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IDENTIFICATION AND PHOTOCHEMICAL TESTS OF FUNGI MUSHROOMS AND HONEY SPECIALLY BANGKA BELITUNG

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ABSTRACT

Pelawan bitter honey and Pelawan mushrooms are two examples of Bangka's unique biodiversity found in Central Bangka Regency. Pelawan mushroom grows in the Bangka Belitung Islands Province, is generally used as a food ingredient, and has the potential to be developed as a source of natural immunomodulators. Phenolic compounds are known to have immunomodulatory activity that can enhance the immune system. In addition, based on preliminary studies, honey is also used by local people to strengthen their immune systems. The purpose of this study was to identify the content of active compounds in Pelawan honey and Pelawan mushrooms. This was an experimental study with multiple stages of testing, including phytochemical screening, thin-layer chromatography testing, and extract standardization testing. The results of the study proved that the phytochemical test of Pelawan honey contained Flavonoid and Alkaloid compounds, and for the Thin Layer Chromatography (TLC) test, it contained Flavonoids and Alkaloids. The Pelawan mushroom phytochemical test contained phenolic and alkaloids with Mayer's reagent; the Pelawan mushroom thin layer chromatography test contained flavonoids with an average Rf value of 0.56, while the standardization of Pelawan mushroom extract produced a total ash content of 28%, drying shrinkage of 40.88 g/mL, specific gravity of 0.67 g/mL, water-soluble essence of 59.1%, and ethanol-soluble essence content of 41.12%. From these results, it could be surmised that honey and fighting fungi have the potential to be developed as immunomodulatory agents.

Keywords: *Flavonoids, Phenolics, Pelawan fungi, Immunomodulators, and Pelawan honey*

INTRODUCTION

The Pelawan tree (*Tristaniopsis merguensis*) is a key species for the sustainability of biodiversity in Central Bangka Regency. Pelawan trees can guarantee the continued growth of the fungus *Heimioporus sp* and the harvest of Pelawan honey in the Biodiversity Park, Central Bangka Regency. Pelawan mushroom is known as the most expensive mushroom in Bangka Belitung Province, maybe even in Indonesia (Akbarini, 2016). Richi (2011) mentions that the Pelawan mushroom is a fungus that forms an ectomycorrhizal symbiosis with the Pelawan tree. Ectomycorrhizae are a type of fungus that envelops the host plant's root cells and has external hyphae that can form macroscopic fruiting bodies inside or on the soil surface. The Pelawan mushroom (*Boletus sp*.) is an ectomycorrhizal fungus that has a symbiosis with the roots of the Pelawan plant (*Tristaniopsis merguensis*). This symbiosis is known to help pelawan plants absorb nutrients so as to increase plant height and chlorophyll content in pelawan leaves. Pelawan mushrooms have high economic value, with a selling price of up to 1,800,000 rupiah per kilogram for mushrooms that have been dried and have been exported to other countries such as Malaysia and Singapore (Tasuruni 2012).

Pelawan mushroom grows in the Bangka Belitung Islands Province and is generally used as a food ingredient (Salma 2013). Rich (2011) mentions that fighting mushrooms contain high levels of protein, are rich in dietary fiber, and are low in fat.

Valine, methionine, threonine, isoleucine, leucine, phenylalanine, and lysine are the essential amino acids contained in Pelawan mushrooms. Pelawan mushrooms also contain natural antioxidants such as phenolic components, carotenes, and lycopene.

Phenolic compounds are known to have immunomodulatory activity that can enhance the immune system. The immune system is a complex interaction of various cells that work together to fight the invasion (entry) of pathogenic microorganisms or other harmful substances that enter the body (Yanuar 2009). The use of immunomodulators is more effective than antibiotics because long-term administration of antibiotics causes pathogenic bacteria to develop resistance. In addition, the use of immunomodulators does not leave residues that are harmful to the environment or humans (Febrianto 2009).

Several plants, such *as Echinacea purpurea, Phylanthus niruri L, Morinda citrifolia,* and *Androphagis andriculatan,* are known to act as immunomodulators. Pelawan mushrooms contain antioxidants in the form of phenolics, carotenes, and lycopene, so they have the potential to be developed as a source of natural immunomodulators. It is hoped that information related to the immunomodulatory ability of fighting fungi can increase the functional value of these fungi.

