Journal of Chemical Natural Resources Vol. 03, No. 02, 2021 | 123 – 130

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Isolation of Flavonoid Compounds from Salam (*Syzygium polyantha Wight*) Leaves

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Abstract. Isolation of flavonoid compounds from Salam (*Syzygium polyantha Wight*) leaves has been done by maceration technique with methanol solvent. Methanol extract obtained was evaporated and dissolved with ethyl acetate. The solution was then concentrated and evaporated. The ethyl acetate fraction was dissolved with methanol and partitioned with n-hexane solvent. The methanol layer was evaporated and then tested with Benedict's reagent and then acidified with HCl 6% while heated. It was then extracted partition with chloroform. The chloroform layer was separated using Column Chromatography with silica gel as the stationary phase and n-hexane: ethyl acetate 90:10, 80:20, 70:30, and 60:40 v/v, respectively as the mobile phase. The pure compound was paste, brownish red, mass = 8.5 mg, and Rf=0.60. It was a positive reaction with flavonoid compound reagents. The compound was further identified by using UV-Visible, FT-IR, and ^{1}H-NMR spectroscopy. Spectroscopy data obtained indicated that the compound is a flavonoid.

Keywords: Salam Leaves, Isolation, Flavonoid

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1 Introduction

Flavonoid is one of the largest groups of natural phenols. According to estimates, approximately 2% of all carbon photosynthesized by plants is converted into flavonoids or compounds closely related to them. Flavonoid compounds are found in all parts of plants including leaves, roots, wood, bark, pollen, flowers, fruits, and seeds. Most of these flavonoids are in plants, except algae (Markham, 1988). Flavonoids contained in plants can be used as a protector of the human body from free radicals and can reduce the risk of cancer and inflammation (Nessa, 2003).

Plants containing flavonoid compounds have usually treating stroke, rheumatism, mastitis, heart pain, launching breast milk, treating constipation, ulcers, treating flu, rheumatism, and cancer properties (Santoso, 2008).

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Journal of Chemical Natural Resources Vol. 03, No. 02, 2021 | 123 – 130

One of the plants found in Indonesia that can be used as medicine is the Salam plant. The leaves of this plant are useful for treating diarrhea, diabetes, itching, and ulcers (Hariana, 2002). Salam plant is known as Gowok (Sunda), Manting (Java), Salam (Madura), Maselangan, Uba Lemongrass (Malay).

Salam plants grow wild in the forest or the mountains or are planted in the yard. The tree is lush, reaching 25 m in height, rooted in mounts, rounded trunk, and smooth surface. Single leaves, opposite, stemmed in length 0.5 to1cm. The leaf blade is oval to elliptical in shape and dark green in color. This plant spreads throughout Java and several other areas (Heyne,1987).

The chemical content of Salam leaves is 0.05% of essential oil which consist of citral, eugenols, tannins, and flavonoids (Hariana, 2002).

From literature studies, research on the content of compounds contained in the Salam leaves have been conducted by the Faculty of Pharmacy ITB in 2001. Preliminary phytochemical screening of Salam leaves found the presence of flavonoids, steroids, triterpenoids, and tannins compounds. The chemical content of water extract of Salam leaf has been studied guided by an anti-inflammatory test on rat legs, the water extract is dried and dissolved with ethanol, the soluble fraction of ethanol is suspected of containing steroidal compounds/triterpenoids and the insoluble fraction of ethanol is suspected of containing the flavonoids (Sugarlini, 2001). The previous researchers have also tested the effect of anti-oxidants ethanol extract of Salam leaves in white rat blood serum induced by CCl_4 (Rony, 2008), and Djoko Wahyono from the Faculty of Pharmacy UGM has tested the hypoglycemic activity of ethanolic extract 30% and 70% of Salam leaf and its effect on parasympathetic stimulation in male rabbits loaded with glucose, the content of compounds identified by thin layer chromatography in the extract of Salam leaf is a flavonoid, using silica gel GF-254 and a mixture of solvent butanol, acetic acid, and water with a ratio of 4: 1: 5 and using glycoside flavonoid rutin as a comparison, from observations with UV light at length a wavelength of 254 nm seen that ethanolic patches of Salam leaf comparable with flavonoid rutin as standard, this indicates that the ethanolic extract of Salam leaf contains flavonoid compounds (Wahyono, 2008).

So far, studies on the isolation of flavonoid compounds from Salam leaves have not been reported in scientific publications.

The preliminary test that the researchers did, namely by phytochemical screening test with concentrated H_2SO_4, $FeCl_3$ 5%, NaOH 10%, and Mg-HCl reagent showed that methanol extract of Salam leaves contains the flavonoid compounds.

