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Research Article

Phytochemical Constituent of Methanol Extract in Bark and Leaves from Gofasa Tree (*Vitex cofassus*) Lives in Halmahera, North Maluku which is Potential as Anti-cholesterol

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ABSTRACT

Research on Phytochemical constituent and Anti-cholesterol Potential of Methanol Extract from Gofasa Tree in Bark and Leaves (Vitex cofassus) from Halmahera has been done. Extraction was carried out by maceration method as much as 3 x 24 hours with methanol solvent and then the phytochemical test was performed on thick extract. Anticolestrol test was carried out using the CHOD-PAP method. The phytochemical test results showed methanol extract from bark of gofasa tree containing alkaloids, flavonoids, and tannins and methanol extract from the leafs of gofasa tree contained alkaloids, steroids, flavonoids, tannins and saponins. Methanol extract of bark and leaves of gofasa tree has the ability to reduce cholesterol levels by 90.69% and 93.95% respectively. This shows the bark and leaves of the gofasa tree has the potential to be developed into an anti-cholesterol drug.

Keywords :Phytochemical constituent, Methanol Extract, Halmahera, Anti-cholesterol.

INTRODUCTION

Indonesia is a rich country in biodiversity. Which is have around 80 percent of the medicinal plants in the world, where 28,000 plant species grow, 1,000 of them have been used as medicinal plants and 31 types of which are used in the herbal, non-herbal, spice and export for industrial needs[1]. In Indonesia the use of plants as traditional medicine has been carried out for thousands of years. However, many of them still have not been studied further to explore the potential of these plants as medicinal raw materials[2]. One of the plants that is often used by people in Indonesia as medicinal plants is *Vitex cofassus* which is known by the community by the name of gofasa, sassuwat, bana or Biti wood trees. *V. cofassus* has large stems, 30-40 meters high and up to 130 cm in diameter. Classification of *V. cofassus* is: Kingdom: Plantae; Division: Spermatophyta; Class: Angiosperms; Order: Tubiflorae; Family: Lamiaceae; Genus: Vitex; Species: *V. Cofassus*[3]. The water for boiling the bark of the gofasa tree often by the people of North Maluku as a cure for jaundice[4]. Methanol extract of gofasa tree leaves has the potential as a ringworm drug, because it can inhibit the growth of T. mentagrophytes at a concentration of 3.2%[2]. The n-hexane extract of gofasa tree bark has been identified as a steroid group and has an LC_{50} value of BSLT (Brine Shrimp Lethality Test) of 74,079 µg / mL which indicates its potential as an anticancer[5]. Ethanol extract of the bark of the gofasa tree stem had an LC50 value of A. salina of 29.51 ppm[6]. So far, the potential of anticolesterol from the bark and leaves of gofasa trees not been studied yet. Therefore, the potential anticolesterol analysis and phytochemical screening of methanol extract from bark and leaves gofasa tree (*V. cofassus*) have to be done.

MATERIAL AND METHOD

Material

Bark and leaves of gofasa tree, methanol p.a, ammonia, chloroform, HCl, reagent Dragendorff, Mayer, Wagner, Libermann-Burchard, diethyl ether, Mg powder, amyl alcohol, distilled water, filter paper, $FeCl_3$, CHOD-PAP reagent.

Sample Preparation

The bark and leaves of the gofasa tree were taken from Halmahera Island in Oba Utara District, and identified species at Herbarium Bogoriense Biology Research Center-LIPI Jakarta. The samples were washed thoroughly, then dried and milled until each sample preparation was in powder form.

Extraction

100 g of bark and leaves powder from *V. cofassus* macerated with 500 mL of p.a methanol solvent as much as 3 x 24 hours. Maserate methanol bark, and leaves were concentrated using a Rotary Evaporator until thickened methanol extract was obtained from each sample.

Phytochemical screening

Phytochemical screening was carried out on root methanol

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Phytochemical constituent	Methanol extract	
	Bark	Leaf
Alkaloid	++	+++
Steroid	-	+
Terpenoid	-	-
Flavanoid	++	+++
Saponin	-	+
Tanin	++	++

extract, bark and *V. cofassus* leaves using standard test procedures[12,13,14,15] to determine the compound content of alkaloid groups, steroids, terpenoids, flavonoids, saponins, and tannins.

