Test the Power of drag Against the growth of bacteria of *Staphylococcus aureus* and *Escherichia coli* Extracts Ethanol Leaf Mangrove *Rhizophora mucronata* and the effects of Antidiabetik on Mice induced Aloksan

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Abstrack: The purpose of this research is to know the component's bioaktif compound, the anti-microbial activity and the effect of antidiabetik from the leaves of the plant Rhizopora mucronata. The sample form of the leaves of the mangrove coastal area derived from Barru. Leaf Rhizopora mucronata is extracted by means of maceration using ethanol 96% for 3x24 hours. The extracts were then tested in phytochemicals, anti-microbial testing and test antidiabetik. Test results of Phytochemical Rhizopora mucronata leaf extract contains positive phenolic moieties. While based on the test results the test against bacteria antimicrobial i.e. Staphylococcus aureus and Escherichia coli, the Rhizopora mucronata leaf extract showed an absence of antimicrobial activity against different types of microbes are examined. While based on a test of ethanol extract of antidiabetik leaves the Rhizopora mucronata can lower blood glucose levels the mice experienced Hyperglycemia after induced by aloksan. The antidiabetik effect is shown by the awarding of the ethanol extract of R. mucronata doses of 625 mg/kgBB.

Keywords: Rhizopora mucronata, phytochemicals, Staphyllococcus aureus, Eischerichia coli, antimicrobial, antidiabetik

INTRODUCTION

Indonesia is a country very rich in natural resources. Both are derived from animal or vegetable. The natural resources abundant widespread biodiversity of Islands ranging from Sumatra to Irian jaya island. One of the natural resources abundant biodiversity is the mangrove forest.

The mangrove forest is a type of forest that grows in this part of the coastline, especially above the swamp – a swamp of brackish water, sheltered beaches, where its existence is inundated when sea water inundation and free of tides when the water recedes. The Mangrove area in the village of Lappa and tongke Tongke-Sub Mangarabombang is one of the mangrove area in South Sulawesi province that consists of 25 species of mangrove and dominated by species of Rhizophora mucronata and *Rhizophora apiculata* Blume Lamark (Ernawati, 2013).

Mangrove plant is a plant that thrives in coastal areas that have the potential for very high bioaktif content. Indonesia with vast waters territory (2/3 of the total area) and the tropical climate is ideal for the growth of mangrove. Indonesia has the largest mangrove forest in the world, with an area of about 3.5 million hectares (Noor et al. 2006). About 202 species of mangroves in Indonesia have been identified and grown with lush.

Mangrove plants have filtered down its activities, i.e. as antiviral, antibacterial, antibisul, and antiinfl amasi (Agoramoorthy et al. 2008; Premanathanet al. 1999). One of the hopes of an alternative source of natural antioxidants are mangrove fruit (r. mucronata Lamk.) because previous research results according to Priyanto (2012) mangrove fruit has a very strong antioxidant activity, is present in large quantities and easily obtainable all along the coast of Indonesia, which has not been utilized optimally.

According Irianti (2008), natural antioxidants actually have long used hereditary, but have not been much researched activity and bioaktifnya content. Sartini et al. (2007) stated that natural antioxidants are antioxidants are commonly isolated from natural sources, mostly derived from herbs and fruits. According to Purwaningsih

(2013), stated that one of the fruits containing high antioxidants from plant mangroves is a mangrove black fruit (*R. mucronata* Lamk.).

This research aims to test the effectiveness of antibacterial against bacterial infection Staphylococcus aureus, Escherichia coli and decrease in blood glucose levels of black mangrove leaves (*R. mucronata* Lamk.).

RESEARCH METHODS

Time and location Research

This research has been carried out in June and August 2016, location research in the biology laboratory of University of Makassar.

Object Of Research

The object of the research is a kind of mangrove *Rhizophora mucronata* plant spp. plant material used are leaves taken from Regency Barru. The retrieval of the material is done by taking a the leaves of *Rhizophora mucronata*, collected and cleaned and allowed to dry aerial or under-anginkan. After the next dry mashed up shaped flour.

