Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

JCNaR
Journal of Chemical Natural Resources

Isolation of Flavonoid Compounds from Suren Leaves (*Toona sureni*)

Iwan Freddy Sidabutar[1], Tonel Barus[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 Suren leaves (*Toona sureni*) has been done using the extraction method. The suren leaves extract was separated by using the column chromatography method. The isolated compound results were yellow paste with weight 47 from fraction 55-67 and Rf = 0.44 of eluent n-hexane: ethylacetate 60:40 (v/v). The identification process was analyzed by UV-Vis, FT-IR spectroscopy, and ^{1}H-NMR spectroscopy. The obtained result shows that the isolated compound was isoflavone.

Keywords: Suren Leaves, Isoflavone, Isolation, Flavonoid

Received [11 December 2020] | Revised [11 January 2021] | Accepted [22 February 2021]

1 Introduction

One of the potentials to be developed from plants is the utilization of their secondary metabolite compounds for medicinal ingredients (Kurz & Constable, 1998). Medicinal plants are all plants, both cultivated and uncultivated, that can be used as medicine, ranging from those visible to those that are visible under a microscope (Hamid et al, 1991). Flavonoid compounds are polyphenolic compounds that have 15 carbon atoms, consisting of two benzene rings linked together by a linear chain consisting of three carbon atoms.

Previous researchers on the *Toona species* have isolated triterpene and phenolic compounds. A total of 15 compounds, namely methyl gallate, gallic acid, kaempferol, quercetin, quercitrin, rutin, kaempferol-glucoside, catechins, epicatechin, stearic acid, palmitic acid, sitosterol, stigmasterol, β-sitosterol-glucoside and stigmasterol-glucoside (Hsieh et al, 2004) and identified from the plant T. *sinensis.* There are 7 polyphenolic compounds using UV absorbance, namely catechins, epicatechins, methyl gallate, rutin, gallic acid, quercitrin, and kaempferol, and it is also indicated that catechins and polyphenols are present in fruits, vegetables, tea, T. *sinensis,* and red wine have prophylactic properties that are very compatible with human health (Hsieh et

*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

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

al, 2006). In 2012, research was conducted on the antioxidant activity and total phenolic compounds of the T. *sinensis* leaf extract (Chen et al, 2012). Anti-neoplastic effect of gallic acid as a major compound of T. *sinensis* leaf extract, cytotoxic activity in human lung cancer cells (Chia et al., 2010).

In the health sector, the red Suren leaves are used as astringents, tonics, and drugs for chronic diarrhea, dysentery, and other intestinal diseases (Edmonds and Staniforth, 1998). Suren leaf extract is known to have an antibiotic effect and has bioactivity as an antimicrobial against *Staphylococcus* bacteria. Suren leaf shoots can also be used to treat kidney swelling. The bark, leaves, and fruits are rich in essential oil content (Yuhernita and Juniarti, 2011).

The leaves, flowers, bark, and stems of T. *sinensis* (A.Juss) are often used as a vegetable pesticide and are a natural ingredient that has the potential to be developed as anti-ovarian cancer, in this previous study the chemical compound content was studied (Sesilia et al, 2006). Suren bark is used by the community for the treatment of diarrhea and dysentery, so the activity of the ethanol extract and boiled water of Suren bark was tested against *Escherichia coli*, *Shigella dysenteriae,* and *Bacillus subtilis* bacterias (Fahwid, 2009), and the bioactivity of the ethanol extract of *Suren beureum* against shrimp larvae (Jamhari, 2009). 2011). A cholestan compound has been isolated from the chloroform extract of the bark of the plant T. *sinensis*, namely 4-methyl-kolest-24-en-3-ol, and its bioinsecticide test against A*rmyworms* (Eka and Hidajati, 2012). The leaves of the T. S*ureni* plant leaves also contain methyl gallic compounds and structure was determined by UV-Vis, FTIR, NMR, and MS spectra and tested the activity of isolated methyl gallic compounds as an antioxidant (Ekaprasada et al., 2009).

From the description above, based on the literature regarding the chemical content found in the Suren leaves and a preliminary test with phytochemical screening for the identification of natural biological compounds, especially secondary metabolites from the methanol extract of the Suren plant leaves are positive with the reagents of phenolic, flavonoid, alkaloid, terpenoid and steroid compounds. So researchers are interested to conduct research on this plant, especially, to determine the secondary metabolites and isolation of flavonoid compounds contained in this plant. The obtained isolated results were characterized using ultraviolet-visible (UV-Visible) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), spectroscopy and fourier transform infrared (FT-IR) spectroscopy.