Pelawan bitter honey is also found in the Biodiversity Park area of Central Bangka Regency. Honey is a thick liquid produced by honey bees that comes from various sources of nectar. Nectar is a bitter or sweet liquid produced by plant nectar glands (SNI, 1994). Pelawan bitter honey is honey produced by *the Apis dorsata* honey bee (a forest bee). The honey bee *Apis dorsata* feeded on the pollen of the Pelawan tree (*Tristaniopsis mergueinsis*). Pelawan bitter honey has a bitter taste but is mixed with a sweet taste like other honeys (Akhbarini, 2016).

Based on a preliminary study of the people in Namang Village, honey was also used to strengthen the immune system by 20% (Mita L., 2017). This was the background of the authors' research on the use of Pelawan mushrooms and Pelawan honey for their benefits as immunomodulators. Therefore, the purpose of this study was to analyze the phytochemical content contained in Pelawan mushrooms and Pelawan honey.

RESEARCH METHODS

Time and Place of Research

This research was conducted from September 2020 to October 2021. This research was conducted at the Pharmacognosy Laboratory of the Pharmacy Study Program as well as the Biology Laboratory, Physics Laboratory and Basic Laboratory of the Faculty of Agriculture, Fisheries and Biology, University of Bangka Belitung.

Object of research

Pelawan mushrooms and Pelawan bitter honey were taken from Pelawan Forest, Namang Village, Central Bangka Regency. The village has a Pelawan plantation forest (*Tristaniopsis merguiensis*), which was protected and cultivated. The forest is a producer of Pelawan mushrooms and Pelawan honey from forest bees (*Apis dorsata*).

Tools and materials

The tools used include analytical scales, test tubes, test tube racks, a micropipette, a stirrer (a spatula), a measuring pipette, disc paper, a beaker, an Erlenmeyer funnel, a Bunsen boiler, an *autoclave*, and a *Dry Head Oven*. The materials used include disc paper, benzoyl peroxide, distilled water, newsprint, brushes, tissue, Pelawan mushrooms, and Pelawan bitter honey. 96% ethanol, heparin, EDTA, CMC-Na, *cotton swab*, alcohol

sweat, 2N HCl, Dragendroff reagent, chloroform, FeCl3, Mayer's reagent, 10% NaOH, Liberman-Buchard reagent, Pb acetate, AlCl3 solution, 10% KOH solution, 5% FeCl3, ethyl acetate, chloroform, methanol, n-Heksan, butanol, glacial acetic acid, H2SO4 10%, tranexamic acid 10%, and *aquadest.*

Sample Preparation

Contrary fungus samples were obtained from the location and immediately brought to the laboratory for further preparation.Pelawan mushroom samples from the field were cleaned of dirt using a tissue or brush. The fighting mushroom fruit bodies werw then sliced to speed up the drying process. Then, sliced mushrooms were dried in an oven at 39 degrees Celsius for 48 hours.

Coarse extraction

The dried samples were crushed using a blender to produce a coarse powder of Pelawan mushrooms. Coarse mushroom powder was macerated in stages with hexane, ethyl acetate, and ethanol solvents. The results of the maceration werw filtered and evaporated using an evaporator at a temperature of 40–60℃ to obtain a thick extract.

Standardization of total ash content

A total of 2 g of Pelawan mushroom ethanol extract that had been crushed and weighed carefully, put into a silicate crucible or platinum crucible that has been ignited and tared, and then the powder was leveled. The crucible was heated slowly to a temperature of 500–6000 °C until the charcoal was used up, cooled, and weighed. If the charcoal cuoldn't be lost in this manner, hot water was added and filtered through ash-free filter paper. The remaining charcoal was ignited, and filter paper was placed in a crucible. The filtrate was put into a crucible, evaporated, and ignited, then weighed to a constant weight. The ash content was calculated as a percentage of the simplicia that had been air-dried (Depkes RI, 2000).

Determination of specific gravity

Specific gravity was determined using 1% extract. empty vial filled with 2 mL of water and marked. Weigh the empty vial (VO) and the vial contained 2 mL of 1% ethanol extract (V1). The specific gravity of the extract was calculated by comparing the weight of a 1% solution of ethanol extract to the weight of water, assuming the specific gravity of water was equal to 1.

Determination of drying shrinkage

To determine drying shrinkage, 1 gram of Pelawan mushroom ethanol extract was carefully weighed in a closed porcelain crucible that had previously been heated at 1050 °C for 30 minutes and tare. Spread it evenly by shaking it until it was a layer 5–10 mm thick. Dried the weight in the oven at 1050 °C until the lid remains open. Furthermore, the closed crucible was removed from the oven, cooled in a desiccator to room temperature, and then recorded. The sample was then returned to the oven, cooled in a desiccator to room temperature, and recorded. Then put it back in the oven at 1050℃ for 1 hour. The procedure was repeated until the difference in weighing results was no more than 0.5 mg per gram of sample after being dried for 1 hour (Depkes RI, 2000).