Research Procedure

This research uses a type of experimental research is conducted with the following steps:

1. Preparation samples

The sample leaves the black mangroves (*Rhizophora mucronata*) which was made into a sample of black mangrove leaves is still fresh and then cleaned and washed and then dried then mashed to form a powder.

2. Extraction

The process of maceration technique done using extraction. As much as 1 kg of dry black mangrove leaves maceration. with solvent ethanol 96% for 3x24 hours. The extract obtained using concentrated water bath to obtain a viscous blackish-green extracts. Next in evaporating and then evaporated at room temperature, so that the obtained extract thick ethanol. This extract is used for testing the effectiveness of antibacterial and blood sugar levels in mice.

3. Test of Phytochemicals

Phytochemical analysis is made on the basis of health (2009) referenced by Tirtana dkk. (2013) are as follows:

- Steroid/Triterpenoid

As many as 3 drops of extract solution of melted into the plate drops then coupled with reactant Lieberman-Burchard. Steroid compounds cause the color green and purple colors evoke a triterpenoid.

- Flavanoid

As many as 3 drops of extract solution of melted into the plate drops then coupled with FeCl3. Falavanoid compounds cause brownish green color.

- Alkaloid

Aqueous extract plus 2 drops of reagents Mayer. Positive results if formed deposits of white/yellow. Aqueous extract plus 2 drops of reagents Wagner. Positive results if formed deposits of Brown.

4. Test Of Antibacterial Activity

Antibacterial test done by the method of disc diffusion (Kirby-Bauer test). OSE sterile put into test tubes containing a bacterial suspension is then smeared on the media NA. After the brand of bacteria to dry out, paper disk (diameter 6 mm) that have been soaked for 1 hour extract drained and placed on top of the media containing the brand of bacteria with a little repressed so that paper disc attached to the surface of the media. Subsequently incubated at a temperature of 37 ° C for 24 – 48 hours. The antibacterial activity was declared positive in form drag zone formed a clear zone surrounding the paper disk.

5. Test Blood Glucose Levels

- Measurement of blood glucose in Mice

Aloksan monohidrat-induced test animals with a dose of 150 mg/kg (Sujono and Munawaroh, 2009) dissolved with sterile Aquabidestilasi for injection injected in intraperitonial. Aloksan monohidrat that has been dissolved should immediately be injected before there is a change of colour from pink into nodes. Dosage aloksan given on the murine standard (200 g) 200 g/1000 g x 150 mg/kg = 30 mg/200 g BB House mouse. Maximum allotment volume on the standard mice are injected in intraperitonial 2.0-5.0 mL. In this study, the concentrations of aloksan given on the murine standard is 30 mg/2 mL.

Blood glucose measurement is performed before the mice induced aloksan (GD0). On the third day, fasting blood glucose levels are measured again when already experiencing rising > 140 mg/dL (Mahendra et al, 2008) stated to have suffered diabetes (GD3) and soon was given a grant of treatment of leaf extract of *R. mucronata* in orally.

- Dosage of ethanol extracts of leaves of *R.mucronata*

A dose of methanol extract of leaves of *R.mucronata* is 312.5, 625, and 1250 mg/kg given per oral per day during 7 days after the third day blood glucose measurements.

- Assay activity decreased blood glucose levels in mice

Animal testing as much as 25 tail mice are grouped into 5 groups of treatment. Prior to the test, mice are fasting for 6 hours. The first thing that must be done before aloksan is a measurement-induced murine blood glucose levels normal mice (GD0). The taking of blood was performed by means of the lateral tail vein of wrenching on mice, blood glucose strips in then. Glucose levels tested using gauges glucose multicheck brand Necso.

Next 25 the House mouse tail has been divided into 5 groups of aloksan induced by a dose of 150 mg/kg body weight injected in intraperitonial. On the third day of fasting blood glucose levels (GD3) measurable return to comparison with GD0, when the increase has occurred becomes > 140 mg/dL then stated to have diabetes. Then each group getting preferential treatment:

Group I: as negative control, given Na-CMC 0.5% daily for 7 days.