2 Materials and Methods

2.1 Equipments

In this study, the equipments used were a graduated cylinder, separating funnel, glass funnel, extractor, chromatographic column, test tube, drip plate, rotary evaporator, bottom flask, UV

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

lamp, spatula, stirring rod, analytical balance, dropper pipette, bath water, vials, thin layer chromatography vessel, a set of FT-IR spectrophotometer, ^{1}H-NMR spectrophotometer, and UV-Visible spectrophotometer.

2.2 Materials

The main materials used were Suren plant leaves, methanol, n-hexane, ethyl acetate (EtOAc), aquadest, silica gel 60(0.063-0.200), $FeCl_3$, sodium hydroxide (NaOH), hydrochloride acid (HCl), Mg powder, potassium chloride (KI), Iodine, $HgCl_2$, bismust nitrate, nitric acid (HNO_3), acetic acid (CH_3COOH), cerium sulfate, sulfuric acid (H_2SO_4), chloroform, silica gel TLC plate 60 F 254, Benedict's reagent.

2.3 Sample Preparation

Suren plant leaf was collected, dried, and mashed. The Suren leaf was determined at the Herbanium Medanense (MEDA) Universitas Sumatera Utara scientifically as certain tree species. As much as 1400 g Suren plant leaf powder was obtained.

2.4 Phytochemical Screening of Suren Leaf (*Toona sureni*

2.4.1 Flavonoids Screening

In order to determine the presence of flavonoid compounds in the Suren plant leaves, a qualitative preliminary test was carried out. As much as 10 g of Suren plant leaf fine powder was extracted by maceration with methanol and then filtered. The filtrate was tested using some reagents, such as (1) filtrate was added with 3 drops of $FeCl_3$ 5% reagent solution, a black colloid was formed (Harborne, 1987), (2) filtrate was added with 0.1 g of Mg powder and 1 mL of HCl 2N to form a pink, orange, red-purple solution (Cannell, 1998), (3) filtrate was added with 3 drops of NaOH 10% reagent solution to form a blue-violet solution (Tobing, 1989), (4) filtrate was added with 3 drops of concentrated H_2SO_4 reagent solution, a yellowish orange solution was formed (Cannell, 1998).

2.4.2 Alkaloids Screening

In order to determine the presence of alkaloid compounds in the Suren plant leaves, a qualitative preliminary test was carried out. As much as10 g Suren plant leaf fine powder was extracted by maceration with methanol and then filtered. The filtrate was tested using some reagents, such as (1) filtrate was added with 3 drops of Bouchardat reagent solution, a brown precipitate was formed (Tobing, 1989), (2) filtrate was added with 3 drops of Wagner's reagent solution to form a brown precipitate, (3) filtrate was added with 3 drops of Mayer's reagent solution to form a yellowish white precipitate, (4) filtrate was added with 3 drops of Dragendorff's reagent solution to form a brownish orange precipitate (Cannell, 1998).

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

2.4.2 Terpenoids Screening

In order to determine the presence of terpenoid/steroidal compounds in the Suren plant leaves, a qualitative preliminary test was carried out. As much as 10 g Suren plant leaf fine powder was extracted by maceration with methanol and then filtered. The filtrate was tested using some reagents, such as (1) filtrate was added with 3 drops of Salkowsky reagent to form a red solution, (2) filtrate was added with 3 drops of Lieberman Burchard's reagent solution to form a bluish-green solution, (3) filtrate was added with 3 drops of $CeSO_4$ 1% reagent solution in H_2SO_4 10% to form a brown solution (Tobing, 1989).

2.5 Maceration Extraction of Suren Plant Leaves

The samples were taken from Tomok Village, Simanindo District, Samosir Regency, North Sumatra. In this study, as much as 1400 g of dried Suren leaves were macerated for ±48 hours with 9.6 L of methanol as solvent at room temperature. The methanol extract was obtained from the maceration process 3 times which has been rotated by the evaporator and evaporated until the solvent evaporates. The extracted result was in the form of a thick greenish-brown liquid. Separation of compounds suspected of tannins was carried out by using ethyl acetate as an aprotic polar solvent. The concentrated methanol extract of 140.9 g was dissolved with ethyl acetate and then stirred until evenly distributed and filtered. The filtrate obtained was evaporated using a rotary evaporator until all the ethyl acetate solvent has evaporated. The residue was redissolved until the filtrate obtained was clear. The residue obtained was 55.79 tannin and the ethyl acetate filtrate obtained was 84.30 g.

