan ketiga digunakan untuk mengukur tingkat pengetahuan para ibu, pilihan jawaban yang salah diberi skor nol sedangkan jawaban benar diberi skor sepuluh.

Ketiga puluh butir soal kemudian diuji validitas dan reabilitasnya. Uji validitas dan reabilitas dilakukan hanya sekali dan langsung mendapatkan hasil yang baik. Hasil uji validitas ketiga puluh butir soal menunjukkan nilai $p < \alpha$ (0,05) untuk masing-masing soal dan dinyatakan valid. Hasil uji reabilitas yang dilakukan pada tiap bagian kuesioner menunjukkan nilai *Cronbach's alpha* > 0.600 dan dinyatakan realibel.

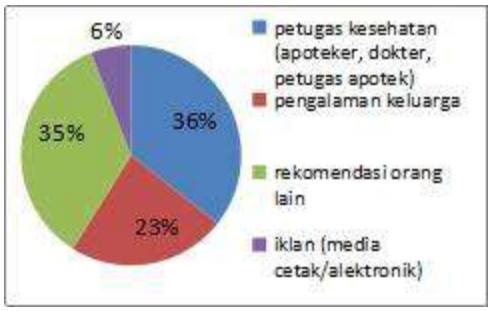
3.2. Gambaran pelaksanaan swamedikasi yang dilakukan para ibu di Kecamatan Cangkringan

Berdasarkan hasil, sebanyak 61% alasan para ibu di Kecamatan Cangkringan melakukan swamedikasi adalah untuk menghemat biaya. Sebanyak 28% para ibu beralasan melakukan swamedikasi untuk menghemat waktu. Sebanyak 6% para ibu melakukan swamedikasi dengan alasan keberhasilan engalaman sebelumnya. Sebanyak 5% para ibu melakukan swamedikasi dengan alasan rekomendasi tenaga kesehatan.

Gambar 1. Alasan para ibu melakukan swamedikasi

Gambar 2. Pertimbangan para ibu dalam memilih obat

Berdasarkan hasil, paling banyak para ibu memilih obat dengan pertimbangan efektivitas dari obat yang akan dibeli. Para ibu yang memilih obat berdasarkan efektivitas sebanyak 45%. Efektif tidaknya suatu obat yang akan dibeli dilatarbelakangi oleh pengalaman sebelumnya dengan menggunakan obat yang sama atau rekomendasi tenaga kesehatan maupun kerabat atau tetangga rumah. Sebanyak 37% para ibu memilih obat dengan pertimbangan berdasarkan gejala penyakit yang dialami. Sebanyak 18% para ibu memilih obat dengan pertimbangan berdasarkan pengalaman baik itu diri sendiri, teman ataupun tetangga.



Gambar 3. Sumber informasi tentang obat

Para ibu umumnya sangat mengetahui informasi obat-obat yang sebelumnya pernah digunakan dan berhasil mengobati gejala dan penyakit. Dari hasil, sebanyak 36% para ibu memperoleh informasi tentang obat dari petugas kesehatan Petugas kesehatan yang biasanya ditemui yaitu bidan, petugas apotek dan dokter. Informasi yang berasal dari petugas kesehatan

umumnya memiliki tingkat keberhasilan terapi yang tinggi dibanding dengan sumber informasi tersier lainnya. Sebanyak 35% para ibu mendapatkan informasi tentang obat dari rekomendasi orang lain. Sebanyak 23% para ibu memperoleh informasi tentang obat dari pengalaman keluarga. Sebanyak 6% para ibu memperoleh informasi tentang obat dari iklan baik itu media cetak maupun elektronik.

Gambar 4. Tempat memperoleh obat bebas dan atau obat bebas

Obat bebas dan obat bebas terbatas dapat diperoleh secara bebas di tempat- tempat penjualan selain apotek. Dari hasil, sebanyak 68% para ibu membeli obat di apotek. Sebanyak 20% para ibu memilih warung sebagai tempat untuk membeli obat. Sebanyak 12% para ibu membeli obat di toko obat. Berdasarkan hasil, sebanyak 54% para ibu biasa membeli obat pereda nyeri seperti asam mefenamat dan ibuprofen. Obat analgesic-antipiretik yang paling banyak dibeli adalah paracetamol.