Alkaloid test, performed on extracts of 0.2 g sample added 5 ml of 25% ammonia and then crushed with mortar. 20 ml of chloroform were added, crushed again and filtered. The filtrate was put into a test tube, added 10% HCl and then shaken. The top solution (chloroform phase) was taken, then divided into test tubes, each added reagent Dragendorff, Mayer and Wager. When red brick sediment are formed with Dragendorff reagents, white sediment with Mayer reagents and brown sediments with Wagner reagents show the presence of alkaloid compounds.

Steroid and Triterpenoid Tests, carried out on extracts of 0.2 g of sample put into erlenmeyer with a closed lid, added 20 ml of diethyleter, macerated for 2 hours then filtered. A total of 5 ml of the filtrate was evaporated in the vaporizer cup until the residue was obtained, then Liebermann-Burchard reagent was added. The formation of red or green color indicates the presence of steroid or triterpenoid compounds.

Flavonoid test, carried out on 0.2 g extract of extract added 0.05 g of magnesium powder (Mg) and 0.2 ml of alcoholic acid (mixture of 37% HCl and 96% ethanol with the same volume), then added 2 ml of amyl alcohol then shake it hard and leave it to separate. The formation of red, yellow or orange in the amyl alcohol layer shows the presence of flavonoid compounds.

Saponin test, carried out on extract of 0.2 g sample added 100 ml of hot water, boiled for 5 minutes, then filtered with filter paper (solution A). 10 ml of solution A is inserted into the test tube and shaken vigorously vertically for 10 seconds. The formation of foam as high as 1-10 cm which is stable for 10 minutes and not lost at the addition of a drop of HCl 2 N, shows the presence of saponin compounds.

Tanin Test, 0.5 gram of sample powder is inserted into the beaker, then 20 mL of distilled water is added and boiled and filtered. After that 0.5 mL of filtrate was added to 0.1% ferricloride and color changes were observed

Anticholesterol Test

The anti-cholesterol in-vitro testing using the Amino cholestrol Amino Phenazone, where cholesterol esters with the help of cholesterol enzymes will be converted to cholesterol and free fatty acids. Cholesterol dioxidase becomes cholestenone and hydrogen peroxide and 4-amino phenazone with the help of the peroxidase enzyme will be converted into quinoneimine pink. First the reagent stamps are measured. Then a standard solution is made by means of 2 ml of reagent and 0.02 ml of standard pipetted into the test tube, the standard solution is incubated at a temperature of 20 ° -25 ° for 10 minutes then measured the absorbance. The experiment was conducted for 60 minutes. Then the sample solution was made by means of 2 ml of reagent and 0.02 ml of sample pipetted into a test tube which was then incubated for 10 minutes at a temperature of 20° - 25°C, then measured the absorbance. The experiment was conducted for 60 minutes. The color intensity formed is proportional to cholesterol concentration and a wavelength of 546 nm.

RESULTS AND DISCUSSION

Phytochemical Profile

To determine the components of chemical compounds contained in the methanol extract of the root of *V. cofassus* tree, phytochemical screening was carried out. The phytochemical profile of the methanol extract of the leaves and skin of the gofasa tree stem is very different as can be seen in table 2.

Alkaloid testing was carried out using reagents specific to containing of compound alkaloids such as Raegen Meyer, Wagner and Dragendorf. The positive results of the alkaloid test are when a brick red mercury (II) iodide is formed if Dragendorff reagents are added, the white mercury complex precipitate if added by Mayer reagents and brown deposits if added Wagner reagent[16]. In the alkaloid test, the amount of sediment and the color change in leaf extract were more than the bark. This shows that the quantity of alkaloid compounds in the leaves of the gofasa tree is more than that of the bark.

Testing for the presence of flavonoids is done by adding Mg powder and alcoholic acid in the form of a mixture of ethanol and HCl, adding HCl to hydrolyze flavonoids into their aglycones by hydrolyzing O-glycosyl. Mixing hydrochloric acid and magnesium powder can reduce carboxy groups in flavonoids so that color changes occur[17,18] In the flavonoid test the color changes in the leaf extract were more concentrated compared to the bark. This shows that the quantity of flavonoids in the leaves of the gofasa tree is more than that of the bark.

