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IDENTIFICATION AND PHYTOCHEMICAL TESTING OF MUSHROOMS AND PELAWAN HONEY TYPICAL BANGKA BELITUNG

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ABSTRACT

Pelawan bitter honey and pelawan mushroom are typical Bangka biodiversity found in Central Bangka Regency. The Pelawan mushroom grows in the Bangka Belitung Islands Province and 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 which can improve the immune system. Apart from that, based on preliminary studies, honey is also used by local people to strengthen the immune system.The aim of this research was to identify the active compound content of Pelawan honey and Pelawan mushrooms. This type of research is experimental research with several test stages including phytochemical screening, Thin Layer Chromatography test and extract standardization test. The research results prove that the phytochemical test of Pelawan honey contains flavonoid and alkaloid compounds, for the Thin Layer Chromatography (TLC) test it contains flavonoids and alkaloids. The phytochemical test for Pelawan mushrooms contains phenolics and alkaloids using Mayer's reagent, the Thin Layer Chromatography test for Pelawan mushrooms contains flavonoids with an average Rf value of 0.56, while the standardization of Pelawan mushroom extract produces a total ash content of 28%, drying loss of 40.88 g. /mL, specific gravity 0.67 g/mL and water soluble essence content of 59.01% and ethanol soluble essence content of 41.12%. From these results it can be suspected that antiviral mushrooms and honey have the potential to be developed as immunomodulatory agents.

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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 Heimioporus sp fungus and the harvest of Pelawan honey in the Biodiversity Park of Central Bangka Regency. Pelawan mushrooms are known as the most expensive mushrooms in Bangka Belitung Province, perhaps even in Indonesia (Akbarini, 2016). Richi (2011) stated that the Pelawan fungus is a fungus that forms ectomycorrhizae in symbiosis with the Pelawan tree. Ectomycorrhiza is a type of fungus that covers the root cells of the host plant and has external hyphae that can form macroscopic fruiting bodies in or on the surface of the soil. The pelawan fungus (Boletus sp.) is an ectomycorrhizal fungus that is in symbiosis with the roots of the pelawan plant (Tristaniopsis merguensis). This symbiosis is known to help pelawan plants absorb nutrients so that they can increase plant height and chlorophyll content in pelawan leaves. Pelawan mushrooms have high economic value with selling prices reaching 1,800,000 rupiah per kilogram for dried mushrooms 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) states that the contrarian mushroom contains high protein, is rich in dietary fiber, and is low in fat. Valine, methionine, threonine, isoleucine, leucine, phenylalanine, and lysine are essential amino acids contained in repellent mushrooms. Anti-aging mushrooms also contain natural antioxidants such as phenolic components, carotene and lycopene.

Phenolic compounds are known to have immunomodulatory activity which can improve the body's 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 dangerous substances that enter the body (Yanuar 2009). The use of immunomodulators is more effective than antibiotics, because giving antibiotics over a long period of time causes pathogenic bacteria to develop resistance. In addition, the use of immunomodulators does not leave residues that are harmful to the environment and 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, carotene, and lycopene, so they have the potential to be developed as a source of natural immunomodulators. Information regarding the immunomodulatory abilities of antifungals is expected to increase the functional value of these fungi.

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

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

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Materials and Methods

Time and Place of Research

This research was carried out from September 2020 to October 2021. This research was carried out 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, Bangka Belitung University.

Object of research

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

Tools and materials

Tools used include analytical scales, test tubes, test tube racks, micropipettes, stirrers (spatulas), measuring pipettes, paper discs, beakers, Erlenmeyer, glass funnels, Bunsen, autoclave, Dry Head Oven.

The materials used include paper discs, 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's reagent, chloroform, FeCl3, Mayer's reagent, 10% NaOH, Liberman-Buchard's reagent, Pb acetate, AlCl3 solution, 10% KOH solution , FeCl3 5%, ethyl acetate, chloroform, methanol, n-Hexane, butanol, glacial acetic acid, H2SO4 10%, tranexamic acid 10%, distilled water.