Gambar 5. Alasan para ibu membeli obat di apotek, toko obat dan warung.

Gambar 6. Jenis obat yang biasa dibeli

Sebanyak 31% para ibu membeli obat maag. Kombinasi alumunium hidroksida dan magnesium hidroksida merupakan obat yang paling banyak dibeli. Sebanyak 12% para ibu biasa melakukan swamedikasi terhadap flu dan batuk. Dan sangat sedikit para ibu membeli obat diare, yaitu sebanyak 3%. Obat diare yang paling banyak dibeli adalah attapulgit dan kaolin-pektin. Dari hasil, biaya yang harus dikeluarkan para ibu paling banyak sebesar lima ribu sampai sepuluh ribu rupiah. Sebanyak 18% mengeluarkan biaya mulai dari sepuluh ribu rupiah sampai tiga puluh ribu rupiah. Sebanyak 16% mengeluarkan biaya kurang dari lima ribu rupiah dan sisanya sebanyak 8% memperoleh obat secara gratis, yang diperoleh dari teman dan tetangga.

Gambar 7. Biaya yang harus dikeluarkan untuk membeli obat

Gambar 8. Tindakan para ibu jika pengobatan gagal dengan swamedikasi.

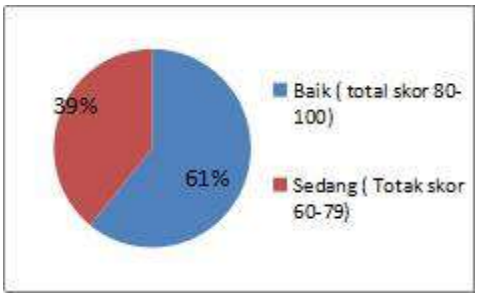
Berdasarkan hasil, hal yang paling banyak dilakukan para ibu ketika penyakit tidak juga sembuh adalah berobat ke dokter. Sebanyak 44% para ibu memilih dokter sebagai rujukan pertama bila penyakit dan gejala penyakit tidak juga sembuh. Keberadaan dokter di sarana pelayanan kesehatan seperti puskesmas tidak selalu ada, hanya pada hari- hari tertentu saja dokter bertugas di tempat tersebut. Selain dokter, sebanyak 37% para ibu memilih berobat ke bidan desa. Sebanyak 19% para ibu memutuskan menggunakan pengobatan tradisional seperti jamu bila dengan swamedikasi gejala penyakit maupun penyakit tidak kunjung sembuh. Para ibu yang memutuskan berobat ke dokter memiliki beberapa alasan. Paling banyak para ibu memutuskan pergi ke dokter jika obat yang dibeli sudah habis tetapi tidak kunjung sembuh.

Gambar 9. Alasan para ibu memutuskan berobat ke dokter

3.3. Gambaran tingkat pengetahuan

a. Tingkat pengetahuan tentang informasi umum obat

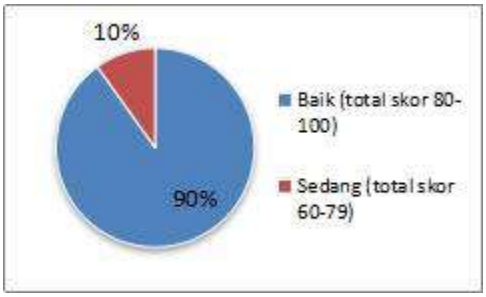
Para ibu yang memutuskan berobat ke dokter memiliki beberapa alasan. Paling banyak para ibu memutuskan pergi ke dokter jika obat yang dibeli sudah habis tetapi tidak kunjung sembuh.