Journal of Chemical Natural Resources Vol. 03, No. 02, 2021 | 123 – 130

Based on the description above, the authors are interested to conduct research on the Salam plant leaves, especially regarding the flavonoid compounds contained in it.

2 Materials and Methods

2.1 Equipments

In this study, equipments used were: measuring cup, beaker glass, Erlenmeyer glass, funnel, separating funnel, extractor, chromatographic column, test tube, drip plate, rotary evaporator Büchi R-114, bottom flask, stative and clamps, UV lamp, spatula, stirring rod, analytical balance, dropper pipette, water bath, vial, thin layer chromatography (TLC) vessel, ultraviolet-visible (UV-Vis) spectrophotometry, fourier transform infrared (FT-IR) spectrophotometry, proton nuclear magnetic resonance (^{1}H-NMR) spectrophotometry, Whatman Filter Paper, TLC Plate, Preparative TLC Vessel

2.2 Materials

The main materials used were Salam leaves, methanol (Me-OH), n-hexane, ethyl acetate (EtOAc), Aquadest, Silica gel 60 (0.063-0.200), $FeCl_3$ 5%, NaOH 10%, Mg-HCl, concentrated H_2SO_4, HCl 6%, chloroform, cotton, silica gel, TLC plate 60 F254, Benedict's reagent.

2.3 Preparation of Salam Leaves

Salam leaves were obtained from Kabanjulu village, DairI regency, North Sumatra. The Salam leaves were dried in the open air, then mashed until 1450 g of. Salam plant leaves were obtained as much as 1450 g.

2.4 Preliminary Test of Salam Plant Leaves Extract

2.4.1 Phytochemical Screening

As much as 10 g of Salam leaves that have been dried, then cut into small pieces and put into Erlenmeyer, then added as ±100 mL of methanol and mixed. The methanol extract was divided into four test tubes, and added each reagent:

The tube I: with $FeCl3_5$% produced a black solution

Tube II: with $H_2SO_{4(p)}$ produced a yellowish orange solution

Tube III: with Mg-HCl produced a light pink solution

tube: with NaOH10% produced a violet-blue solution

Journal of Chemical Natural Resources Vol. 03, No. 02, 2021 | 123 – 130

2.4.2 Thin Layer Chromatography (TLC) Analysis

TLC analysis was performed on chloroform extract using silica gel $60F_{254}$ (Merck) stationary phase. This analysis was intended to search for suitable solvents for Column Chromatography.The mobile phase used was a mixture of n-hexane:ethyl acetate with a ratio of 90:10 ; 80:20 ; 70:30 ; 60:40 (v/v), respectively.

As much as 10 mL of mobile phase solution, n-hexane: ethyl acetate (90:10 (v/v)) was introduced into the chromatographic vessel, then saturated. Next, dripped the chloroform concentrated extract on the activated TLC plate. The plate was inserted into a vessel that contains a saturated solvent, then closed and eluted. The eluted plate was removed from the vessel, then dried and fixed with $FeCl_3$ 5% reagent. The color spots arising were observed and calculated the Rf value obtained. The same treatment was carried out for the ratio of solvent n-hexane: ethyl acetate was 80:20; 70:30; 60:40 (v/v), respectively.

The TLC analysis results showed that Salam leaves contain flavonoid compounds. A good separation result was given by the mobile phase n-hexane: ethyl acetate of 60:40 (v/v).

2.5 Salam Leaves Extraction

As much as 1450 g of Salam leaves were macerated with 5 L methanol until all samples were soaked and left for 48 hours. The results maceration was accommodated and concentrated using a rotary evaporator to obtain a concentrated methanol extract. Then, the methanol extract was evaporated until all the methanol solvent has evaporated. Next, the methanol fraction was dissolved with ethyl acetate to block the tannin compound and filtered. The filtrate was then evaporated by the rotary evaporator until the solvent evaporated. Then, the ethyl acetate fraction was dissolved with methanol and partitioned repeatedly with n-hexane until the n-hexane layer was clear. The methanol layer was separated from the n-hexane layer, then re-concentrated with a rotary evaporator and evaporated so that a concentrated extract of the methanol layer was obtained, then a sugar content test with Benedict reagent was then hydrolyzed using HCl 6% while heating for 45 minutes, cooled and filtered, then extracted partition with chloroform for 3 times. After that, evaporated to obtain a concentrated extract of chloroform was as much as 532 mg.

2.6 Isolation of Flavonoid Compounds by Column Chromatography

Isolation of flavonoid compounds by column chromatography was performed on chloroform concentrated extract that has been obtained. The stationary phase used was silica gel 60 (0.063-0.200) and the mobile phase used was n-hexane 100%, a mixture of solvent n-hexane: ethyl acetate with a ratio of 90:10, 80:20, 70:30, and 60:40 (v/v), respectively.

