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Triterpenoid compound from metanol extract of mangrove leaves (*Sonneratia alba*) and anti-cholesterol activity test

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Abstract:

Mangrove plant *(Sonneratia alba)* is easily found in West and North Indonesia. Mangrove plant has the potential of being a herb medicine. Mangrove plant variously used in ethnomedicine to treat various deseases like wounds, diarrhea, and fever. In previously sutudy, leaf extract of mangrove plants reported have anti-cholesterol activity. This plant is widely used to treat various deseases like wounds, diarrhea, and fever. The purpose of this study was to isolate triterpenoid compound from the ethyl acetate fraction of mangrove leaves and anticholesterol activity test. Extraction was done by maceration method using methanol 96% as solvent. Isolation was carried out by column chromatography using a combination of *n*-hexane, ethyl acetate, and methanol solvents. The elucidation of the structure was determined by analysis of IR, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, and MS spectroscopies as well as by comparisons with the literature. Anticholesterol activity test was carried out in vitro. The results showed that triterpenoid compounds (lupeol) were able to reduce cholesterol from concentrations of 5, 10, 20, 40, 60, and 80 ppm are 13.7; 29.4; 49.0; 60,1; 70.2; and 77.0% respectively. Therefore, isolates compound (lupeol) have anti-cholesterol activity.

Keywords:

Anti-cholesterol; mangrove; triterpenes

Introduction

Mangrove plants (*Sonneratia alba*) are a group of plants that have high or shrubs and grow in tropical and subtropical coastal areas. This plant has a distinctive morphological features and can survive in environments with high salinity (Analuddin et al. 2018). Some mangroves have been used as herbs and extracts have biological activity in humans, animals, and phatogen bacteria.

Sonneratia alba is one of mangrove plants in the family of *lythraceae*. *Sonneratia alba* widely distributed in the coastal regions of Southeast Asia and the Indian Ocean. This plant has been used traditionally in coastal communities of Indonesia to treat of wounds, diarrhea, and fever deseases (Prabhu & Guruvayoorappan, 2012). In previous study, phytochemical investigation *Sonneratia alba* has been reported contained triterpenoid, steroid, and flavonoid compounds (Kumar et al. 2011; Musa et al. 2018).

The trend of eating practical and instant foods like fast food and preserved food has developed rapidly in the community. The types of this food can harm the human bodies, because it contains saturated fatty acids and high cholesterol. Cholesterol is an essential compound for the body to synthesize important substances, such as cell membranes, and insulating materials for nerve cells, as well as sex hormones and kidney children. Based on WHO data (2011) cardiovascular disease is the biggest cause of death in worldwide. Of the 57 million deaths of the world population, 17.3 million (30%) deaths are caused by cardiovascular diseases, especially heart attacks (7.3 million) and strokes (6.2 million). It is estimated that in 2030 that 23.6 million people worldwide will die of cardiovascular disease.

The treatment that has been done to reduce cholesterol levels is by using synthetic drugs. Synthetic drugs have various disadvantages, among others, expensive prices, side effects caused, and discomfort in treatment. According to

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this reason, searching drug compounds from nature are still needed to do (Masek et al. 2017). Mangrove plants are a very important biological resource in Southeast Sulawesi because they support the lives of endemic animals such as Anoa (*Buballus sp*) and Sulawesi Eagle and contain antioxidants and nutrients that are potential to be utilized for human welfare (Septiana et al. 2016).

Mangroves grow in extreme environments because of their ability to produce secondary metabolites and act as antioxidants to adapt to various extreme environmental factors on the beach. Analudin, (2018) reported that mangrove leaf tea extract (*Sonneratia alba*) was able to reduce cholesterol levels in mice in the range of 33.33 to 53.67%. Triterpenoid compounds are known to have various activities such as anticancer, antitumor, antibacterial, anti-inflammatory, and anti-cholesterol. Based on the background above, it is necessary to isolate the triterpenoid compound from the leaves of mangrove plant and anticolesterol activity test of the isolated compound.

Materials and Methods

Material and instrumentation

The research specimen is mangrove leaves collected from Dulupi vilage, Boalemo district, Gorontalo province, Indonesia in march 2017. The chemicals used in this research were ethyl acetate, *n*-hexane, methanol, distilled water, silica gel G60 (70-320 mesh), thin layer chromatography (TLC), silica plate, octadecylsilane (ODS) RP- 18, 10% H2SO4 in ethanol, alcohol 70%, and cholesterol salt. Instrument were used erlenmeyer, micro tubes, micro pipets, spectrometer uv-vis, IR, 1-D and 2-D NMR and evaporator.