2.6 Partition Extraction of Suren Plant Leaves

Partition extraction of Suren plant leaves was carried out on 84.30 g of ethyl acetate extract was dissolved with methanol, then put into a separating funnel and partitioned repeatedly with n-hexane with a ratio of methanol extract: n-hexane 1:2. Two layers were formed, the bottom layer was the methanol extract layer and the top layer was the n-hexane extract layer. The partition was performed 10 times until the n-hexane layer was clear.

2.7 Hydrolysis

Hydrolysis of Suren leaf tissue on the concentrated extract as a result of partitioning was 43.13 g in an acid condition. Hydrolysis of methanol extract was added with HCl 2N and then heated for ± 30 minutes in a water bath. Then cooled and filtered before being re-partitioned with chloroform (Harborne, 1987).

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

2.8 Thin Layer Chromatography (TLC) Analysis

Thin layer chromatographic analysis was carried out on the chloroform extract of Suren plant leaves using silica gel 60 F254 as the stationary phase and the mobile phase used was a mixture of n-hexane: ethyl acetate with solvent ratios 90:10, 80:20, 70:30, 60:40 (v/v), respectively. This analysis was intended to find a suitable solvent in column chromatographic analysis. In order to see the change in elution, the thin layer chromatogram was marked with an upper and lower limit using a pencil. The concentrated extract of chloroform was applied to the initial line of the thin layer plate, then put into a chamber containing the eluent and allowed to move until the eluent moved up to the upper limit.

The thin layer chromatogram was irradiated with an ultraviolet lamp to see the spots of the compound, then marked with a pencil and the Rf value was calculated. Furthermore, the thin layer was fixed with $FeCl_3$ 5% solution resulting in a black spot on the thin layer chromatogram indicating that it contained phenolic compounds positively. Observe the color of the spots that arise and calculate the Rf value obtained. The same procedure was performed for each eluent ratio used to determine the results of the thin layer chromatogram separation.

2.9 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 was silica gel 60 (0.063-0-200) and the mobile phase was a mixture of solvent n-hexane: ethyl acetate with a ratio of 90:10, 80:20, 70:30, 60:40 (v/v), respectively. The stage isolation of the chromatographic column was carried out in a dry way where the chromatographic column which had been assembled and equipped with cotton at the lower limit, was added with n-hexane 100% solvent to the ¾ of the volume of the used column. As much as 50 g of silica gel was slowly added, and eluted with n-hexane solvent until the silica gel solid in the column was evenly distributed and free of air bubbles. Cotton was added to the top layer of silica gel as the sample boundary area with silica gel. entered 1. 3 g of Suren plant leaf chloroform concentrated extract into a chromatographic column that has been mixed with 10 g of silica gel, then added the mobile phase n-hexane: ethyl acetate (90: 10) v/v slowly, and adjusted so that the phase flow out from the column as much as the addition of the mobile phase from above. The polarity was increased by adding the mobile phase n-hexane: ethyl acetate with a ratio of 80:20, 70:30, and 60:40 (v/v), respectively. The results obtained are accommodated in vials every 5 mL.

2.10 Faction Merger

The fractions obtained from column chromatography were then combined with the same Rf value. Fractions were combined using thin layer chromatography analysis of the ethyl acetate fraction. Fraction 55-68 using the stationary phase silica gel 60 F254 and the mobile phase used was a mixture of n-hexane: ethyl acetate with a solvent ratio of 60:40 (v/v). This analysis was

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

intended to find the same Rf value for each fraction of column chromatography results. In order to see the change in elution, the thin layer chromatogram was marked with an upper and lower limit using a pencil. The fraction was spotted on the initial line of the thin layer plate, then inserted into the chamber containing the eluent and left until the eluent moves up to the upper limit.

The thin layer chromatogram was irradiated with an ultraviolet light to see the compound spots, then the thin layer was fixed with $FeCl_3$ 5% solution to produce a black spot on the thin layer chromatogram which showed that it contained phenolic compounds positively. The color of the spots observed that arise and calculated the Rf value obtained. The same Rf value was then combined into one fraction and then in TLC analysis again.