Sample Preparation

Samples of pelawan fungus were obtained from the location and immediately taken to the laboratory for further preparation.Pelawan fungus samples from the field are cleaned of dirt using a tissue or brush. The fruiting body of the pelawan mushroom is then sliced to speed up the drying process. The sliced mushrooms are then dried using an oven at 39oC for 48 hours.

Coarse extraction

The dried samples were then ground using a blender to produce a coarse powder of pelawan mushroom. The coarse powder of pelawan mushrooms was macerated in stages with hexane, ethyl acetate and ethanol solvents. The maceration results are filtered and evaporated using an evaporator at a temperature of 40-60oC until a thick extract is obtained.

Standardization of total ash content

A total of 2 g of Pelawan mushroom ethanol extract which has been crushed and weighed carefully, is put into a silicate crucible or platinum crucible which has been ignited and tared, then the powder is evenly distributed. The crucible is slowly heated to a temperature of 500-6000C until the charcoal runs out, cooled and weighed. If the charcoal cannot be removed using this method, add hot water, filtered through ash-free filter paper. The remaining charcoal is ignited and the filter paper is placed in a crucible. The filtrate is put into a crucible, evaporated and ignited, then weighed until the weight remains constant. Ash content is calculated as a percentage of simplicia that has been dried in air (Ministry of Health of the Republic of Indonesia, 2000).

Determination of specific gravity

Determination of specific gravity was carried out using 1% extract. Fill the empty vial with 2 mL of water and mark it. Weigh the empty vial (VO) and the vial containing 2 mL of 1% ethanol extract solution (V1). The specific

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gravity of the extract is calculated by comparing the weight of the 1% ethanol extract solution to the weight of water, assuming the specific gravity of water is equal to 1.

Determination of drying shrinkage

Determination of drying loss by carefully weighing 1 gram of ethanol extract of pelawan mushroom in a porcelain crucible with a lid which has previously been heated at 1050C for 30 minutes and has been stored. Flatten by shaking until it forms a layer 5-10 mm thick. Dry in the oven at 1050C until the weight remains with the lid open. Next, the closed crucible is removed from the oven and cooled in a desiccator to room temperature and then recorded. Then put it back in the oven, cool it in a desiccator to room temperature and then record it. Then put it back in the oven at 1050C for 1 hour. This procedure was repeated until the difference in the weighing results was no more than 0.5 mg per gram of sample after drying for 1 hour (Ministry of Health of the Republic of Indonesia, 2000).

Determination of water and ethanol soluble essence content

A total of 5 grams of ethanol extract of pelawan mushroom was macerated for 24 hours with 100 mL of water-chloroform (for water soluble essence content) and 100 mL of 96% ethanol (for ethanol soluble essence content) using a clogged flask while shaking repeatedly for the first 6 hours. Then left for 18 hours and filtered. Next, 20 mL of the filtrate was evaporated to dryness in a flat-bottomed shallow dish that had been tarred, the residue was heated at a temperature of 1050C until the weight remained constant.

Phytochemical Test of Pelawan Mushroom and Pelawan Bee Honey

Phytochemical screening was carried out qualitatively on Pelawan mushrooms 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 wool and put in equal amounts in test tubes I and II, then 3 drops of Dragendorf's reagent were added to solution I and 3 drops of Meyer's reagent were added to solution II. If a precipitate forms between the two reagents, it indicates the presence of alkaloids (Sulistyani, 2012).

Saponins

The sample is placed in a test tube and distilled water is added, shaken vigorously for 30 seconds, then left in an upright position for 30 minutes. If constant foam appears on the surface and does not disappear after dropping dilute HCl, this indicates the presence of saponin (Sulistyani, 2012).

