Journal of Chemical Natural Resources Vol. 02, No. 02 ,2020 | 132 – 140

JCNaR
Journal of Chemical Natural Resources

Isolation of Flavonoid Compounds from Guava Plant Leaves (*Syzygium malaccense (L.) Merr. & Perry*)

Satriani Marito Lubis[1], Phillippus Siregar[2], and Sovia Lenny[3*]

[1,2,3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jalan Bioteknologi No.1 Kampus USU Medan 20155, Indonesia

Abstract. Isolation of flavonoid compound from the leaves of guava (*Syzygium malaccense (L.) Merr. & Perry*) has been done using the extraction method. Extract guava leaves separated by using the Column Chromatography method with silica gel as the stationary phase and n-hexane: ethyl acetate (60:40) v/v as the mobile phase. The result of the isolation compound was a yellow crystal with a mass of 39 mg from fractions 325-435, a melting point of 157-159^{o}C, and an Rf of 0.35. The compound was further identified analysis by spectroscopy analysis using Ultraviolet-Visible (UV-Vis), Fourier Transform Infra-Red Spectroscopy (FT-IR), and Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR). The spectroscopy data of the isolated compound indicated that the compound is a flavonoid named flavonol.

Keywords: Leaves of Guava, Isolation, Flavonol, Flavonoid

Received [18 May 2020] | Revised [16 June 2020] | Accepted [27 July 2020]

1 Introduction

Flavonoids are compounds containing C15 consisting of two phenolic nuclei linked by three carbon units (Sastrohamidjojo, 1996). More than 4000 types of flavonoids have been identified, and some of them play a role in the coloring of flowers, fruits, and leaves. Various edible plants and fruits contain many flavonoids, where higher concentrations are in the leaves and peels than in the deeper tissues. Flavonoids can act as antioxidants, inhibit the development of cardiovascular disease, inhibit tumor cell attacks, as well as anti-cancer, and others (Winarsi, H. 2005).

Syzygium malaccense belongs to the Myrtaceae family. This type of plant has a dual function, namely as an ornamental plant or a shade plant as well as a fruit plant and medicinal plant. Guava is a tree plant; its height can reach approximately 6-15 m and can grow well in open or slightly protected places from the sun, both in the lowlands and in the highlands, namely at an

*Corresponding author at: Department of Chemistry, Faculty of Mathematics and Natural Sciences Universitas Sumatera Utara, Medan, Indonesia.

E-mail address: sofia1@usu.ac.id

Journal Homepage: http://jcnar.usu.ac.id

altitude of 1 – 1,200 m above sea level (Suryowinoto, 1997). Guava or guava dersana is an annual fruit plant originating from the Indo-China region, Malaysia, the Philippines, and Indonesia. Other literature concludes that guava originated in Malaysia. In Indonesia, the distribution of guava is concentrated on the island of Java (Prihatman, 2000). This tree gives one of the best types of guava. More than others, this fruit has a shape like an apple. They are pink with black stripes. Guava is planted everywhere and is most fruitful in moist soil (Kloppenburg, 1988).

This plant from a medical point of view can be used as medicine, such as the leaves can be used to cure oral infections and bronchitis, the bark is used to cure throat infections, coughs, and stomach ailments, and the root bark can be used to cure dysentery, a decoction of fruit leaves or the seeds can treat fever. In Brazil, various parts of this plant are used for constipation, diabetes, coughs, headaches, and other ailments (Whistler et al., 2006). Steeping from the bark can be used as a sore throat remedy, and the leaves have been widely used as traditional medicine. The fruit can be consumed when it is still raw, ripe, or preserved (Little, EL et al., 1989). Various parts of this plant are used in traditional medicine, and some of them have been shown to have antibacterial properties. Each part of the stem, leaf, and root of the guava plant is used to treat various types of different diseases in several countries, including countries outside Asia (Orwa et al, 2009).

The preliminary test that the researchers did, namely the phytochemical screening test with reagent H_2SO_4(p), 5% $FeCl_3$, 10% NaOH, and Mg-HCl, showed that the methanol extract of guava leaves contains flavonoid compounds. From the description above and based on the literature regarding the chemical found in the guava plant, the researchers are interested in researching the leaves of the guava plant, especially regarding the flavonoid compounds contained in it.

