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ISOLATION AND IDENTIFICATION SECONDARY METABOLITE COMPOUNDS EXTRACT OF N-HEXANE FROM LEAF OF *MANILKARA ZAPOTA*

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ABSTRACT

This research aims to isolate and identify the secondary metabolite compound contained in the n-hexane extract of leaf of Manilkara Zapota. Isolation is done in several stages; extraction, fractionation, purification, and identification. The result was obtained as pure an isolate in white needle crystal with a melting point of 188-190°C. The isolate gives a positive response to Lieberman-Burchard reagent test. Isolate was identified by analyzing the infrared spectrum which showed the wave number (cm-1) are: 1026,13 (CO); 1458.18 and 1367.53 (CH2 and CH3); 1647,21 (C = C); 2947,23 (C-H); 3442,94 (OH alcohol). Base on reagent test and FTIR data, it is suggested, that isolate is terpenoid compound.

KEYWORDS: *Isolation, M. Zapota, FTIR, Terpenoids*

INTRODUCTION

The diversity of fauna and flora is seen as a strategic asset and very valuable when viewed from the chemical terms of the natural materials it contains. Exploration of biological natural resources, especially plants for treatment is still underway. Chemical compounds that are bioactive can be produced from various types of plants. The presence of bioactivity, the plant has a prospect for use in the field of treatment. The secondary metabolite compounds are small molecules, specific, have varying structures, each compound has different functions or roles. Secondary metabolite compounds function to defend themselves. Secondary metabolites are biomolecules that can be used as lead compounds in the discovery and development of new drugs (Atun, 2010)

The selection of natural ingredients for research can be derived from materials traditionally used by communities to cope with disease (ethnopharmacology) and also derived from bioactive ingredients (Wahyuningsih, 2006). Therefore, studies continue to be done as an effort to uncover various chemical compounds contained in plants that are efficacious as a drug ingredient.

One of Indonesia's tropical plants is a Sapotaceae-fed plant that is divided into 53 genera, comprising 1,250 species with worldwide distribution, mainly in the tropics and subtropics of Asia and South America (Kohatri, et al, 2010). One of the plants that are pregnant with Sapotaceae and potentially medicinal is M. Zapota. Commonly known by different names based on the area where it grows, as in Indonesia is known as sawo, Filipino (tsiko), Malaysia (ciku), India (chiko or sapota) and many more the name of this plant based on the country where it grows (Dalimartha, 2006). In the field of health, M. Zapota leaves not known to the public in general, only in certain areas. Often used as a fever

remedy and other injuries, commonly also used as a cough and cold medicine. The study by Jain et al. (2011) on the Evaluation of Analgesic Activity of Manilkara zapota (leaves) obtained alkaloid compounds and flavonoids in which this compound acts as a painkiller. In addition, the study found steroid compounds in petroleum ether and ethanol extracts in M. Zapota leaf (Manilkara zapota). Research conducted by Habib (2010) entitled in vivo anti-inflammatory and anti pyretic activities of Manilkara zapota leaves in albino wistar rats obtained flavonoid compounds namely flavones and flavonols with C- and O-glycosides, isoflavones C- and O- glycosides, flavanone C - and O-glycosides, chains with C- and O-glycosides, and dihydrochlala, anthocyanin, auron O-glycosides.

Based on the above description, the researcher isolated and identified the secondary metabolite compound from n-hexane leaf extract of M. Zapota, where the leaves are part of the plant which is often used as a medicinal material and why n-hexane solvent used to contract non-polar compounds contained in leaf M. Zapota because n-hexane has the selectivity in extracting non-polar compounds. In addition, n-hexane is also safe to use and easily evaporate, so it is used as a solvent to extract the leaves of M. Zapota.

MATERIALS AND METHODS

Material

Materials used include M. zapota leaves, organic solvents such as methanol (CH3OH), n-hexane (C6H14), ethyl acetate (CH3COCH2CH3), chloroform (CHCl3) p.a. Merck, reagent Liebermann-Buchard ((CH3CO) 2O, sulfuric acid (H2SO4), Wagner and Mayer reagents, 1% iron (III), chloride (FeCl3) 1%, cerium sulfate (CeSO4) 10% in 2 N sulfuric acid, impregnation of the sample using silica gel Merck G 60 H catalog number 7733, silica gel Merck G 60 (70-230 mesh) catalog number 7730 for vacuum liquid column chromatography (KKCV), and silica gel Merck G 60 (230-400 mesh) catalog number 7734 for Flash column chromatography (KKF), as well as the silica gel aluminum KLT plate Merck G 60 GF254 catalog number 7730