The phytochemical test of the Tanin group compound showed a positive change in the color of the extract to be darker or blackish green or become blue in ink. The use of 1% FeCl3 solution in testing tannin compounds to determine the presence of phenol groups in a sample or extract. Color changes that occur become darker or blackish green because tannins will form complex compounds with Fe3 + ions[19]. There are similarities in the same color changes on methanol extracts of bark and gofasa leaves when tested for tannins. So it can be assumed that the quantity of tannin in the methanol extract of the bark and leaves of the gofasa tree is the same

Identification of steroids and terpenoids showed positive results with the formation of red. This is because of the lengthening of the conjugation from and resulting in the appearance of the red color[7]. Steroid and terpenoid tests on bark extract did not change color, whereas in leaf extract there was a change in color which indicated the presence of steroids.

Table 1: Anticholesterol Test Results of Wood Skin and Gofasa Tree Leaves.

Sample	Cholesterol Levels before testing (mg / dL)	Cholesterol Levels after testing (mg / dL))	Percent of Decrease (%)
Bark	378	35,2	90,69
Leaves	292	17,67	93,95

In the saponin test using root extract, stem bark and Gofasa leaves after strong shaking on the extract, it was seen only on the part of the leaves of Gofasa which formed foam, this foam happened because the sugar chain contained in the extract broke. To prove that the foam formed is the result of a broken sugar chain, dilute HCl can be added, if the saponin the foam will remain stable. A solid foam formation when extracting or when concentrating plant extracts is evidence of saponins. In the Gofasa tree, the presence of Saponins is only present in the leaves, the possibility of saponins found in the leaves is steroid-type saponins, this is indicated by steroid group compounds contained in leaf extract.

Anticholesterol activity

Anticholesterol testing of methanol extract of bark and gofasa leaves was carried out using the CHOD-PAP method. Making the sample solution is done the same as making a standard solution. But in this sample solution pipetted into the test tube is 2ml of reagent solution and 0.02ml of sample, then this standard solution is incubated for 10 minutes at room temperature then followed by measuring the absorbance value using a spectrophotometer. Reading the absorbance value in the sample solution was also carried out 6 times to produce a constant absorbance value. The incubation process is carried out because the reagents contained in the sample solution contain enzymes that require a certain amount of time to react optimally, so that incubation time is needed. Anticolesterol test results showed methanol extract of bark and leaves of Gofasa tree.

The anti-cholesterol properties of the methanol extract of the leaves and bark of the gofasa tree are due to the chemical components contained there in. Tannin compounds in the body can trigger mucosal proteins that are on the surface of the small intestine so that it can reduce the effectiveness of cholesterol and fat absorption[8].

Can be seen in table 1, the percentage decrease in cholesterol levels by methanol extract leaves higher than the methanol extract of bark. One reason is the higher quantity of flavonoid compounds in methanol extract of leaves compared to methanol extract of bark. Flavonoid compounds have cardioprotective effects reducing the risk of atherosclerosis and increasing cholesterol efflux in the liver and increasing cholesterol changes to bile acids. In addition, flavonoids can also reduce LDL (Low Density Lipoprotein) and triglycerides in the body. In addition, flavonoids can increase the density of LDL receptors in the liver and bind apolipoprotein B[9,10].

The presence of saponin steroid compounds in leaf methanol extract is also a cause of a decrease in cholesterol levels by higher leaf methanol extract compared to methanol extract of bark. Steroid saponin compounds in the body function as lowering cholesterol levels by inhibiting the absorption of cholesterol in the intestine through competition with cholesterol in the absorption process in the intestine, thereby helping to reduce the amount of cholesterol entering the bloodstream and accelerate cholesterol excretion[10,11].

CONCLUSION

Methanol extract of gofasa tree bark contains alkaloids, flavonoids, and tannins while methanol extract of gofasa tree leaves contains alkaloids, steroids, flavonoids, tannins, and saponins.

Methanol extract of bark and leaves of gofasa tree has potential as an anti-cholesterol because it can reduce cholesterol levels by 90.69% and 93.95% respectively.

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