Determination of water and ethanol soluble extracts

As much as 5 grams of ethanol extract of Pelawan mushrooms were macerated for 24 hours with 100 mL of water-chloroform (for water-soluble essence content) and 100 mL of 96% ethanol (for ethanol-soluble extract content) in a clogged flask while shaking repeatedly for the first 6 hours. Then it was left for 18 hours and filtered. The filtrate was then evaporated to dryness in a shallow, flat-bottomed tareed cup, and the residue was heated at 1050 °C until a constant weight was reached.

Pelawan Mushroom Phytochemical Test and Pelawan Bee Honey

Phytochemical screening was carried out qualitatively on Pelawan mushroom species and Pelawan bitter honey to determine the groups of alkaloids, saponins, flavonoids, phenolics, triterpenoids, and glycosides.

Alkaloids

Put the sample in a test tube and add 1% HCL (10 mL) for 10 minutes over a boiling water bath. Then the suspension was filtered with cotton and put in the same number of test tubes I and II, and then 3 drops of Dragendorf reagent were added to solution I and 3 drops of Meyer's reagent were added to solution II. If the two reagents form a precipitate, it indicated the presence of alkaloids (Sulistyani, 2012).

Saponins

The sample put into a test tube with added distilled water, shaken vigorously for 30 seconds, then left upright for 30 minutes. If constant foam appears on the surface and did not disappear after dropping dilute HCl, it indicated the presence of saponins (Sulistyani, 2012).

Flavonoids

Take 1 mg of the sample, put it in a test tube, and dissolve it in 1-2 mL of 50% hot methanol. After that, add the Mg metal and 4-5 drops of concentrated HCl. A positive result if a red or orange solution indicated the presence of flavonoids (Latifah , 2015).

Phenolic

A sample of 50 g was added with 10 drops of FeCl3 at 1%. Pelawan bitter honey contained phenol if it produces green, red, purple, blue, or dark black colors (Najoan, 2016).

Triterpenoids

A sample of 50 g was added along with 10 drops of glacial CH3COOH. The solution was shaken gently and left for a few minutes. The triterpenoids had a red or purple color (Najoan, 2016).

Glycosides

0.1 mL of the sample was evaporated over a water bath, then 5 mL of anhydrous acetic acid P was added and 10 drops of P sulfuric acid were dropped. The color changed.

RESULTS AND DISCUSSION

Yield of Pelawan mushroom extract

Calculation of the results of 200 g of Pelawan mushroom extract simplicia powder was observed; if a blue or green color changed, it indicated the presence of glycosides.

Thin-Layer Chromatography Test
First, preparation of the stationary phase A silica gel G60 F254/TLC plate with a length of 8 cm and a width of 2 cm was washed with methanol, then activated in an oven at 1000 °C for 10 minutes. As much as 10 mg of the extract was dissolved in 1 ml of ethanol and then spotted on the stationary phase.

Identification of Flavonoid Compounds
The mobile phase was glacial acetic acid : butanol, and : water (1:4:5), with ammonia vapor as the visible stain. A positive reaction was indicated by the formation of a yellow-brown stain after being steamed with ammonia in visible light and a blue color at UV 36nm, confirming the presence of flavonoids (Marliana, 2005).

Identification of Steroid Compounds
The mobile phase used was chloroform-methanol (9:1), with the Liberman-Buchard reagent staining agent accompanied by heating at 1050 °C for 5 minutes. A positive steroid reaction was indicated by the presence of a blue-green stain (Kristanti et al., 2008).

Identification of Tannin Compounds
The mobile phase was methanol-water (6:4) with 5% FeCl3 reagent. A positive reaction was indicated by the formation of a black stain (Banu and Nagarajan, 2014).

Identification of Anthraquinone Compounds
The mobile phase used was n-hexane-ethylacetate (3:7) with a 10% KOH solution in methanol as a stain remover. A positive reaction was indicated by the formation of yellow, brown yellow, red, and purple stains (Banu and Nagarajan, 2014). After soaking in 96% ethanol for 2000 mL, an extract yield of 32.2 g (16.1%) was obtained. The yield of Pelawan mushroom extract was presented in Table 4.