2.11 Purification

The paste obtained from isolation by column chromatography was redissolved with ethyl acetate. Then slowly added n-hexane until two layers were formed. Then the upper solution was decanted. Then the remaining solvent was evaporated to obtain a paste that was completely free of solvents until a pure compound was obtained (Jacobs, 1974).

2.12 Purity Test of Isolation Results Using Thin Layer Chromatography

The purity test of isolated compounds was carried out by thin layer chromatography. The isolated compound obtained was dissolved with ethyl acetate solvent, and elucidation was carried out using an eluent made with several solvent ratios, namely n-hexane: ethyl acetate with the ratio of 60:40 (v/v), chloroform: methanol with a ratio of 80:20 (v/v), ethyl acetate: methanol with the ratio of 90:10 (v/v). In order to see the change in elucidation, the thin layer chromatograms were marked with the upper and lower limits using a pencil. The isolated compound was spotted on the lower boundary of the thin layer plate, then put into a chamber containing the eluent and allowed to move up to the upper limit.

The thin layer chromatogram was irradiated with an ultraviolet lamp to see the spots of the compound, then marked with a pencil and the Rf value was calculated. Furthermore, the thin layer was fixed with $FeCl_3$ 5% solution resulting in a single black spot on the thin layer chromatogram indicating that it contained flavonoid compounds positively. The color of the resulting stain was observed and the Rf value obtained was calculated. The same procedure was done for each eluent ratio used to determine the results of the thin layer chromatogram separation. The separation results of the thin layer chromatogram from the pure isolation were obtained in one spot.

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

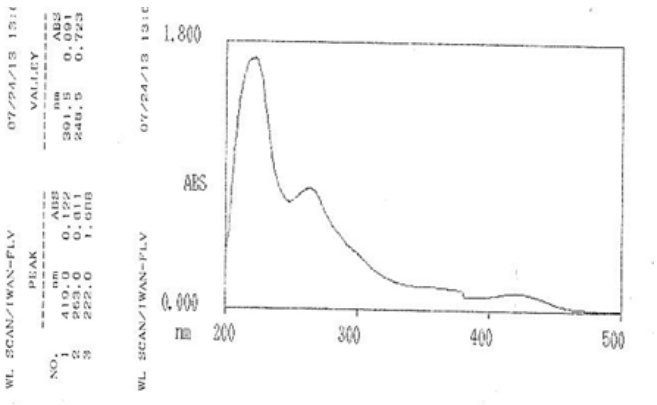
2.13 Identification of Isolated Compounds

The identification of isolated compounds was analyzed using UV-Visible spectroscopy, FT-IR spectroscopy, and ^{1}H-NMR spectroscopy.

3 RESULT AND DISCUSSION

3.1 Research Results

The identification results of secondary metabolites were carried out by using several identification reactions that describe most of the natural compound groups from the Suren leaves indicating that the methanol extract was positive for phenolic, flavonoid, alkaloid, terpenoid, and steroid reagents. The resulted isolated flavonoid compounds from the Suren plant



leaves were in the form of a yellow paste with a mass of 47 mg, and the Rf value of about 0.44 was obtained from the mobile phase of n-hexane: ethyl acetate (60:40) (v/v).

Figure 1. UV-Vis spectrum of isolated compounds

The UV-Vis test results indicated in figure 1 show that the isolated compounds with methanol as a solvent gave the maximum wavelength. Band 1 and 2 at 263 nm and 222 nm, respectively, showed the maximum wavelength of isolated compounds.

The FT-IR test results displayed in figure 2 show that the ten typical bands found in isolated compounds were the band at 3471.87 to 3236.55 cm^{-1}, which demonstrated the presence of OH groups, the band at 2939.52 to 2877.79 cm^{-1}, which showed aliphatic CH bonds, the band at 1724.36 cm^{-1}, which assigned C=O bonds from ketones, the band at 1604.77 cm^{-1}, which indicated the aliphatic of C=C bonds, the band at 1512.19 cm^{-1}, which showed the aromatic of C=C bonds, the band at 1442.75 to 1355.96 cm^{-1}, which assigned $–CH_3$ bonds, the band at 1232.51 cm^{-1}, which demonstrated C-O bonds, the band at 1170.79 cm^{-1}, which showed C-CO-C bonds from ketones, the band at 1099.43 to 1026.13 cm^{-1}, which indicated the asymmetric of C-O-C bonds, and the band at 966.34 to 858.32 cm^{-1}, which assigned the aromatic of =C-H bonds (Pavia, 1979).