Group II: as a positive control, given the glibenklamid with a dose of 0.45 mg/kg per day for 7 days.

Group III: ethanol extracts were given leaves of *R.mucronata* with doses of 312.5 mg/kg per day for 7 days.

Group IV: ethanol extracts were given leaves of *R.mucronata* with doses of 625 mg/kg per day for 7 days.

Group V: ethanol extracts were given leaves of *R.mucronata* with doses of 1250 mg/kg per day for 7 days.

After 7 days the granting of extracts, measured blood glucose levels back murine (GD10) for comparison with kenol (GD0) day and third day (GD3), what is happening decreased blood glucose levels.

RESULT AND DISCUSSION

Phytochemical Compounds Test

Based on a test of phytochemicals which have been done, ethanol extract of r. mucronata positive contain metabolite sekuder. This can be seen in table 1.

Table 1. The results of The Test of ethanol extract of r. mucronata

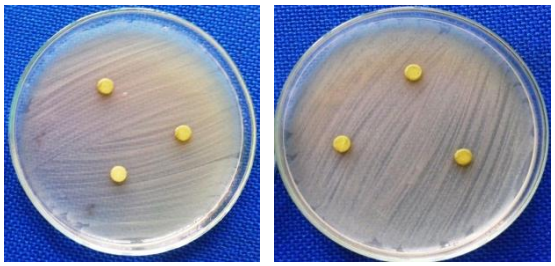
Reactant	Observations	Description
$FeCl_3$	green → brownish-green	(+) Flavonoid
Liebermann-Burchard	green → clear yellow	(-) Steroid
Mayer	green → clear yellow	(-) Alkaloid
Wagner	green → brown	(-) Alkaloid

From the table it can be seen that the ethanol extrack *R.mucronata* positive contains secondary metabolites i.e. flavanoid. It is characterized by changing the color of extracts from green to green-brown colour when reacted with FeCl3. Based on previous research that has been done by Nurdiani et al. (2008) showed that the leaves and bark Rhizophora mucronata bioaktif contain saponins, tannins, and flavonoids.

The identification of the presence of steroid compounds in the extract of r. mucroata done by reacting with reactant extract Liebermann-Burchard. The test result shows the extract of r. mucronata doesn't contain steroid compounds.The identification of the presence of steroid compounds in the extract of r. mucroata done by reacting with reactant extract Liebermann-Burchard. The test result shows the extract of r. mucronata doesn't contain steroid compounds. Whereas the testing of plant extracts on the alkaloid is done with two reactant test phytochemicals i.e. reactant Mayer and Wagner. On the reactant Mayer is characterized by the formation of a white precipitate, while Wagner's perekasi is characterized by the formation of deposits of brown to yellow. On *R.mucronata* extracts do not contain the alkaloid compounds because of the test result shows no sediment formation of phytochemicals white on reactant Mayer and Brown to yellow deposits on the reactant Wagner.

Test of Antibacterial Activity of Ethanol Extracts of Leaves of Mangrove *Rhizophora mucronata* Against Growth of Bacteria Test of *Staphylococcus aureus* and *Escherichia coli*

Positive test results of the antimicrobial activity is characterized by the formation of a clear zone around the disk containing the paper extract.



(a) (b)

Figure 1. Test the power of ethanol extracts of drag r. mucronata against bacteria bakteri (a) *Staphyloccus aureus* (b) *Escherichia coli*

Test results of antimicrobial activity against the bacteria s. aureus and e. coli does not indicate the presence of antibacterial activity (clear/cloudy zone zone = 0 mm). This is allegedly due to concentration of flavanoid found in the extracts are not enough damage the cell membranes of bacteria so that the bacteria could still reproduce his cell.

Assay Activity Decreased blood Glucose Levels of Ethanol Extract Rhizopora *mucronata* against Mice induced Aloksan

Testing the effect of ethanol extracts of leaves *Rhizopora mucronata* against blood sugar levels in mice males (*Mus musculus*) can be seen in table 2.