2 Materials and Methods

2.1 Equipments

The tools used in this study were: distillation apparatus, measuring cup, beaker, Erlenmeyer, glass funnel, separating funnel, extractor, chromatography column, capillary tube, test tube, rotary evaporator Buchi B-480, bottom flask, UV lamp, spatula, analytical balance, dropper, water bath, vial, TLC vessel, UV-Visible spectrophotometer, IR spectrophotometer and ^{1}H-NMR spectrometer, melting point apparatus.

2.2 Materials

The materials used were guava plant leaves, methanol, n-hexane, ethyl acetate, chloroform, aquadest, silica gel 60 G, $FeCl_3$ 5%, NaOH 10%, Mg powder, $HCl_{(P)}$, $H_2SO_{4(P)}$, HCl 6 %,

Journal of Chemical Natural Resources Vol. 02, No. 02 ,2020 | 132 – 140

Benedict's reagent, Whatman filter paper no.42, Silica gel TLC plate 60 F254, and aluminum foil.

2.3 Preparation of the sample

The sample was the leaves of the guava plant which were obtained from the campus area of the University of North Sumatra, Padang Bulan, Medan. The leaves of the guava plant were dried in the open air without being exposed to direct sunlight, then crushed to obtain 1030 g of guava leaf powder.

2.4 Preliminary test on the guava leaf extract

The leaf powder of the guava plant is identified by using the following phytochemical screening method. To determine the presence of flavonoid compounds in the leaves of the guava plant, a preliminary qualitative test was carried out first, where 10 grams of dried guava leaf powder was put into an Erlenmeyer and soaked in ± 100 ml of methanol, then allowed to stand for 1 night and then filtered. and the extract was divided into 4 test tubes to add reagents: a) Tube I: with 5% FeCl3 produces a black solution; b)Tube II: with Mg powder, and HCl(p) produces a pink solution; c) Tube III: with 10% NaOH produces a dark green solution; d) Tube IV: with H_2SO_4(p) produces a yellowish orange.

2.5 Extraction of Guava Leaf Plants

The leaf powder of the guava plant was weighed as much as 1030 g, then macerated with ± 3 L of methanol until all samples were submerged and left for ± 48 hours, repeated four times. The macerate was collected and concentrated using a rotary evaporator to obtain a concentrated methanol extract. Then evaporated until all the methanol solvent evaporates. Then the tannin was blocked by dissolving the methanol fraction with ethyl acetate and filtered, and this process was carried out continuously until the ethyl acetate was quite clear. The filtrate is then rotated on an evaporator and then evaporated until all the ethyl acetate solvent has evaporated. Then the ethyl acetate fraction was dissolved with methanol and partitioned with n-hexane, then the methanol layer was separated from the n-hexane layer. This partitioning process is repeated until the n-hexane layer resulting from the partitioning process is quite clear. After the n-hexane layer produced was quite clear, the methanol layer was concentrated again with a rotary evaporator and then evaporated to obtain a concentrated extract of the methanol layer. The methanol fraction was then hydrolyzed by adding 6% HCl while stirring and heated for ± 45 minutes. Then cooled and filtered and the filtrate obtained was partitioned with chloroform repeatedly extracted ± 3 times. The chloroform layer obtained was concentrated again to obtain a concentrated chloroform extract of 1.24 g. The methanol layer was concentrated again with a rotary evaporator and then evaporated to obtain a concentrated extract of the methanol layer. The methanol fraction was then hydrolyzed by adding 6% HCl while stirring and heated for ± 45 minutes. Then cooled and filtered and the filtrate obtained was partitioned with chloroform

Journal of Chemical Natural Resources Vol. 02, No. 02 ,2020 | 132 – 140

repeatedly extracted ± 3 times. The chloroform layer obtained was concentrated again to obtain a concentrated chloroform extract of 1.24 g. The methanol layer was concentrated again with a rotary evaporator and then evaporated to obtain a concentrated extract of the methanol layer. The methanol fraction was then hydrolyzed by adding 6% HCl while stirring and heated for ± 45 minutes. Then cooled and filtered and the filtrate obtained was partitioned with chloroform repeatedly extracted ± three times. The chloroform layer obtained was concentrated again to obtain a concentrated chloroform extract of 1.24 g.