Instrumentation

The tools used in this research are glass tools, the chamber as KLT container, capillary tube as a bottle, vacuum liquid column chromatography (KKCV) and column compression chromatography (KKT) for sample fractionation. Then some instruments such as Hahn Shin® HS2005VN evaporator, Stuart® hot plate, Memmert® oven, UV VL-4 LC 254-356 nm lamp, Cheetah® FA2204B analytical balance sheet, Kris Chef® balance, Stuart micro melting point and electromechanical ® SMP11 as well as FTIR SHIMADZU Prestige-21 spectrometer

METHODS

Sample Preparation

First, the leaf is dried by air aerated for ± 2 weeks then dried leaf samples are milled using a blender and pounded to obtain a fine powder of 4.96 kg.

Extraction

The extraction is done by maceration technique. A total of 4.96 kg of leaf powder of M. Zapota macerated with methanol as much as 60 L for 3x24 hours. The extract obtained was concentrated using an evaporator until approximately one-quarter of the initial volume (viscous extract) was obtained. The liquid extract was extracted liquid by using a

separating funnel with a non-polar solvent n-hexane at a ratio of 1: 1 (n-hexane: methanol extract). The obtained n-hexane extract was evaporated to obtain the n-hexane extract.

Fractionation

The n-hexane extract was fractionated by thin layer chromatography (TLC). A small amount of extract was stained on a plate of TLC silica gel G 60 GF254 with an n-hexane combination of the eluted solution: ethyl acetate, chloroform: ethyl acetate in various rats then detected under UV light 254 and 365 nm and followed by spraying of cerium sulfate solution, CeSO4 10% when heated. The n-hexane extract was then impregnated with Silica gel G 60 H catalog 7733 in the sample ratio: silica gel (1: 3). This fractionation step is performed by vacuum liquid column chromatography (KKCV) method. The eluent volume for each elution is 100 mL.

Furthermore, the fraction which formed a good splitting pattern is selected for further fractionation and firstly identified with TLC further fractionated by press column chromatography with 100% n-hexane else, the combination of n-hexane: ethyl acetate to 100% ethyl acetate, then ethyl acetate: acetone up to 100% acetone and finally with 100% methanol. as a mobile phase. The fractions having the same stain profile were combined and then evaporated and then returned to the TLC with eluent n-hexane: ethyl acetate (9: 1).

Purification

Fractions that exhibit good stain patterns and form powders are purified by using n-hexane solvent. The purified compound was then continued on the KLT process of three kinds of eluents: ethyl acetate: n-hexane (1:99), ethyl acetate: chloroform (1:19), and chloroform: n-hexane (1: 9). The next purification step is melting point test using Melting Point SMP11

Identification

The identification was done by using Liebermann-Burchard reagents (terpenoids and steroids), FeCl3 (flavanoid), Wagner and Mayer (alkaloids) to determine the class of isolates. Further identification was performed with the Shimadzu Prestige-21 FTIR spectrometer to determine functional groups.

RESULTS AND DISCUSSIONS

Results

Extraction

Extraction is 4.9 kg of leaves M. Zapota macerated with methanol solvent for 3 x 24 hours, macerate obtained as much as 35 liters, then evaporated to produce methanol condensed as much as 1.5 liters of dark brown. The result of a partition with n-hexane obtained by green n-hexane extract as much as 1.5 liters and extract methanol as much as 1.5 liters, then extract n-hexane evaporated and obtained n-hexane thick extract as much as 15.2507 g.

Isolation and Purification

The n-hexane condensed ecstasy was identified using TLC with several eluent tests used on TLC, a combination of n-hexane: ethyl acetate. The results of TLC are obtained by combining n-hexane: ethyl acetate which gives a good and clear separation pattern. The n-hexane extract (15.2507 g) was fractionated by KKCV with eluent n-hexane, n-hexane: ethyl acetate, ethyl acetate, ethyl acetate: acetone, acetone, and methanol in an enhanced polar sequence

obtained by KKCV yield of 33 fractions than the incorporation of the obtained fractions identified through TLC yields 7 major fractions as shown in Table 1 and the chromatogram shown in Figure.1.