Table 4. Yield of Pelawan mushroom extract

Pelawan Mushroom Powder Tristaniopsis merguensis(g)	**Amount of solvent (mL)**	**Amount of condensed extract (g)**	**Yield of Pelawan mushroom extract**Tristaniopsis merguensis(%)
200	2000	32,2	16,1

(Source: Primary data that has been processed)

In several studies, the yield percentage of an ethanol extract was higher than that of other extracts such as ethyl acetate and n-hexane. This was because ethanol had a low molecular weight so that it could form hydrogen bonds, mix with water, and dissolve in it up to infinite solubility. This explained the selection of ethanol as a very appropriate solvent in this study. According to Romandanu *et al*., (2014), ethanol had a very strong polar group; ethanol (C2H5OH) had another name, ethyl alcohol, which was a very polar molecule because of the presence of a hydroxyl group (-OH) with a very high electronegativity of oxygen, which caused hydrogen bonds to occur so that it could bind to other molecules. polar and ionic molecules.

Standardization of total ash content

Standardization of medicinal plant extracts in Indonesia is one of the important stages in the development of native Indonesian medicines. Total ash content, specific gravity, drying shrinkage, water-soluble extract, and ethanol content are non-specific parameters of extracts. The purpose of this test was to introduce as much as possible subjectively using the five senses by describing shape, color, smell, and taste (Anonymous, 2000; Anonymous, 2008).

For one hour, the total ash content was tested using a furnace tool at a temperature of 500 oC. As a result, a total ash content of 28% was obtained. The determination of the total ash content was useful to provide an overview of the mineral content of the extract, starting from the initial process to the formation of the extract, so that the total ash content parameter was related to the purity and contamination of the extract (Anonymous 2000).

Determination of drying shrinkage

In the drying shrinkage test, the remaining substance was measured after drying. The results of this drying shrinkage test yielded a percentage of 40.88%. The drying shrinkage could be used to provide a maximum limit (range) for the amount of compounds lost during the drying process (Ministry of Health, 2000).

Standardization of specific gravity

A pycnometer was used to calculate specific gravity. As a result, the specific gravity of the extract was 0.67 g/mL. The determination of specific gravity served to describe the amount of mass per unit volume to provide a boundary between liquid extracts and thick extracts, besides the fact that specific gravity was related to how to know the purity of a substance, which was determined by its specific gravity (Ministry of Health, 2000).

Determination of water and ethanol soluble extracts

The determination of water-soluble extracts was useful in determining the amount of compounds extracted from a simplicia with water (Fauzi, 2013). The determination of extracts dissolved in ethanol seeks to ascertain the number of compounds that could be extracted from a simplicia using ethanol (Fauzi, 2013). In this study, the water-soluble essence content was 59.01%, and the ethanol-soluble extract content was 41.12%.

Results of Standardization of Pelawan Honey and Pelawan Fungus Phytochemical Screening

Table 5 shows that Pelawan honey positively contained secondary metabolites, namely flavonoids and alkaloids, while Pelawan mushroom extract positively contained phenolic and alkaloid secondary metabolites. The results of the phytochemical screening research could be seen in Table 5.

Table 5. Results of Phytochemical Screening of Ethanol Extract Pelawan Honey and Pelawan Mushrooms

Type Sample	Compound	Reactor	Test results			Information
			U1	U2	U3	
Petra Honey	Alkaloids	HCl, P. Dragendorf, and P. Mayer	+	+	+	Ring formed
	Flavonoids	Pb acetate and NaOH	+	+	+	Orange
	Glycosides	As.acetic anhydrous P	-	-	-	Red
	Saponins	-	-	-	-	No foam
	Triterpenoids	Glacial CH3COOH	-	-	-	No changes
Fighting Mushroom	Phenolic	FeCl3 1%	+	+	+	Blackish green
	Alkaloids	HCl, and P. Mayer	+	+	+	A slight precipitate formed
	Alkaloids	HCl, P. Dragendorf	-	-	-	No changes
	Flavonoids	Pb acetate and NaOH	-	-	-	No changes
	Glycosides	As.acetic anhydrous P	-	-	-	No changes
	Triterpenoids	Glacial CH3COOH	-	-	-	No foam

(Source: Primary data that has been processed)

Information:
U1 : First iteration
U2 : Second repetition
U3 : Third repetition
(+) : Positive test result
(-) : Negative test result