Gambar 10. Tingkat pengetahuan tentang informasi umum obat

b. Tingkat pengetahuan tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi

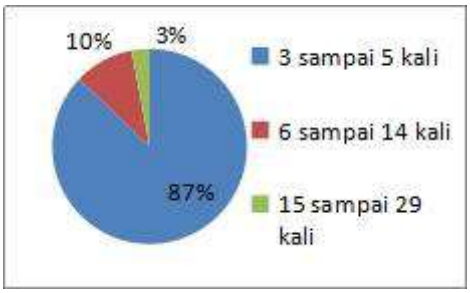
Berdasarkan total skor jawaban responden pada kesepuluh soal dibagian ketiga kuesioner tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi didapat sebanyak 90% termasuk kategori berpengetahuan baik sedangkan 10% termasuk dalam kategori berpengetahuan sedang. Tidak ditemukan ibu yang masuk dalam kategori berpengetahuan buruk.



Gambar 11. Tingkat pengetahuan tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi

c. Tingkat kejadian swamedikasi dalam tiga bulan terakhir.

Berdasarkan hasil, tingkat kejadian swamedikasi yang dilakukan para ibu di kecamatan cangkringan sangat beragam. Pada penelitian ini, tingkat kejadian yang diukur yakni dalam 3 bulan terkahir. Sebanyak 87% responden masuk pada kategori sangat jarang melakukan swamedikasi, sebanyak 10% termasuk kategori jarang melakukan swamedikasi, dan 3% responden masuk dalam kategori sering melakukan swamedikasi.

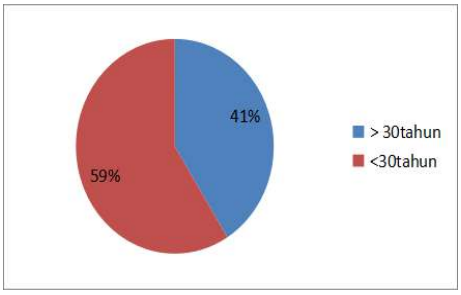


Gambar 12. Distribusi tingkat kejadian swamedikasi

3.4. Faktor yang mempengaruhi tingkat pengetahuan para ibu

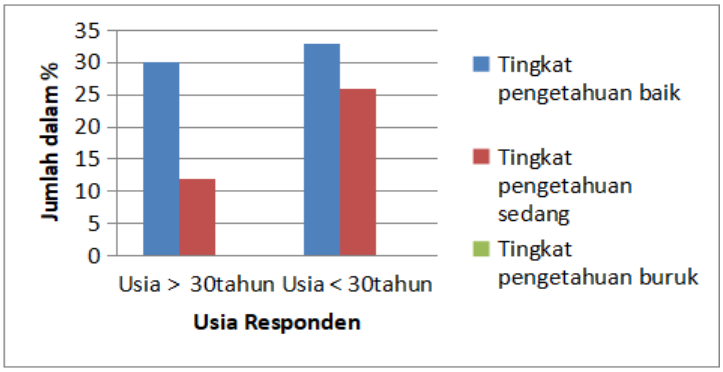
a. Usia

Berdasarkan hasil, diperoleh usia responden yang berada diatas 30 tahun sebanyak 41% dan sebanyak 59% responden berada pada kisaran umur dibawah 30tahun. Para ibu yang berada di kisaran umur kurang dari 30 tahun termasuk dalam kategori ibu muda, dengan jumlah anak 1-2 orang.