Flavonoids

A sample of 1 mg is taken, put in a test tube, dissolved in 1-2 mL of 50% hot methanol. After that, Mg metal and 4-5 drops of concentrated HCl are added. Positive results if a red or orange solution is formed indicates the presence of flavonoids (Latifah , 2015).

Phenolic

A sample of 50 g was added with 10 drops of 1% FeCl3. Pelawan bitter honey contains phenol which produces a green, red, purple, blue or dark black color (Najoan, 2016).

Triterpenoids

A sample of 50 g was added with 10 drops of glacial CH3COOH. The solution was shaken

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gently and left for several minutes. Triterpenoids gave a red or purple color (Najoan, 2016).

Glycosides

A sample of 0.1 mL was evaporated on a water bath, then 5 mL of anhydrous acetic acid P was added and 10 drops of sulfuric acid P were added. The color change was observed, if a blue or green color change occurred, it indicated the presence of glycosides.

Thin Layer Chromatography Test

Preparation of the Silica gel G60 F254 stationary phase/TLC plate with a length of 8 cm and a width of 2 cm, then washed with methanol, then activated in an oven at 1000C for 10 minutes. A total of 10 mg of extract was dissolved in 1 ml of ethanol then added to the stationary phase.

Identification of Flavonoid Compounds

Mobile phase of glacial acetic acid: butanol: water (1:4:5), with visible ammonia vapor stains. A positive reaction is indicated by the formation of a yellow-brown stain after evaporating ammonia when observed with visible light and blue at UV 366 nm confirming the presence of flavonoid content (Marliana, 2005).

Identification of Steroid Compounds

The mobile phase used was Chloroform-methanol (9:1), with a Liberman-Buchard reagent stain appearing accompanied by heating at a temperature of 1050C for 5 minutes. A positive reaction to steroids is indicated by a blue-green stain (Kristanti et al., 2008).

Identification of Tannin Compounds

Methanol-water mobile phase (6:4), with a stain appearance of 5% FeCl3 reagent. A positive reaction is 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 stain appearance of 10% KOH solution in methanol. A positive reaction is indicated by the formation of yellow, brown, red, purple stains (Banu and Nagarajan, 2014).

Results and Discussion

Calculating the yield of 200 g of Pelawan mushroom extract simplicia powder soaked in 2000 mL of 96% ethanol solvent resulted in an extract yield of 32.2 g (16.1%). The yield results of Pelawan mushroom extract are presented in Table 4.

Table 4. Yield Results of Pelawan Mushroom Extract

Pelawan Mushroom Powder*Tristaniopsis merguensis*(g)	**Amount of solvent (mL)**	**Amount of thick extract (g)**	**Pelawan Mushroom extract yield***Tristaniopsis merguensis*(%)
200	2000	32.2	16.1

(Source: Primary data that has been processed)

In several studies, the percentage yield of ethanol extract was greater than that of other extracts such as ethyl acetate and n-hexane. This is because ethanol has a low molecular weight so it is able to make hydrogen bonds, can mix and dissolve with H2O up to infinite solubility. This makes the choice of ethanol as a very appropriate solvent in this research. According to Romandanu et al. (2014), ethanol has a very strong polar group, ethanol (C2H5OH) has another name, ethyl alcohol, which is a very polar molecule because of the presence of a hydroxyl group (-OH) with a very high oxygen electronegativity which causes

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hydrogen bonds to occur so that it can bond with the molecule. 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 original Indonesian medicines, total ash content, specific gravity, drying loss, water soluble essence and ethanol content are non-specific extract parameters. The aim of this test is a simple initial recognition subjectively possible using the five senses by describing shape, color, smell and taste (Anonymous, 2000; Anonymous 2008).

Total ash content testing using a Furnace at a temperature of 500°C for 1 hour. As a result, a total ash content of 28% was obtained. Determining the total ash content is useful for providing an overview of the mineral content of the extract, starting from the initial process until the formation of the extract so that the total ash content parameter is related to the purity and contamination of the extract (Anonymous 2000).