Table 2. Blood glucose average in mice male (Mus musculus) at GD0, GD3, and GD10

No	Treatment	Blood glucose average (mg/dL)			The percentage decrease in blood glucose levels (mg/dL) mice male (Mus musculus)
		GD0	GD3	GD10	
1	Negative control	119[a]	202,25[a]	177[b]	2,77%
2	Positif control	98,25[a]	225[a]	97[a]	14,05
3	EERm 312,5 mg/kg BB	108,25[a]	329,5ab	168[b]	17,73%
4	EERm 625 mg/kg BB	113,75[a]	480[b]	99,75[a]	41,75%
5	EERm 1250 mg/kg BB	126[a]	280,75[ab]	65[a]	23,69%

Description: the same Letter in a column indicates "different is not real". The letters that differ in one column shows "real different". A different letter between one column with another column showing "real different" EERm (Rhizopora mucronata Ethanol Extracts).

The data obtained were tested with test data distribution Homogenity of Variances, and continued with test Duncan to compare between each treatment. Based on the results can be seen fasting blood sugar levels (min 0) for all treatment has typically been a normal fasting blood sugar levels i.e. < 140 mg/dL. After induction of aloksan blood sugar levels in all treatment groups experienced a fairly high rise with an average rise in blood glucose levels ± 190.45. Aloksan beta cells can cause damage due to the onset of oxidative stress. Glutation dialuric acid is reduced to, so redox recycling process produces reactive oxygen species that would damage the cell beta (Arulanandraj et al., 2011). On GD10 visible blood glucose levels on a positive control is not unlike real with blood glucose levels at 625 and 1250 EERm Group mg/kgBB and differs markedly with the negative control group and EERm 312.5 mg/kgBB. While the Group EERm 312.5 mg/kgBB is no different with the negative control group. This indicates that group EERm 625 and 1250 mg/kgBB has the effect of antidiaetik

almost the same with the positive control (glibenklamid). Effect of antidiabetik owned by the extract of leaves of r. mucronata flavanoid compounds due to allegedly contained in the extract.

On diabetes mellitus increase free radicals, so plants that have antioxidant activity are expected to fight free radicals and lipid peroxidase (Li et al., 2004; Modak et al., 2007). Flavonoids, alkaloids, steroidal glycosides diterpenoid, and hypoglycemic effects. Chances are the mechanism is by increasing peripheral glucose metabolism and insulin release (Trease and Evans, 2002). Antioxidants that are able to neutralize radikan free can prevent diabetes mellitus, even reducing the severity of complications arising (Modak et al., 2007).

Flavonoids may improve pancreatic beta cell function. Flavone C-glycoside can inhibit aldose reductase (Li et al., 2004). Flavonoids is a scavenger of active oxygen species, inhibit the formation of nitrate, and a metal fastener. These compounds can undergo autooksidasi to generate hydrogen peroxide if there are metal. Other capabilities are increasing the activity of the enzyme cells (Farghaly and Hassan, 2012).

In this study the results obtained that the awarding of the Rhizopora mucronata leaf extract can lower blood glucose levels the mice experienced Hyperglycemia after induced by aloksan. The antidiabetik effect is shown by the awarding of the ethanol extract of r. mucronata doses of 625 mg/kgBB.

CONCLUSION

Based on the research that has been done can be inferred that

1. Extract the ethanol *Rhizopora mucronata* positive compounds bioaktif flavanoid containing marked with brownish green after extracts was reacted with FeCl3 1%.
2. Extract the ethanol *Rhizopora mucronata* was not able to inhibit the growth of bacteria *Eischerichia coli* and *Staphyllococcus aures*.
3. Ethanol extracts of leaves Rhizopora mucronata can lower blood glucose levels the mice experienced Hyperglycemia after induced by aloksan. The antidiabetik effect is shown by the awarding of the ethanol extract of r. mucronata doses of 625 mg/kgBB.

ADVICE

Research needs to be done of the effects of the extract of the leaves of the Rhizopora mucronata against pancreatic histopathology, immunohistochemical examination, or marker more like effect against beta cells repair, HOMA B, and HOMA IR as a beta cell dysfunction alert and insulin resistance.

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