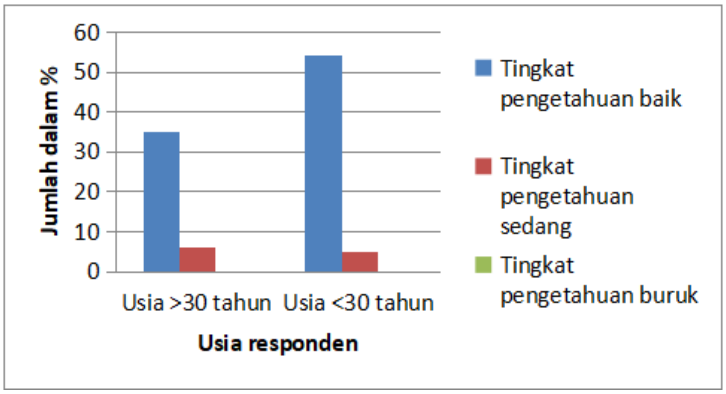


Gambar 13. Distribusi usia para ibu

Pada kuesioner bagian kedua tentang Informasi Umum Obat, untuk kelompok para ibu usia diatas 30 tahun ada sebanyak 30% yang memiliki pengetahuan baik dan 12% yang berpengetahuan sedang. Sedangkan kelompok para ibu kategori usia kurang dari 30 tahun, ada sebanyak 33% yang masuk dalam kategori pengetahuan baik dan 26% yang memiliki tingkat pengetahuan sedang.



Gambar 14. Distribusi tingkat pengetahuan para ibu tentang informasi umum obat berdasarkan usia.



Gambar 15. Distribusi tingkat pengetahuan para ibu tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi berdasarkan usia

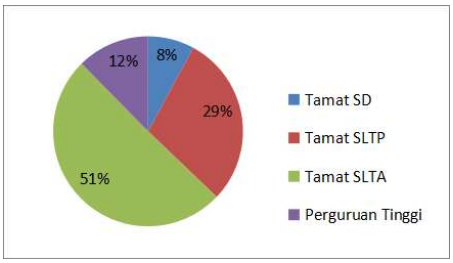
Pada kuesioner bagian ketiga tentang Keluhan dan Penyakit yang dapat diatasi dengan Swamedikasi, untuk kelompok para ibu usia diatas 30 tahun ada sebanyak 35% yang memiliki pengetahuan baik dan 6% yang berpengetahuan sedang. Sedangkan kelompok para ibu kategori usia kurang dari 30 tahun, ada sebanyak 54% yang masuk dalam kategori pengetahuan baik dan 5% yang memiliki tingkat pengetahuan sedang. Hasil ini sangat bertolak belakang dengan teori. Umur merupakan faktor internal individu yang dihitung sejak lahir yang menentukan faktor predisposisi untuk terjadinya perubahan pengetahuan dan perilaku. Faktor umur biasanya dikaitkan dengan kematangan fisik dan psikis seseorang (Videbeck, 2008).

b. Tingkat pendidikan

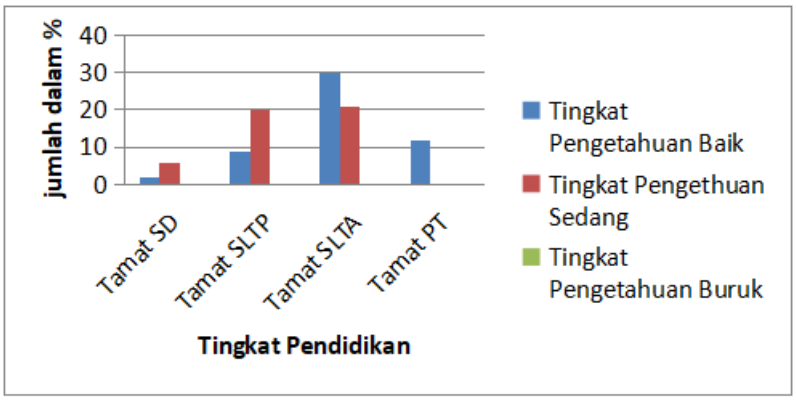
Berdasarkan hasil, paling banyak para ibu menempuh tingkat pendidikan akhir tamat SLTA yakni sebanyak 51%, disusul para ibu dengan tingkat pendidikan akhir tamat SLTP sebesar 29%.

Para ibu dengan tingkat pendidikan akhir tamat perguruan tinggi sebanyak 12%, sedangkan sebanyak 8% ibu menamatkan pendidikan pada tingkat sekolah dasar.