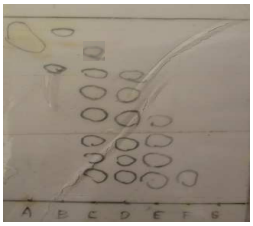

Figure 1: Chromatogram of A-G Combined Faction Eluen: Ethyl Acetate: n-Hexane (1: 9)

Table 1: Merger Result of KKCV Fraction Based on Stain Profile on TLC Chromatogram

Fraksi	Komponen	Berat (g)	Warna Fraksi
1-2	A	0,6387	Kuning
3-4	B	1,1513	Orange
5-8	C	8,5151	Hijau tua
9-12	D	0,3604	Hijau tua
13-16	E	0,5552	Hijau tua
17-19	F	0,2652	Hijau tua
20-33	G	0,2563	Hijau tua

The second major fraction (B) (1,1513 g) is further fractionated using the method of KKT with its mobile phase using 100% n-hexane eluent, a combination of n-hexane: ethyl acetate to 100% ethyl acetate then ethyl acetate: acetone up to 100 % acetone and lastly with 100% methanol, yielded 24 fractions. The fraction of the results of the KKT identified with KLT obtained 11 major fractions of the merger result based on the staining pattern shown on the TLC as shown in Table 2. The combined fraction is identified by TLC with ethyl acetate: n-hexane ratio (1: 9). The chromatogram is shown in Figure.2. The fractions were evaporated. The B4 fraction (0.1167 g) forms a yellow powder

Table 2: Results of Merger of Fraction of Results of KKT

Fraction	Component	Weight (g)	Color
1	B_1	0,0366	Clear
2	B_2	0,3912	Orange
3	B_3	0,0133	Yellow
4	B_4	0,1167	Yellow
5	B_5	0,3604	Orange
6	B_6	0,0197	Orange
7	B_7	0,0031	Greenish yellow
8-10	B_8	0,0306	Greenish yellow
11	B_9	0,1036	Green
12-17	B_{10}	0,0569	Clear green
18-24	B_{11}	0,0054	Clear

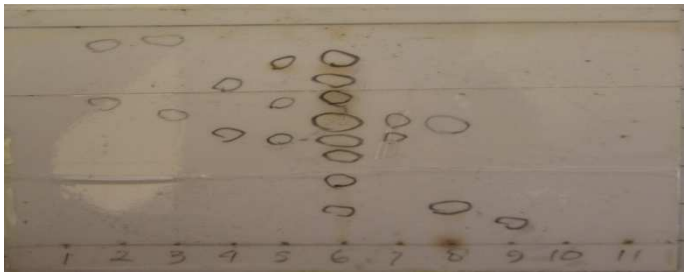

Figure 2: Chromatogram of Combined Fraction B1-B11 KKT Results Eluen: Ethyl Acetate: N-Hexane (1: 9)

The B4 fraction (0.1167 g) was purified by n-hexane resulting in a white powder of 0.0506 g with a melting point of 188 C0-190 C0 as shown in Figure 3

Figure 3: Powder of B4 Fraction after Purification

The TLC analysis showed a stain on three different eluents: chloroform eluent: n-hexane (1: 9), ethyl acetate: chloroform (1:19), and ethyl acetate: n-hexane (1:99). The chromatogram is as shown in Figure 4.

(a) (b) (c)

Figure 4: Chromatogram of B4 Fraction Result of TLC (a) Chloroform: N-Hexane (1: 9) (b) Ethyl Acetate: Chloroform (1:19) (c) Ethyl Acetate: N-hexane (1:99)

Figure 5: Color Test Results (a) Before the Addition of Reagents (b) After the Addition of Reagents

The isolate obtained was tested with several reagents to determine the type of the compound. The color test results can be seen in figure 5. The data can be seen in Table 3.

Table 3: Result of Color Test on Pure Isolate of B4 Fraction

Reagents	Observation	Information
Liebermann-Burchard	Pink	(+) Terpenoids
$FeCl_3$	Yellow	(-) Flavonoids
Meyer	Clear	(-) Alkaloids
Wagner	Red	(-) Alkaloids

Identifikasi

The identification is continued by using the FTIR spectrometer. The infrared spectra of the B4 fraction isolate compound are shown in Figure 6 and the data of the wave number, the shape of the band, the intensity and the corresponding clusters are presented in Table 4