Determination of drying shrinkage

In the drying shrinkage test, the residual substance after drying is measured. The results of this drying shrinkage test obtained a percentage of 40.88%. Knowing the drying loss can provide a maximum limit (range) of the amount of compounds lost in the drying process (Ministry of Health, 2000).

Standardization of Specific Gravity

Specific gravity is determined using a pycnometer. As a result, the specific gravity of the extract was obtained0.67g/mL. Determining specific gravity serves to describe the amount of mass per unit volume to provide a boundary between liquid extract and thick extract, apart from that, specific gravity is also related to how to determine the purity of a substance whose specific gravity is determined (Ministry of Health, 2000).

Determination of water and ethanol soluble essence content

Determining water-soluble essence content is useful in knowing the number of compounds that can be extracted with water from a simplicia (Fauzi, 2013). Determining the concentration of essence that dissolves in ethanol aims to determine the number of compounds that can be extracted with ethanol from a simplicia (Fauzi, 2013). In this study, the water soluble essence content was 59.01%, and the ethanol soluble essence content was 41.12%.

Results of Standardization of Phytochemical Screening of Pelawan Honey and Pelawan Mushroom

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

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

Type Sample	Compound	Reactor	Test results			Information
			U1	U2	U3	
Pelawan Honey	Alkaloids	HCl, P. Dragendorf, and P. Mayer	+	+	+	A ring is formed
	Flavonoids	Pb acetate and NaOH	+	+	+	Orange
	Glycosides	Acetic anhydrous P	-	-	-	Red
	Saponins	-	-	-	-	Doesn't foam
	Triterpenoids	CH3COOH Glacial	-	-	-	No changes
Combat Mushrooms	Phenolic	FeCl3 1%	+	+	+	Blackish green
	Alkaloids	HCl, and P. Mayer	+	+	+	A slight precipitate is formed
	Alkaloids	HCl, P. Dragendorf	-	-	-	No changes
	Flavonoids	Pb acetate and NaOH	-	-	-	No changes
	Glycosides	Acetic anhydrous P	-	-	-	No changes
	Triterpenoids	CH3COOH Glacial	-	-	-	Doesn't foam

(Source: Primary data that has been processed)
Information:
U1 : First repetition
U2 : Second repetition
U3 : Third repetition
(+) : Positive test result
(-) : Negative test result

ExistencePhenolic compounds in pelawan mushroom extract are characterized by a black color change in the test sample but are not too concentrated after being added withFeCl3 1%. The existence of sPhenolic compounds in an extract are declared positive if they are presentthe color changes from bluish black to dark black when 1% FeCl3 is added. Phenolics can react with 1% FeCl3 to form a deep red, purple, blue or black color because FeCl3 reacts with the –OH aromatic group (Habibi et al. (2018);Haryati et al. 2015). Meanwhile,Flavonoid compounds are declared positive if the color changes to reddish black, yellow or orange during testing (Tarukbua et al. 2018).After adding HCl and magnesium powder, the color of the extract will change to reddish black, indicating positive results 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 is to reduce the benzopyrone core contained in the flavonoid structure so that red or orange flavilium salts are formed. Alkaloid testing is carried out byDragendorff's reagent andMayer's reagent. Positive results were shown in the test using Mayer's reagent, namely that there was a white precipitate. According to Tarukbua et al. (2018), a positive test for alkaloids using Mayer's reagent is indicated by the formation of a white precipitate in the solution.