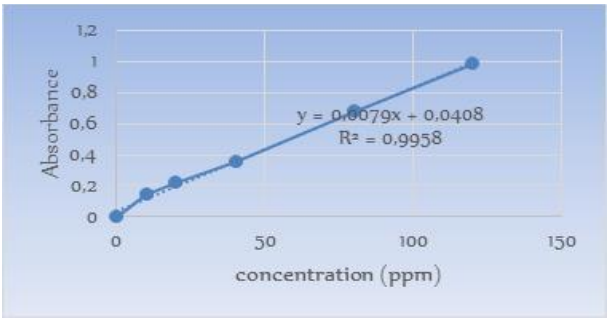

Fig 1. Calibration curva of anti-cholesterol measurement

Extraction and purifycation compound

Dried mangrove leaves (1.5 kg) were soaked in 5 L methanol solvent for 2 days. The shole mixture was then filtered through filter paper and the filtrate was then evaporated under reduce pressure at 55 °C using a Buchi rotary evaporator.

The extracts were filtered through a cotton plug followed by Whatman filter paper and then concentrated by using a rotary vacum evaporator to provide of methanol crude extract. The methanol extract (10 g) was subjected to liquid chromatography over silica gel using a gradient elution mixture of n-hexane-EtOAc (10:0-0:10) as an eluting solvent, yielding 7 fractions (A–G). Fraction C (0.15 g) was subjected to column chromatography over silica gel using a mixture of *n*-hexane: EtOAc (9:1) as an eluting solvent, affording 30 fractions (E01– E30) and give pure isolated. The purification results of these compounds were determined by TLC on silica gel and ODS with several solvent systems and showed a single spot.

Cholesterol determination

Reductions of cholesterol levels of isolated compound were determined by comparison with the blank absorbance (cholesterol solution). The stock cholesterol solution was made by mixing cholesterol salt (100 mg) in 100 mL ethanol (1000 ppm) at 45 °C in water bath. To 0.025, 0.05, 0.1, 0.2, 0.3, and 0.4 mL of cholesterol solution in a cuvette (BRAN® UV cuvette) were added acetic anhydride (2 mL) and H_2SO_4 (0.1 mL) and diluted with ethanol to a final volume of 5 mL, respectively (blank solutions). To 0.5 mL of cholesterol solutions were added 0.025, 0.05, 0.1, 0.2, 0.3, and 0.4 mL of isolated compound (1000 ppm), acetic anhydride (2 mL) and H_2SO_4 (0.1 mL) and diluted with ethanol to a final volume of 5 mL, respectively. After being incubated in the dark for 15 mins at room temperature, the absorbance of the solutions were measured at 423 nm (Ochani & D'Mello, 2009; Bachmid et al. 2015). Experiments were carried out independently three times and averages are presented. Statistical analysis was performed by unpaired two-tailed t test (Excel). Differences were considered significant at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Test for triterpenoid with Liebermann-Burchard reaction

A few crystals of compound 1 and 2 were dissolve in chloroform and a few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of anhydride acetid. In this case isolated compound turned to violet blue and finally formed green color which indicates the presence of triterpenoid (Shanmugapriya et al. 2012; Situmeang et al. 2016; Febrina et al. 2017; Simorangkir et al. 2017).

Results

The leaves of *S. alba* was dried and successively extracted with methanol 96%. Therefore, the subsequent phytochemical analysis was focused on the methanol extract, which was chromatographed over a coloumn packed with silica gel G60 with gradient elution. The fractions were repeatedly subjected to normal-phase and reverse-phase column chromatography, yielding one triterpenoid pentacyclic. The compound (20 mg), appeared as white needles.

The results of the phytochemical screening using Liebermann-Burchard reagent showed that the content of pure isolates was thought to be a triterpenoid compound with reddish discoloration. The IR result (KBr) shown in Table 1. Anti-cholesterol activity test result shown in Table 2. Calibration curva of anti-cholesterol measurement shown in Fig 1.