The isolation stage was done by stringing the chromatographic column apparatus. Firstly, silica gel 60 (0.063-0.200) was fertilized using n-hexane, stirred until homogeneous, and then inserted into the chromatographic column. Then eluted by using n-hexane 100% to solid and homogeneous silica gel. As much as 532 mg of chloroform extract of Salam leaves was put into the chromatographic column containing silica gel slurry, then added the mobile phase n-hexane: ethyl acetate 90:10 slowly, and arranged so that the phase flow out of the column was as much as the addition of the mobile phase from above. The polarity was enhanced constancy by adding the mobile phase n-hexane: ethyl acetate in the ratio of 80:20, 70:30, and 60:40 (v/v), respectively. The results obtained were accommodated in vials every 5 mL, then in KLT and combined fractions with the same Rf value, and then tested with $FeCl_3$ 5%. Then it was evaporated until a paste formed.

2.7 Purification (Recrystallization)

The paste obtained was re-dissolved with ethyl acetate, while stirring until all the paste was completely dissolved. Then, added slowly n-hexane until the deposition of impurities at the bottom of the container. The upper layer solution was decanted and evaporated the remaining solvent from the paste was until the paste obtained was completely free of solvents.

2.8 Purity Test of Isolation Results with Thin Layer Chromatography (TLC)

The paste purity test was done by TLC using silica gel 60 F_{254} as the stationary phase and the mobile phase was n-hexane: ethyl acetate of 60:40 (v/v). As much as 10 mL of mobile phase solution was inserted into a chromatographic vessel, then saturated. The paste previously dissolved with ethyl acetate was dripped on the TLC plate. The KLT plate was placed into the chromatographic vessel that has been saturated. After the mobile phase solvent seeped up to the marked line, the TLC plate was removed from the vessel, dried, and fixed using $FeCl_3$ 5% reagent in methanol to produce black spotting which indicated the presence of flavonoid compounds.

2.9 Identification of Isolated Compound Result

The isolated compound results were characterized by using UV-Vis, FT-IR, and $_1$H-NMR spectrophotometry.

Journal of Chemical Natural Resources Vol. 03, No. 02, 2021 | 123 – 130

3 RESULT AND DISCUSSION

The isolation results of flavonoid compound from Salam (*Syzygium polyantha Wight*) were obtained using mobile phase n-hexane: ethyl acetate of 60:40 (v/v) showed that it was positive for flavonoid reagent.

The UV-Vis test result indicated in figure 1 shows that the maximum wavelength at 215 nm and 276 nm, which demonstrated the UV-Vis spectrum of flavonoid compound:

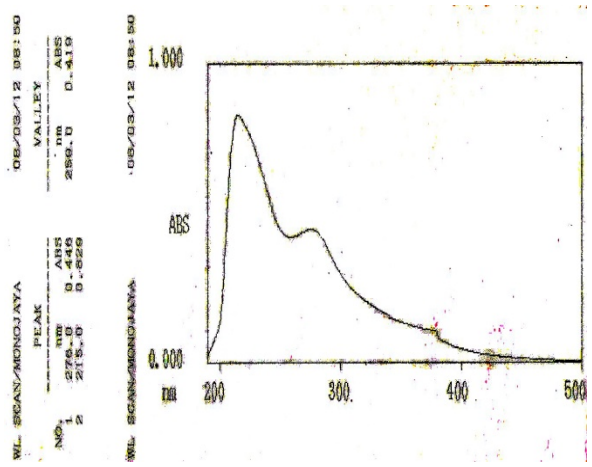
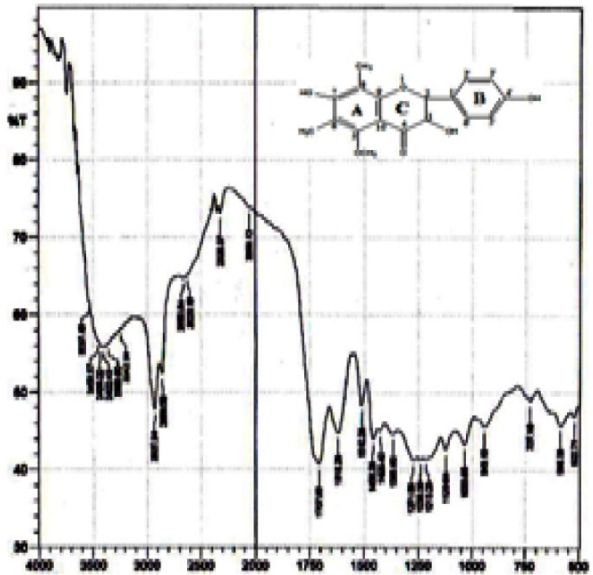