Pada kuesioner bagian kedua tentang Informasi Umum Obat, untuk kelompok para ibu yang menamatkan pendidikan di bangku SD yang memiliki pengetahuan baik sebanyak 2% dan 6% yang berpengetahuan sedang. Kelompok para ibu yang menamatkan pendidikan pada jenjang SLTP ada sebanyak 9% yang masuk dalam kategori pengetahuan baik dan 20% yang memiliki tingkat pengetahuan sedang. Kelompok para ibu yang menamatkan pendidikan pada jenjang SLTA, ada sebanyak 30% berpengetahuan baik dan 21% berpengetahuan sedang. Kelompok ibu yang menamatkan pendidikan di jenjang Perguruan Tinggi (PT), seluruhnya berpengetahuan baik yakni sebanyak 12%.



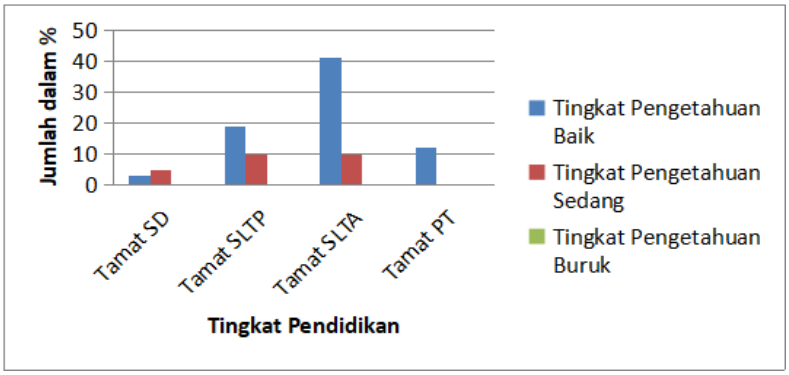
Gambar 16. Distribusi tingkat pendidikan para ibu



Gambar 17. Distribusi tingkat pengetahuan para ibu tentang informasi umum obat berdasarkan tingkat pendidikan

Pada kuesioner bagian ketiga tentang Keluhan dan Penyakit yang dapat diatasi dengan Swamedikasi, untuk kelompok para ibu yang menamatkan pendidikan di bangku SD ada sebanyak 3% yang masuk kategori berpengetahuan baik dan 5% yang berpengetahuan sedang. Kelompok para ibu yang menamatkan pendidikan di bangku SLTP, ada sebanyak 19% berpengetahuan baik dan 10% pengetahuan sedang. Kelompok para ibu yang menamatkan pendidikan pada jenjang

SLTA ada sebanyak 41% yang memiliki tingkat pengetahuan baik dan 10% masuk kategori berpengetahuan sedang. Sedangkan kelompok ibu yang menamatkan pendidikan jenjang Perguruan Tinggi, seluruhnya berpengetahuan baik yakni sebesar 12%. Berdasarkan hasil penelitian ini dapat disimpulkan bahwa semakin tinggi tingkat pendidikan seseorang, semakin tinggi pengetahuannya serta semakin berhati-hati dalam penggunaan obat dalam pengobatan sendiri.

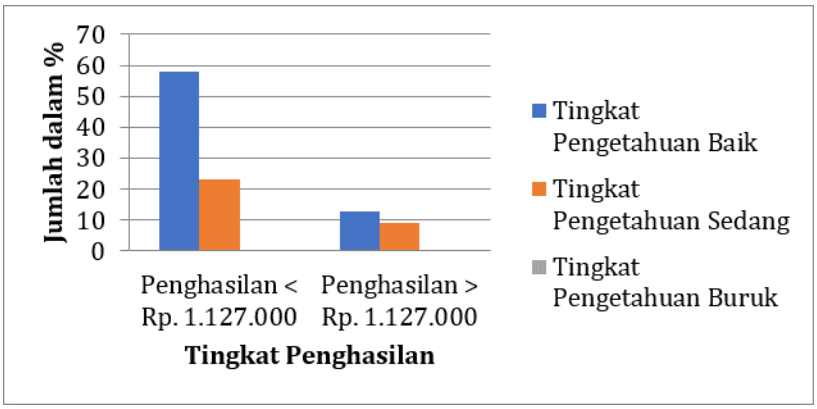


Gambar 18. Distribusi tingkat pengetahuan para ibu tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi berdasarkan tingkat pendidikan