Existence The phenolic compounds in pelawan mushroom extract were characterized by the occurrence of a black color change in the test sample but were not too concentrated after being added with FeCl3 at 1%. The existence of sphenolic compound in an extract was stated positively if they were present. The color changed from bluish black to dark black when 1% FeCl3 was added. Because FeCl3 reacted with aromatic -OH groups, phenolics could react with 1% FeCl3 to form deep red, purple, blue, or black colors (Habibi et al. (2018); Haryanto et al. 2015). Meanwhile, flavonoid compounds werw declared positive if, during the test, there was a reddish-black, yellow, or orange color change (Tarukbua et al. 2018). After adding HCl and magnesium powder, there would be a change in the color of the extract to a reddish black, indicating a positive result for flavonoids (Rumagit et al. 2015). According to Ergina *et al*. (2014), the purpose of adding a few drops of HCl solution and Mg powder was to reduce the benzopirone nucleus contained in the flavonoid structure to form red or orange flavone salts.

Dragendorff and Mayer's reagents were used to test for alkaloids. Positive results were shown in the test using Mayer's reagent, namely the presence of a white precipitate. According to Tarukbua et al. (2018), a positive test for alkaloids using Mayer's reagent was characterized by the formation of a white precipitate in the solution.

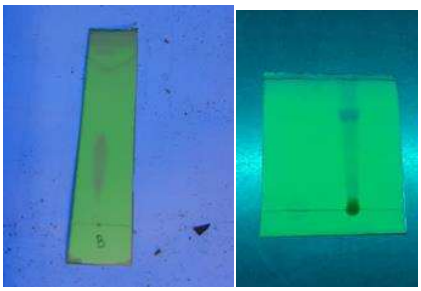
The result above showed that honey and Pelawan mushrooms could produce secondary metabolites. Secondary metabolites were a group of compounds that were widely used by organisms for self-protection from the environment and from attack by other organisms, and these compounds were usually biosynthetic products derived from primary metabolites that were generally produced by organisms (Murniasih 2003).

Phenolic compounds had various benefits, including antioxidant activity, cholesterol lowering properties, and other used for health (Awika & Rooney, 2004). Previous studies demonstrated the potential of phenolics as immunomodulatory agents. For example, the phenolic content in red ginger rhizome was thought to act as a regulator of immunity through immune cell regulatory mechanisms, pro-inflammatory cytokine synthesis, and gene expression (da Cunha et al, 2019). In addition, its activity could also inhibit certain enzymes involved in the production of free radicals (ROS), such as NADPH oxidase (NOX) and xanthine oxidase (Yahfoufi et al, 2018).

The fungal cell wall component generally consisted of two important components, namely chitin and -glucans (beta glucan), the presence of which caused mushrooms to be important in terms of health and the treatment of various diseases.In addition, mushrooms also had many other components such as polysaccharides, agaritin, ergosterol, and polyphenols, which were usually referred to as biological response modifiers (BRMs) because they could modulate the immune system to fight cancer cells and other diseases. In vitro and in vivo studies had supported this (Baldassano et al. 2017; Chen et al 2004; Gao and Zhou 2002; Pelley and Strickland 2000).

Thin Layer Chromatography Test

The TLC (Thin Layer Chromatography) test aimed to see the purity level of the active compound content of honey and Pelawan mushrooms with the help of a capillary tube on the TLC plate. The TLC process used n-hexane and ethyl acetate as the eluent (mobile phase) with a ratio of 7:3 .



(a) (b)

Figure 1. TLC Test (a) Pelawan Honey, (b) Pelawan Mushroom.

The results obtained in the Pelawan honey TLC test obtained an Rf value of 0.54. The Rf value showed that Pelawan honey contained active The Rf value showed that Pelawan honey contained active compounds, namely flavonoids and alkaloids, which were marked by the stain spots obtained, while the Pelawan mushroom extract produced an Rf value of 0.56, which indicated that the active compounds contained were phenolics and alkaloids.

CONCLUSION

From the results of photochemical tests, it was known that pelawan honey contains flavonoid compounds and alkaloids, while the KLT test results contained flavonoids and alkaloids where the average Rf value was 0.54. The standardization of extracts against Pelawan mushroom extract included a total ash content of 28%, a drying shrinkage of 40.88 g/mL, a specific gravity of 0.67 g/mL, a water-soluble juice content of 59.01%, and an ethanol-soluble juice content of 41.12%, a phytochemical test of fighting fungi contained phenolics and alkaloids, and a confirmed KLT test contained flavonoids. The test results showed that mushrooms and honey had the potential to be further researched and developed for use as immunomodulatory agents.

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