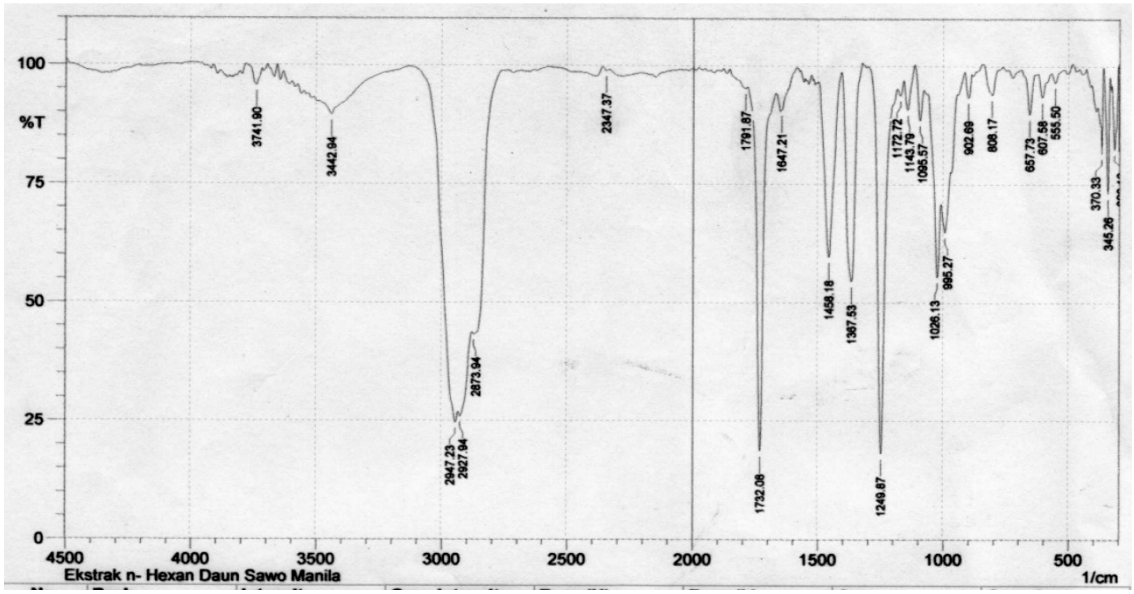

Figure 6: FTIR Spectra

DISCUSSIONS

Isolation and Purification

The 15, 2507 g of n-hexane viscose extract was fractionated by KKCV method firstly identified with TLC to find out the amount of compound contained in the extract marked by the number of stains seen on the chromatogram. In addition, TLC also aims to find out the eluent appropriate for KKCV. The TLC results show that eluent n-hexane: ethyl acetate provides a clear stain appearance and a good separation pattern so that the eluent is used for fractionation on the KCV.

Fractionation with KKCV uses silica gel as a stationary phase and a variety of eluents by SGP (Step Gradient Polarity), ranging from nonpolar eluent to polar as a mobile phase. The gradual elution is intended to allow all non-polar and polar compounds to be fractionated and for cost and work efficiency. Eluate obtained as many as 33 fractions grouped into 7 joint fractions (fractions A to G). Fraction B has formed a powder on the glass wall after the solvent evaporates.

Fraction B (1,1513 g) was chosen for further fractionation with consideration of orange powder formed in the glass of B fraction. The stationary phase used in the KKT was silica gel while its mobile phase used eluent from 100% n-hexane, n-hexane: ethyl acetate then ethyl acetate 100%, ethyl acetate: acetone then 100% acetone, lastly with 100% methanol. Eluent obtained as many as 24 fractions grouped into 11 joint fractions (fractions B1 to B11). After all the combined fractions evaporate the solvent, the B4 fraction is formed a powder.

The B4 fraction (116.7 mg) was chosen to be purified due to consideration of the stain appearance on the TLC plate which showed a slight stain compared to the other fraction so it was expected to obtain pure isolate. The solvent used to purify is a solvent which can dissolve the impurities but not dissolve the powder. The fraction of B4 is purified by n-hexane resulting in a white powder. The purification is repeated until a chromatogram is obtained with one spot on the powder. The powder obtained is 50.6 mg.