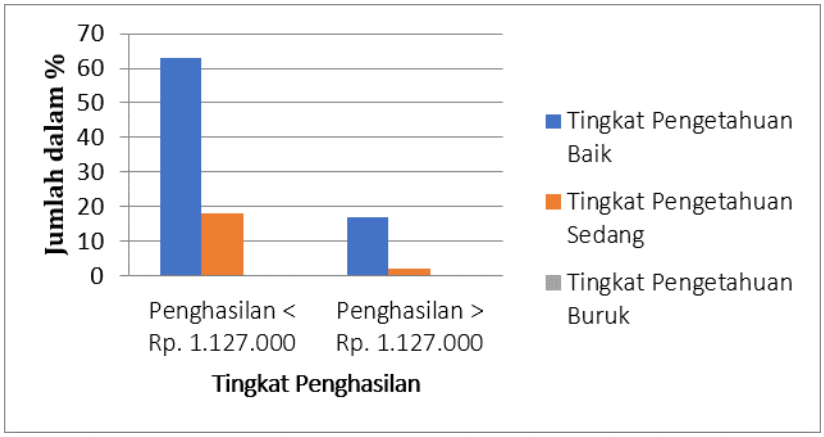
c. Tingkat pendapatan

Berdasarkan hasil, didapat sebanyak 81% para ibu berpenghasilan kurang dari Upah Minimum Rakyat (UMR) Kabupaten Sleman, yakni sebesar satu juta seratus dua puluh tujuh rupiah. Sisanya sebanyak 19% berpenghasilan lebih dari satu juta seratus dua puluh tujuh rupiah. Pada kuesioner bagian kedua tentang Informasi Umum Obat, untuk kelompok para ibu yang berepenghasilan kurang dari UMR ada sebanyak 58% berpengetahuan baik dan 23% yang berpengetahuan sedang. Kelompok para ibu yang berpenghasilan lebih dari UMR ada sebanyak 13% berpengetahuan baik dan 6% berpengetahuan sedang.

Gambar 19. Distribusi tingkat pendapatan per bulan



Gambar 20. Distribusi tingkat pegetahuan para ibu tentang informasi umum obat berdasarkan tingkat penghasilan



Gambar 21. Distribusi tingkat pengetahuan para ibu tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi berdasarkan tingkat penghasilan

Pada kuesioner bagian ketiga tentang Keluhan dan Penyakit yang dapat diatasi dengan swamedikasi, untuk kelompok para ibu yang berepenghasilan kurang dari UMR ada sebanyak 63% berpengetahuan baik dan 18% yang berpengetahuan sedang. Kelompok para ibu yang berpenghasilan lebih dari UMR ada sebanyak 17% berpengetahuan baik dan 2% berpengetahuan sedang. Berdasarkan hasil, diketahui bahwa yang berpendapatan lebih tinggi umunya memiliki pengetahuan lebih baik, ini bertolak belakang dengan teori. Pendapatan tidak berpengaruh langsung terhadap pengetahuan seseorang. Namun bila seseorang berpendapatan cukup besar maka dia akan mampu untuk menyediakan atau membeli fasilitas – fasilitas sumber informasi. Dari data, kemudian dilakukan analisis regresi logistik untuk mengetahui adakah hubungan antara variable *independent* dan variable *dependent* secara serentak (Sugiyono, 2007).