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

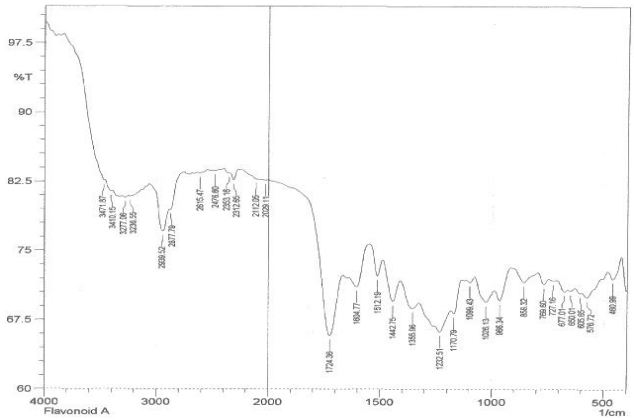


Figure 2. Infrared Spectrum of isolated compounds

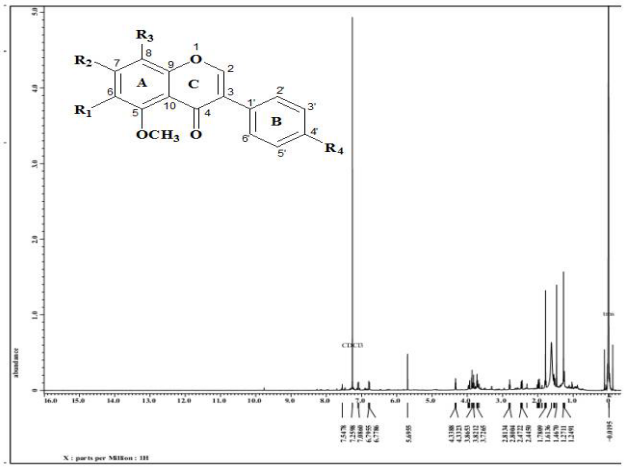


Figure 3. ^{1}H-NMR spectrum of isolated compounds

The ^{1}H-NMR test results presented in figure 3 show that the isolated compounds using chloroform and TMS solvents as standards provide chemical shift signals. The chemical shift in the δ = 7.5478 ppm region with a singlet peak, which indicated a proton from H-2 in ring C of flavonoid compounds, the chemical shift in the δ = 7.1029 to 7.0860 ppm region with doublet peak, which showed protons from H-2' and H-6' in ring B of flavonoid compounds, the chemical shift in the δ = 6.7955 to 6.7786 ppm doublet peak, which showed protons from H-3' and H-5' in ring B of flavonoid compounds, the chemical shift in the δ = 5.6955 ppm singlet peak, which assigned the proton of the vinyl compound, the chemical shift in the δ = 3.8653 ppm singlet peak, which indicated a proton from –OCH_3 on the C-5 atom of ring A of flavonoid compounds, the chemical shift in the δ = 3.8212 ppm singlet peak, which demonstrated a proton from –OCH_3, the chemical shift in the δ = 1.7809 ppm singlet peak, which indicated a proton from CH_3, the chemical shift in the δ = 1.4670 ppm singlet peak, which showed a proton from CH_3 and the chemical shift in the δ = 1.2802 ppm singlet peak, which assigned a proton from CH_3.

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 43 – 52

Based on the test results above, the obtained isolated compound was a flavonoid compound of the isoflavone group with hydroxyl, methoxy, vinyl, and methyl substituents, which the obtained structure assumed in figure 4.

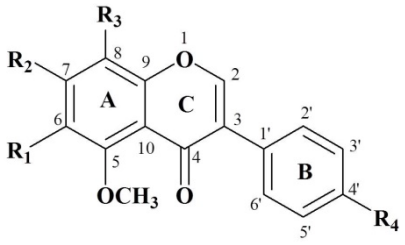


Figure 4. Isoflavone structure

4 Conclusion

In conclusion, isolation of flavonoid compounds from Suren plant leaves was successfully conducted. This is supported by the identification results obtained from phytochemical screening and also by the characterization from UV-Vis, FT-IR, and ^{1}H-NMR. The isolated compound is found in the flavonoid compound of the isoflavone group. In this study, TLC analysis showed that the isolation obtained from 1400 g Suren plant leaves in the 55-67 fraction was a yellow paste about of 47 mg and the Rf value =0.44 with n-hexane eluent: ethylacetate ratio of 60:40 (v/v).