Isolated compound: white needles. ^{1}H-NMR (500 MHz, CDCl3) δ: 2.22 (2H, m, H1), 1.65 (2H, m, H2), 3.15 (1H, dd, J 15.0, 8.4 Hz, H3), 0.70 (1H, d, H5), 1.42 (2H, m, H6), 1.44 (2H, m, H7), 1.07 (1H, H9), 140 (2H, m, H11), 1.41 (2H, m, H12), 0.75 (1H, s, H13), 1.20 (2H, m, H15), 1.39 (2H, m, H16), 0.96 (1H, d, H18), 2.23 (1H, d, H19), 2.25 (2H, m, H21), 2.22 (2H, m, H22), 0.94 (3H, s, H23), 0.96 (3H, s, H24), 0.85 (3H, s, H25), 0.75 (3H, s, H26), 1.00 (3H, s, H27), 1.59 (3H, s, H28), 4.58 & 4.60 (2H, s, H29), 1.69 (3H, s, H30). 13CNMR (125 MHz, CDCl3) δ: 39.7 (CH2, C1), 28.1 (CH2, C2), 79.7 (CH, C3), 40.1 (Cq, C4), (CH, C5), 19.6 (CH2, C6), 35.7 (CH2, C7), 43.3 (Cq, C8), 56.9 (CH, C9), 38.4 (Cq, C-10), 26.9 (CH2, C11), 28.8 (CH2, C12), 40.2 (CH, C13), 48.6 (Cq, C14), 30.9 (CH2, C15), 38.3 (CH2, C16), 49.2 (Cq, C17), 52.1 (CH, C18), 50.5 (CH, C19), 152.2 (Cq, C20), 35.5 (CH2, C21), 42.2 (CH2, C22), 31.8 (CH3, C23), 16.2 (CH3, C24), 16.9 (CH3, C25), 16.7 (CH3, C26), 15.2 (CH3, C27), 19.5 (CH3, C28), 110.2 (CH2, C29), 22.2 (CH3, C30).

Table 1
IR spectrum (KBr)

Wavelength (cm^{-1})	Signal	Fuction Group
3590	strenght	OH-
2970	strenght	C-H Sp^3
1687	weak	C=C
1236	strenght	C-O
strenght	medium	C-H

Table 2
Decrease cholesterol Levels in Isolates compound

concentration (ppm)	Absorbance	cholesterol	decrease (%)
5	0,722	86,23	13,7
10	0,598	70,53	29,4
20	0,443	50,91	49,0
40	0,356	39,90	60,1
60	0,276	29,77	70,2
80	0,222	22,94	77,0
control (-)	0,83	99,90	0,10

Discussion

The IR spectrum (KBr) of isolated showed characteristic absorption frequencies at 3590 and 1236 cm^{-1}. This spectrum indicated of the O-H and C-O bond vibrations; the absorption observed at 897cm^{-1} was due to an unsaturated out of plane C-H vibration (Souza et al. 2011; Situmeang et al. 2018). The C=C vibrations was shown around 1687cm^{-1} as weakly intense band; stretching and bending vibrations due to methyl groups were represented by the bands at 2935cm^{-1} and 1462cm^{-1} and the signal at 1385cm^{-1} was due to methylenic vibration (Situmeang et al. 2018).

The ^{1}H-NMR spectrum showed revealed the presence of seven singlet methyl protons at δ_H 0.75, 0.85, 0.94, 0.96, 1.00, 1.59 and 1.69 ppm (integrated for 3H). A sextet of one proton at δ 2.23 ppm ascribable to 19β -H is characteristic of lupeol. The H-3 proton showed a multiplet at δ 3.15 ppm while a pair of broad singlets at δ_H 4.58

and δ_H 4.60 (1H, each) was indicative of olefinic protons at (H-29). The methylene proton Sp^3 showed at δ_H 1.20, 1.39, 1.40, 1.41, 1.42, 1.44, 1.65, 2.22, and 2.25 ppm. These assignments are in good agreement for the structure of Lup-20(29)-en-3β-ol) compound (Silva et al. 2012; Prakash & Prakash, 2012).

The ^{13}C NMR spectrum showed which seven methyl groups at [δc: 31.8 (C-23), 19.5 (C-28), 16.8 (C-25), 16.7 (C-26), 16.2 (C-24), 15.2 (C-27) and 22.2 (C-30)]; the signals due to an exomethylene group at [δc: 110.2 (C-29) and 152.0 (C- 20)]; ten methylene, five methine and five quaternary carbons were assigned with the aid of DEPT 135° spectrum (Waliullah et al. 2011). The deshielded singnal at δc 79.0 was due to C-3 with a hydroxyl group attached to it (Li et al. 2018). The forgoing spectral analysis and comparison with reported data, led us to propose the structure of isolated compund as a pentacylic triterpenoid (Fig **2**).

The confirmation of the structure of isolated was accomplished through the 2D-NMR experiments (COSY and HMBC). The ^1H-^1H COSY spectrum of isolated compound exhibited some cross peaks such as between δ_H 2.23, H-19 and one Sp^3 methylene proton signal (δ_H 2.25, H-21) and another Sp^3 methine proton signal (δ_H 0.96, H-18); and between oxygenated methine proton signal (δ_H 1.69, H- 30 and Sp^3 methylene signal (δ_H 1.65, H-2)[16–19].

In the HMBC spectrum, the methine proton signal at δ_H 3.15 (H-3) showed cross peaks with a methyl carbon signal (δc 31.8, C-23) by *J*2 correlation and a methyl carbon signal (δc 19.6, C-6) by *J*3 correlation. The sextet methyl signal at δH 2.23 (H-19) showed cross peaks with two methylene carbon signals δc 35.5 (C-21) and δc 110.2 (C-29)], a methine carbon signal [δc 52.1 (C-18), a methyl carbon signal [δc 22.2 (C-30)] and a quaternary carbon signal [δc 152.2 (C-20)]. HMBC spectrum of isolate compound shown in Fig 3.