2.6 Thin Layer Chromatographic Analysis

Thin layer chromatographic analysis was carried out by inserting 10 ml of n-hexane: ethyl acetate (90:10) v/v mobile phase solution into the chromatography vessel, then saturated. The concentrated extract of chloroform was put on the activated TLC plate. The plate is put into a vessel that already contains a saturated solvent, then closed and eluted until the solvent reaches a predetermined limit. The eluted plate was removed from the vessel, then dried and fixed with a 5% FeCl3 reagent. Observe the color of the spots that arise and calculate the Rf value obtained. The same treatment was carried out for the solvent ratio of n-hexane: ethyl acetate in the ratio (80:20 v/v; 70:30 v/v; 60:40 v/v; 50:50 v/v). From the results of the TLC analysis, it was shown that the leaves of the guava plant contained flavonoid compounds. Good separation results were given to the results of the elution process of the n-hexane: ethyl acetate eluent system with a ratio of (60:40) v/v.

2.7 Isolation of Flavonoid Compounds by Column Chromatography

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

First, a chromatographic column was assembled. Then 60 g of silica gel (0.063-0.200mm) was slurried with 60 g using n-hexane, stirred until homogeneous, and then slowly inserted into the chromatography column. Then it was eluted using 100% n-hexane until the silica gel was solid and homogeneous. After the chromatographic column is ready to be assembled, 1.24 g of concentrated extract of guava leaf chloroform extract, which has been obtained in 10 g of silica gel, is slurried and put into a chromatographic column that already contains silica gel slurry, then the mobile phase is added n-hexane: ethyl acetate (90:10) v/v slowly, and set so that the flow of mobile phase out of the column is as much as the addition of mobile phase into the column. Improved polarity by adding n-hexane mobile phase: ethyl acetate in the ratio (80:20) v/v, (70:30) v/v, and (60:40) v/v. Each fraction that comes out of the column is accommodated in a vial of 5 ml. In TLC, the fractions with the same Rf value are combined, then evaporated to form crystals

Journal of Chemical Natural Resources Vol. 02, No. 02 ,2020 | 132 – 140

2.8 Purification

The crystals obtained from isolation by column chromatography were redissolved with ethyl acetate until all the crystals were completely dissolved. Then added n-hexane slowly until precipitation occurs. Then the upper solution was pipetted, then the remaining solvent was evaporated to obtain crystals that were completely free of solvent (Jacobs, 1974).

2.9 Purity Test of Isolation Results with Thin Layer Chromatography and Melting Point Determination

The crystal purity test was carried out by thin-layer chromatography using silica gel 60 F254 as the stationary phase and n-hexane: ethyl acetate (60:40) v/v as the mobile phase. Add 10 ml of the mobile phase solution into the chromatography vessel, then saturate. The isolated crystals were dissolved with ethyl acetate and then smeared on the TLC plate. The TLC plate was inserted into the saturated chromatography vessel. After the mobile phase solvent seeps up to a predetermined limit, the TLC plate is removed from the vessel, dried, and fixed using a 5% $FeCl_3$ reagent in methanol producing black spots indicating the presence of flavonoid compounds. Test the purity of the isolated crystals by determining the melting point of the crystals using the Melting Point Apparatus.

2.10 Characterization of Isolated Compound

All characterization was obtained from the Chemical Research Center Laboratory–LIPI, PUSPITEK Serpong, Tangerang. The UV-Visible and ^{1}H-NMR spectrophotometers were investigated by using methanol and acetone as a solvent, respectively. The FT-IR Spectrophotometer was prepared by using KBr.

3 Result and Discussion

3.1 The FTIR result analysis of cellulose and carboxymethyl cellulose

The phytochemical screening of methanol extract from guava plant leaves (*Syzygium malaccense (L.) Merr. & Perry*) showed that the sample was a positive result for flavonoid reagents. The isolation of flavonoid compounds from guava plant leaves was obtained using the mobile phase of n-hexane: ethyl acetate (60:40) v/v, and the compound obtained was in the form of yellow crystals, with a mass of 39 mg, a melting point of 157 -159^{o}C, and Rf of 0.35. The UV-Visible spectrophotometer analysis on the isolated crystals with methanol solvent, which gives the maximum wavelength can be seen in figure 1.