References

Cannell, R.JP. 1998. Natural Products Isolation. New Jersey: Humana Press Inc.

Chen, CH, Lin, CY, Lin, LC, Wan, TC, 2012. Antioxidation activity and total phenolic contents of various Toona Sinensis extracts. African Journal of Biotechnology vol 11(73).

Chia, YC, Rajbanshi, R., Calhoun.C., Chiu.RH 2010. Anti-Neoplastic Effects of Gallic Acid, a Major Component of Toona sinensis Leaf Extract, on Oral Squamous Carcinoma Cells.Molecules 15118377.

Edmonds, JM and Staniforth, M., 1998. Toona Sinensis: Meliaceae. Curties's Bot.Mag.15.

Eka, WE and Hidajati, N., 2012. Kolestan Compounds From Chloroform Extract of Toona Sinensis (A.Juss) Roem Stem Bark and Bioinsecticide Test. UNESA Journal of Chemistry Vol 1. Surabaya.

Ekaprasada, MT, Nurdin, H., Ibrahim, S., Dachriyanus. 2009. Antioxidant Activity of Methyl Gallate Isolated from the Leaves of Toona Sureni. Indo.J.Chem,2009, 9(3) 457-460 .

Fahwid, RS 2009. Antibacterial Activity Test of Ethanol Extract and Boiled Water of Ingul Bark (Toona Sinensis M. Moem) Against Several Bacteria. Essay. Medan, Indonesia: USU Faculty of Pharmacy.

Hamid, A., Hadidi, EA, Rostiana, O. 1991. Efforts to Preserve Medicinal Plants in Balittro. In the proceedings for the Utilization of Medicinal Plants and Tropical Forests in Indonesia. Cooperation with the Department of Forest Resources Conservation, Faculty of Forestry, IPB, and Latin Bogor: Bogor.

Hsieh, TJ, Liu, TZ, Chia, YC, Chem, CL, Lu, FJ, Chuang, MC, Mau, SY, Chen, SH, Syu, YH, Chen, CH, 2004. Protective effect of methyl gallate from Toona Sinensis (Meliaceae) against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells. Food Chem. Toxicol.

Hsieh, MM, Chen, CY, Shu LH, Hsieh, SF, Lee, B.PH., Li, CT., Hsieh, TJ 2006. Separation Of Phenols from the Leaves of Toona Sinensis (Meliaceae) by Capillarity Electrophoresis.Journal of the Chinese Chemical Society.

Harborne, JB 1987. Phytochemical Methods Leading the Modern Way of Analyzing Plants. 2nd issue. Translation of Kosasih Padmawinata and Iwang Soediro. Bandung: ITB Publisher.

Jacob, TL 1974. Laboratory Practice of Organic Chemistry. Fifth Edition. New York: Macmillan Publishing Co Inc.

Jamhari, AR Bioactivity of Suren Beureum (Toona sinensis Roemor) Ethanol Extract Against Shrimp Larvae. Bogor Agriculture University. Published thesis.

Kurz, WG and Constable, F. 1998. Production and Isolation of Secondary Metabolites. Translator, Widianto, MB Bandung: ITB Press.

Markham, KR 1988. How to Identify Flavonides. Kosasi Translation. Padmawinata. Bandung: ITB Press.

Pavia, LD 1992. Introduction to Spectroscopy a Guide for Students of Organic Chemistry. Philadelphia: Saunders College.

Sisilia, EP, Fidrianny, L., and Nawawi, A. 2006. Chemical content of suren leaves. (Toona sinensis (Adr.Juss) MJ. Roemer) ITB School of Pharmacy.http://material-alam.fa.itb.ac.id.

Tobing, RL 1989. Chemistry of Natural Materials. Department of Education and Culture. Directorate General of Higher Education. Jakarta: Educational Personnel Education Institution Development Project.

Yuhernita and Juniarti. 2011. Analysis of Secondary Metabolic Compounds From Methanol Extract of Surian Leaves With Potential As Antioxidants. Makara, Science, Vol.15,No.1. Indonesia.