Figure 1 UV-Vis spectrum of an isolated compound

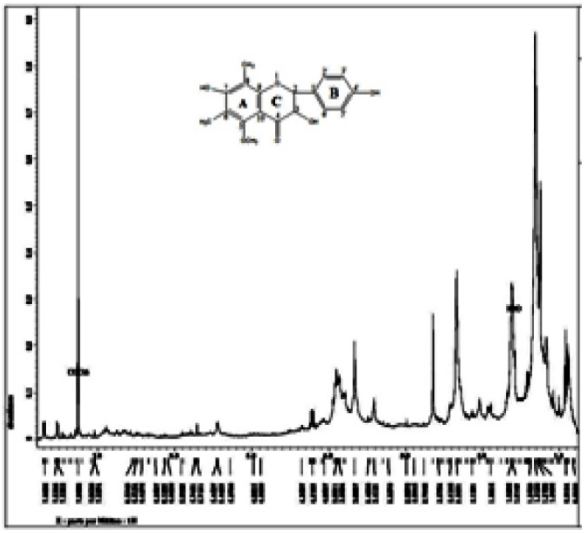
In addition, the FT-IR test result presented in figure 2 shows that the band at 3537.45 to 3242.34 cm^{-1}, which assigned the presence of OH groups, and the band at 2927.94 to 2856.94 cm^{-1}, which showed the aliphatic –CH bonds, the band at, 1707 cm^{-1}, which indicated C=O bonds, the band ar 1618.28 to 1425.40 cm^{-1}, which assigned the presence of C=C of the aromatic system, the band at 1356.60 cm^{-1}, which demonstrated CH_3 bonds, the band at 1271.09 cm^{-1}, which showed the alcohol C-O bonds, the band at 1213.23 cm^{-1}, which indicated the presence of -C-C



(O)-C of ketone, the band at 1120.64 cm^{-1}, which asymmetric C-O-C bonds, the band at 1033.85 cm^{-1}, which presented the symmetric C-O-C bonds, the band at 943.19 cm^{-1}, which assigned the aromatic C-H bonds. (Donald. L.Pavia, 1979).

Figure 2. FT-IR spectrum of an isolated compound

The ^{1}H-NMR test results displayed in figure 3 show that the chemical shift in the region of δ = 3.6637 ppm with the peak of the singlet, which indicated the proton of-OCH_3 at C5, the chemical shift in the region of δ = 2.382 ppm with the peak of the singlet, which assigned the proton of-CH_3 at C8, the chemical shift in the region of δ = 2.6442 ppm with the peak of the singlet, which indicated the proton of CH_3 at C6, the chemical shift in the region of δ = 4.3537 ppm with the peak of the doublet, which showed the proton of H3, the chemical shift in the region of δ = 5.4433-5.4537 ppm with the peak of the doublet, which indicated the proton of H2, the chemical shift in the region of δ = 7.5303-7.5368 ppm with the peak of the doublet, which presented the protons of –C-CH=CH-C from H3'-H5', the chemical shift in the region of δ = 7.6938-7.7055 ppm with the peak of the doublet, which presented the protons –C-CH-CH-C



from H2'-H6' and the chemical shift in the region of δ = 2.9779 ppm with the peak of the singlet, which demonstrated the proton of OH at C3 and also the chemical shift in the region of δ = 5.7131 ppm with the peak of the singlet, which indicated the proton of OH at C4' and C7'.

Figure 3. ^{1}H-NMR chromatogram of an isolated compound

Based on the test results obtained above, Salam (*Syzygium polyantha Wight*) leaves contain the flavonoid compound which assumed that the compound was dihydroflavonol as shown in figure 4..

Journal of Chemical Natural Resources Vol. 03, No. 02, 2021 | 123 – 130

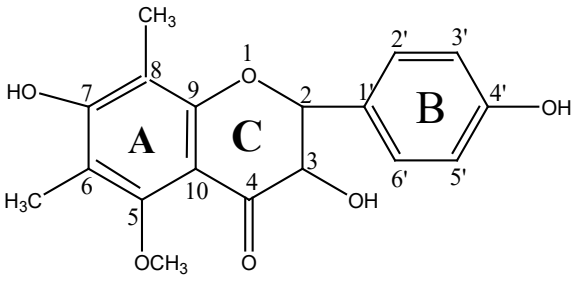


Figure 4. Dihydroflavonol

4 Conclusion

In conclusion, the isolation results obtained from 1,450 g of Salam (*Syzygium polyantha Wight*) plant leaves were 8.5 mg, and the Rf value = 0.60 was a brownish red showing that the positive result on the flavonoid reagent. It was also supported by UV-Vis, FT-IR, and ^{1}H-NMR spectrophotometry to show that the isolated compound from Salam leaves was a flavonoid compound from dihydroflavonol group.

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