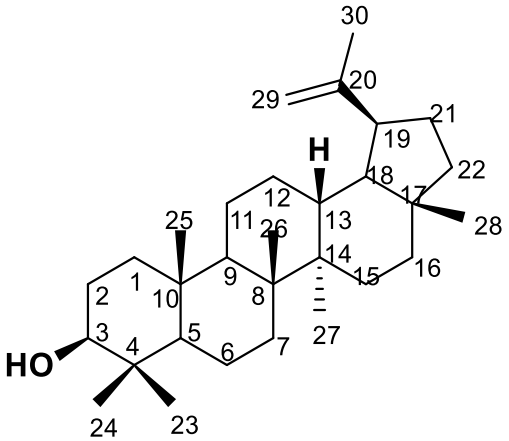

Fig 2. structure of Lup-20(29)-en-3β-ol) (Silva et al. 2012).

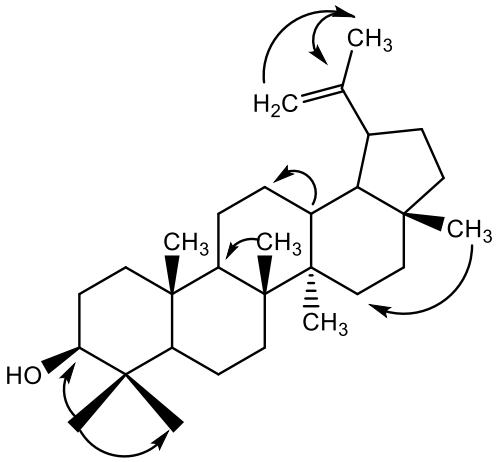

Fig 3. HMBC correlation of isolate compound (Prakash & Prakash, 2012).

Measured of cholesterol level reduction was carried out by UV-Vis spectrometer. Measurements begin with the search for wavelengths maximum, with the aim of knowing the wavelength that produces maximum absorbance and to determine the time of measurement of the results of the reaction. The cholesterol (standart) concentration used in this study was 100 ppm. The experiment was preceded by measuring the initial concentration of the cholesterol solution. The measurement of the initial concentration every time the measurement aims to determine the concentration of cholesterol solution quantitatively before being added with triterpenoid compound isolates. In the experiments of triterpenoid compounds isolates were measured activity against cholesterol reduction with the aim to determine whether the triterpenoid compound isolates had activity against cholesterol reduction or not. Extracts of triterpenoid compounds in the same concentration series are 5, 10, 20, 40, 60, and 80 ppm respectively. From each concentration series added 0.5 mL of triterpenoid compound isolates were then put in a test tube which had added 100 ppm cholesterol standard.

After reacting the test solution is left in a dark place protected from light for 15 minutes according to the time taken, left in a dark place protected from light because the solution of cholesterol is photodegradation is not stable to light and will turn into cholesterol (Anggraini & Ali, 2017). The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 423 nm. After the absorption of the test solution is read then the percent reduction in cholesterol is calculated by means of the initial cholesterol uptake before being added to the sample reduced by cholesterol uptake after being added to the sample solution then divided by the initial cholesterol uptake and multiplied by one hundred percent.

Based on the calculation of the percentage of decline produced, triterpenoid isolates with a concentration of 5 ppm can reduce cholesterol by 13.7%, at a concentration of 10 ppm can reduce by 29.4%, with a concentration of 20 ppm can reduce cholesterol by 40.9%, at a concentration of 40 ppm can reduce cholesterol by 60.1%, at a concentration of 60 ppm can reduce cholesterol by 70.2%, and at a concentration of 80 ppm can reduce cholesterol by 77.0 ppm. This is related to the ability of triterpenoid compounds to reduce cholesterol content. Research by Bachmid et al. (2015) reported that ethanol extract of patikan leaves containing triterpenoid compounds were able to reduce doses of 10 and 30 mg / kgBW with anti-cholesterol activity with a decrease in cholesterol levels of 12 and 71%.

Conclusion

The results of the phytochemical screening using Liebermann-Burchard reagent showed that the content of pure isolates was thought to be a triterpenoid compound with reddish discoloration. The isolates compound known as triterpenoid pentacyclic (Lup-20(29)-en-3β-ol). The results showed that triterpenoid compounds (lupeol) were able to reduce cholesterol from concentrations of 5, 10, 20, 40, 60, and 80 ppm are 13.7; 29.4; 49.0; 60,1; 70.2; and 77.0% respectively.

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