Berdasarkan hasil analisis untuk tingkat pengetahuan para ibu tentang informasi umum obat, menggunakan 202 sampel dengan nilai *Cox and Snell* sebesar 0,501 yang berarti 50,1% variasi dari tingkat pengetahuan tentang informasi umum obat dapat dijelaskan oleh variable *independent* yang digunakan. Nilai *Naqelkerke* sebesar 0,765 yang berarti 76,5% variasi dari tingkat pengetahuan para ibu tentang informasi umum obat dapat dijelaskan oleh variabel *independent* yang digunakan. Nilai *overall percentage* sebesar 81,7 yang bermakna persentasi variable yang dapat diprediksi sebesar 81,7%. Dari hasil, tingkat pendidikan memiliki nilai sig. < 0,05 yang berarti berpengaruh signifikan. Hasil *Odds-ratio* yang paling besar pada variable tingkat pendidikan sebesar 4,894. Dari data dapat disimpulkan bahwa tingkat pendidikan berpengaruh signifikan terhadap tingkat pengetahuan para ibu tentang informasi umum obat dengan peluang kejadian 4 kali dibanding variable lainnya.

Hasil analisis untuk tingkat pengetahuan para ibu tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi, menggunakan 202 sampel menunjukkan nilai *Cox and Snell* sebesar 0,441 yang berarti 44,1% variasi dari tingkat pengetahuan tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi dapat dijelaskan oleh variabel *independent* yang digunakan. Diperoleh pula nilai *Naqelkerke* sebesar 0,711 yang berarti 71,1% variasi dari tingkat pengetahuan para ibu tentang informasi umum obat dapat dijelaskan oleh variabel *independent* yang digunakan. Nilai *overall percentage* sebesar 89,1 yang bermakna persentasi variable yang dapat diprediksi sebesar 89,1%. Hasil, tingkat pendidikan memiliki nilai sig. < 0,05 yang berarti berpengaruh signifikan. Hasil *Odds-ratio* yang paling besar pada variable tingkat pendidikan sebesar 6,027. Berdasarkan data dapat disimpulkan bahwa tingkat pendidikan berpengaruh signifikan terhadap tingkat pengetahuan para ibu tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi dengan peluang kejadian 6 kali dibanding variable lainnya.

Kesimpulan

Gambaran pelaksanaan swamedikasi yang dilakukan para ibu di kecamatan cangkringan : Paling banyak alasan para ibu melakukan swamedikasi adalah untuk menghemat biaya, pertimbangan para ibu dalam memilih obat adalah berdasarkan efektivitas, sumber informasi mengenai obat paling banyak diperoleh dari petugas kesehatan, para ibu banyak memperoleh obat di apotek, alasan terbanyak para ibu membeli obat di apotek adalah karena obat yang dibutuhkan selalu tersedia, jenis obat yang paling banyak dibeli adalah obat penghilang nyeri, paling banyak para ibu mengeluarkan biaya sebesar Rp. 5.000- Rp.10.000, para ibu akan berobat ke dokter bila

keluhan dan penyakit tidak juga sembuh dengan swamedikasi, alasan terbanyak para ibu memutuskan berobat ke dokter karena obat yang dibeli sudah dihabiskan tetapi tidak kunjung sembuh. Tingkat pengetahuan para ibu tentang informasi umum obat, sebanyak 61% ibu tergolong pengetahuan baik dan 39% ibu tergolong pengetahuan sedang. Tingkat pengetahuan para ibu tentang keluhan dan penyakit yang dapat diatasi dengan swamedikasi, sebanyak 90% ibu tergolong pengetahuan baik dan 10% ibu tergolong pengetahuan sedang. Faktor yang mempengaruhi tingkat pengetahuan ibu antara lain usia, tingkat pendidikan dan tingkat penghasilan. Factor yang paling mempengaruhi tingkat pengetahuan para ibu adalah tingkat pendidikan. Perlu penelitian tambahan menggali lebih banyak informasi tentang pengobatan sendiri (selfmedication), dan kemungkinan-kemungkinan ada faktor lain yang lebih berpengaruh terhadap tingkat pengetahuan para ibu.

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