Journal of Chemical Natural Resources Vol. 02, No. 02 ,2020 | 132 – 140

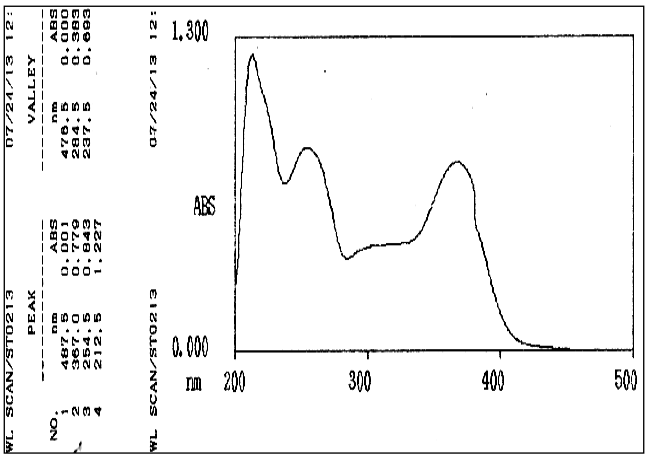

Figure 1. The UV-Visible spectrum of the isolated compounds

From the UV-Visible spectrophotometer analysis with methanol as a solvent in Figure 1, the wavelengths (λ) of band I and band II were 367.0 and 254.5 nm. The FT-IR spectrophotometer of the isolated crystal produced absorption bands in the wavenumber region can be seen in Figure 2.

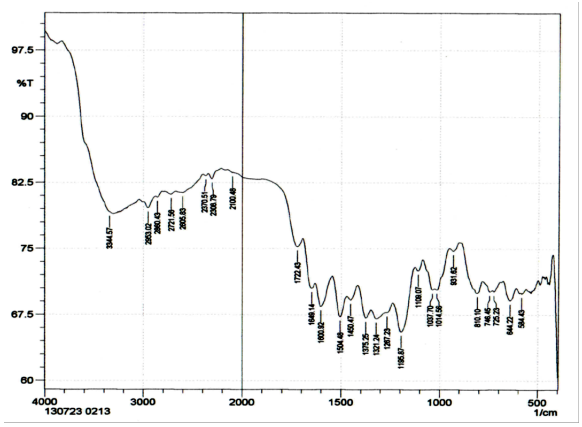

Figure 2. The FTIR spectrum of the isolated compounds

The results of the analysis of the FT-IR Spectrophotometer on the isolated crystals produce absorption bands in the wavenumber region as follows:

1. At a wavenumber of 3344.57 cm^{-1}, it shows the presence of stretching vibration –OH.

2. At a wavenumber 2953.02 - 2721.56 cm^{-1}, it shows the presence of aromatic -CH stretching vibrations.

3. At a wavenumber of 1600.92 cm^{-1}, it shows the presence of stretching vibrations of C=O from ketones.

4. A wavenumber 1504.48 - 1450.47 cm^{-1} shows the stretching vibration of C=C from the aromatic system.

Journal of Chemical Natural Resources Vol. 02, No. 02 ,2020 | 132 – 140

5. At a wavenumber of 1375.25 cm^{-1}, the medium peak indicates a buckling vibration -CH3.
6. At a wavenumber of 1267.23 cm^{-1}, it shows CO stretching vibrations from the alcohol group.
7. A wave number 1195.87 cm^{-1} shows the stretching vibration -CC(O)-C- of the ketone.
8. At a wave number 1109.07 - 1037.7 cm^{-1}, it shows that there is a non-symmetrical COC stretching vibration.
9. At a wavenumber of 1014.56 cm^{-1}, it shows a symmetrical COC stretching vibration.
10. At wave number 931.62 – 810.1 cm^{-1}, it shows the stretching vibration = CH benzene.

The Proton Core Magnetic Resonance Spectrometer (H-NMR) analysis of the isolated compounds using acetone and TMS solvents as standards that provide chemical shift signals in the region can be seen in figure 3.

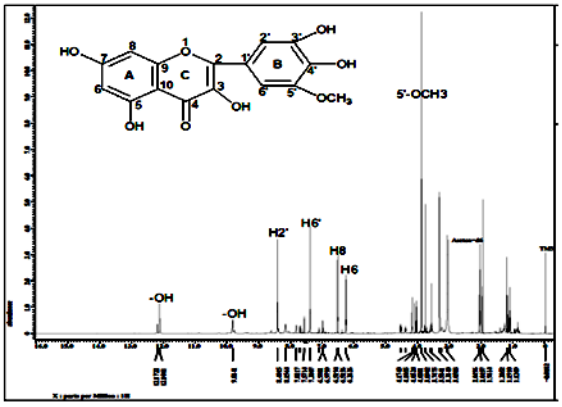

Figure 3. ^{1}H-NMR spectrum of the isolated compounds

The results of the analysis of the Proton Core Magnetic Resonance Spectroscopy (1H-NMR) isolated compounds using acetone solvent gave a chemical shift (ppm) as follows:

1. The chemical shift in the =6.2574-6.2626 ppm doublet peak indicates the H-6 proton in ring A
2. The chemical shift in the =6.5156-6.5194 ppm doublet peak indicates the H-8 proton in ring A
3. The chemical shift in the =7.3807 ppm singlet peak indicates the H-6' proton in the B ring
4. The chemical shift in the =8.4015 ppm singlet peak indicates the H-2' proton in the B ring
5. The chemical shift in the =3.8902 ppm singlet peak indicates a proton from the methoxy group –OCH_3

Journal of Chemical Natural Resources Vol. 02, No. 02 ,2020 | 132 – 140

6. The chemical shift in the =12.1061 ppm singlet peak indicates a proton from the –OH-5. group

7. The chemical shift in the =9.8141 ppm singlet peak indicates a proton from the –OH group

From the results of the above discussion, based on phytochemical screening, and the results of data analysis and interpretation carried out on the UV-Visible spectrum, FT-IR spectrum, and 1H-NMR spectrum, it can be concluded that the crystals isolated from the leaves of the guava plant (*Syzygium malaccense (L.) Merr. & Perry*) are a flavonoid compound of the Flavonol group. The structure of flavonols is suspected from isolated compounds as shown in figure 4.

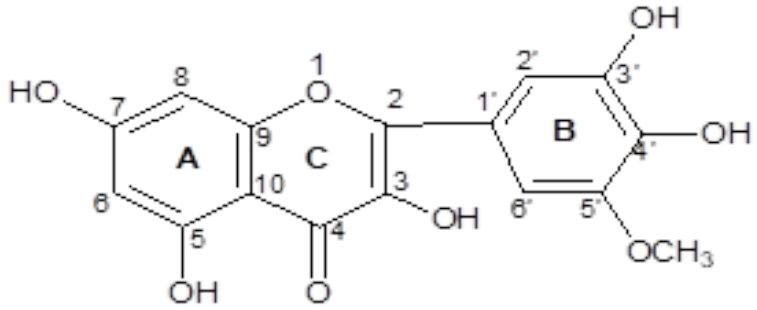

Figure 4. The structure of flavonols suspected from isolated compounds

4 Conclusion

The isolation results obtained from 1030 grams of guava leaves (*Syzygium malaccense (L.) Merr. & Perry*) were yellow crystals with a mass of 39 mg, a melting point of 157 -159^{o}C, and Rf of 0.35. Based on the results of phytochemical screening of flavonoids against crystals isolated from the leaves of the guava plant (*Syzygium malaccense (L.) Merr. & Perry*), the results showed positive results for flavonoid compounds. The results of the analysis using UV-Visible Spectrophotometry, Infrared Spectrophotometry (FT-IR) and Proton Core Magnetic Resonance Spectrometry ($_{1}$H-NMR) showed that the compound isolated from the leaves of the guava plant (*Syzygium malaccense (L.) Merr. & Perry*) was suspected to be flavonoid compounds of the flavonol group.

References

Kloppenburg, J. 1988. *Complete Instructions on Plants in Indonesia and Their Benefits as Traditional Medicines*. Volume 1. Second Printing. Yogyakarta: CD. RS. Bethesda and Andi Offset.

Markham, KR 1988. *How to Identify Flavonoids. Translation of Kosasih Padmawinata*. Bandung: ITB Press.

Orwa, C. et al. 2009. *Agroforestree Database: a tree reference and selection guide version 4.0.*

Pavia, LD 1979.*Introduction to Spectroscopy a Guide for Students of Organic Chemistry*. Philadelphia: Saunders College.

Sastrohamidjojo, H. 1996. *Synthesis of Natural Materials*. Yogyakarta: Publisher Gadjah Mada University Press.

Silverstein, RM 1981. *Spectrometric Investigation of Organic Compounds*. Translation of AJ Hatomo and Anny Viktor Purba. 4th Edition. Jakarta: Erlangga Publisher.

Supratman, U. 2010. *Elucidation of Structure of Organic Compounds*. Bandung : Widya Padjadjaran.

Suryowinoto, SM et al. 1997. *Exotic Flora, Shade Plants*. Yogyakarta: Canisius Publisher.

Whistler, WA, and Elevitch, CR 2006. *Syzygium malaccense (Malay Apple)*. USA: Permanent Agriculture Resources.