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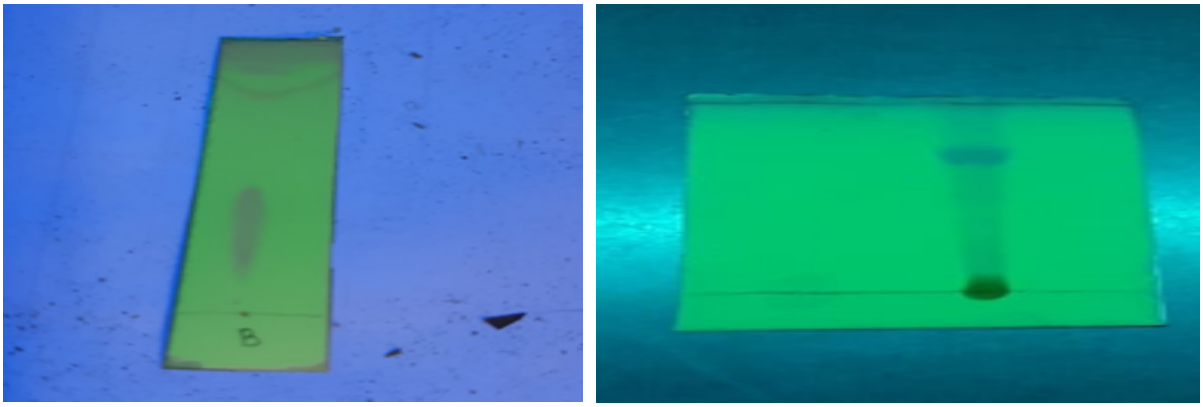
The test results above show that honey and Pelawan mushrooms can produce secondary metabolite compounds. Secondary metabolites are a group of compounds that are widely used by organisms for self-protection from the environment or from attacks by other organisms and these compounds are usually the result of biosynthetic derivatives of primary metabolites which are generally produced by organisms (Murniasih 2003).

Phenolic compounds have various benefits including antioxidant activity, cholesterol lowering properties and other uses for health (Awika & Rooney, 2004). Previous research shows the potential of phenolics as immunomodulatory agents. For example, the phenolic content in red ginger rhizomes is thought to act as a regulator of immunity through mechanisms of immune cell regulation, pro-inflammatory cytokine synthesis, and gene expression (da Cunha et al, 2019). Apart from that, its activity can 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 components of fungal cell walls generally consist of two important components, namely chitin andβ-glucans (beta glucan), the presence of this beta-glucan causes mushrooms to have significance in health aspects and the treatment of several types of diseases. Apart from that, mushrooms also have many other components such as polysaccharides, agaritin, ergosterol, polyphenols which are usually referred to as biological response modifiers (BRMs), because they can modulate the immune system to fight cancer cells and other diseases. In vitro and in vivo studies have 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 Chromtography) test aims to see the level of purity of the active compound content of honey and pelawan mushrooms, with the help of a capillary tube on a TLC plate. The TLC process uses the eluent (mobile phase) n-hexane and ethyl acetate in a ratio of 7: 3.



(a) (b)

Figure 1. TLC test (a) Pelawan honey, (b) Pelawan mushroom.

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The results obtained from the TLC test for pelawan honey showed an Rf value of 0.54. The Rf value shows that pelawan honey contains active compounds, namely flavonoids and alkaloids, which are indicated by the spots obtained, while the pelawan mushroom extract produces an Rf value of 0.56, which shows that the active compounds contained are phenolics and alkaloids.

Conclusion

From the results of the photochemical test, it is known that pelawan honey contains flavonoid and alkaloid compounds, while the TLC test results contain flavonoids and alkaloids where the average Rf value is 0.54. Extract standardization of Pelawan mushroom extract includes a total ash content of 28%, drying loss of 40.88 g/mL, specific gravity of 0.67 g/mL and water soluble essence content of 59.01% and ethanol soluble essence content of 41.12 %, The phytochemical test of the contrarian fungus contained phenolics and alkaloids, while the TLC test confirmed it contained flavonoids, marked by reddish yellow spots where the average Rf value was 0.56. The test results show that anti-fungal and honey have the potential to be researched and developed further as immunomodulatory agents.

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