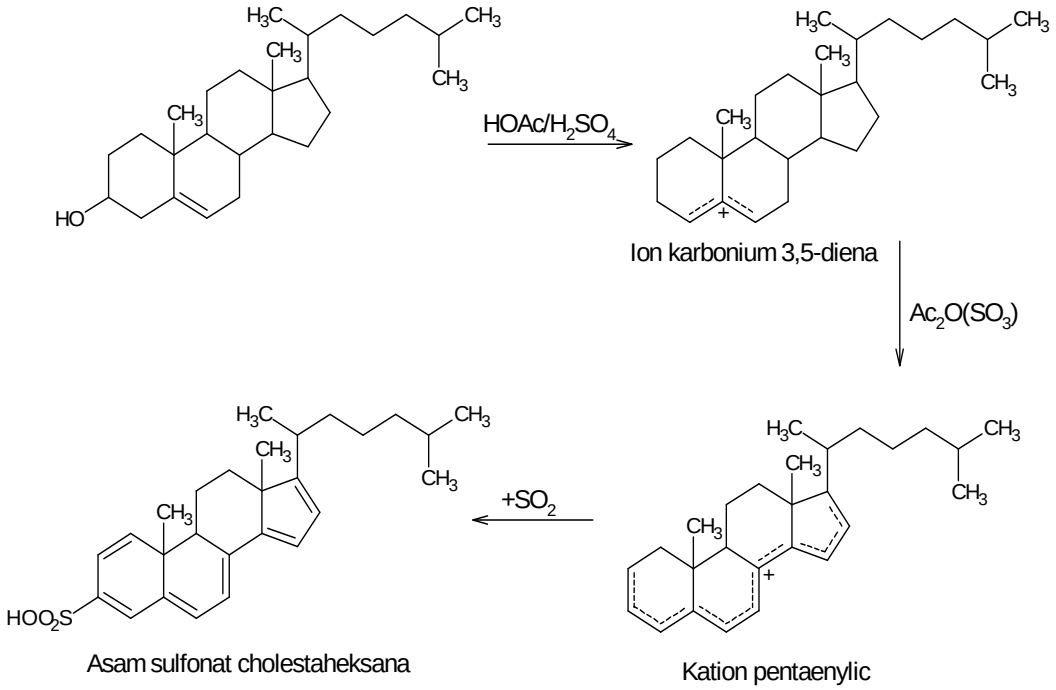

Figure 7: Liebermann-Buchard Reaction

The obtained isolate was then tested for purity by KLT of three different eluents with different solvents and comparisons. This is done to ensure the purity of an isolate indicated by the appearance of a spot on each TLC. TLC analysis showed a stain on three different eluents: chloroform eluent: n-hexane (1:19), ethyl acetate: chloroform (1:19) and ethyl acetate: n-hexane (1:99). The elution stain was sprayed with a 2% CuSO4 stain removal reagent and heated to obtain a purplish-colored stain. Thus, it can be concluded that the isolate of a pure B4 fraction is TLC.

Pure isolates were tested for melting point. The melting point test indicates that B4 isolate starts melting at 188 ° C and melts overall at 190 ° C. Based on the literature study, it was found that the pure compound had a melting point route of no more than 2 (Firdaus, 2011).

Identification followed by color test using multiple reagents. The test results showed that isolate B4 gave a positive reaction of the terpenoid to Liebermann-Buchard reagent which was characterized by the change of color from

pink to pink as seen in Figure 5. Testing of steroids and triterpenoids in glacial CH3COOH with concentrated H2SO4 is based on the ability of steroid and triterpenoid compounds to form blue or green for steroids, and red or purple for triterpenoids. Steroids and triterpenoids are compounds that can be extracted with non-polar or semi-polar solvents (Harbone, 1987). This proves that the compound obtained is terpenoid in accordance with previous research on M. Zapota plants that mostly obtain terpenoid compounds.

FTIR Spectroscopy Test

Identification of isolate groups was performed using FTIR spectrometer with KBr pellet method. The spectral IR spectral analysis of B4 provides absorption at the wavelength (3442,94 cm-1) region marked by a moderately wide band of moderate intensity identified as a vibration of OH (not NH) strain. This bonding bond is thought to be the vibration of the OH group undergoing the intermolecular hydrogen bonds This assumption is supported by the presence of moderate intensity absorption at 1249,87 cm-1 and 1026,13 cm-1 regions identified as vibration C-O alcohol.

The sharp intensity with the strongest intensity at the 1732.08 cm-1 wavelength region is allegedly due to the functional group C = O of a carboxylic acid, this data is supported by a sharp vibration-absorbing vibration band with a strong intensity at the wave region 2947,23 cm-1 and 2873.94 cm-1 indicating the presence of an aliphatic CH-group of carboxylic compounds.

The emergence of sharp absorption bands with strong intensity at the 1647.21 cm-1 waveform region indicates a double bond C = C. This data is reinforced by the C-H bending vibration of 1458.18 cm-1 and 1367.53 cm-1 wave numbers indicating the presence of the dimethyl geminal group and the wavelength number of 995.27 cm-1 representing the bending vibration of the alkene as the hallmark of the terpenoid compound.

The literature study shows that the functional groups as described above are functional groups of terpenoid group compounds with the characteristics of bound OH groups, CH aliphatic, C = O carboxylates, double bonds C = C and C-O alcohols. Interpretation of IR uptake of wave numbers from the B_4 leaf fraction of M. zapota can be seen in Table 3.4.

Table 4: Absorption of IR Fraction B_4 from M.zapota Leaves with Possible Cluster Function

Wavenumber (cm^{-1})	Functional groups
3442,94	-OH
2947,23 dan 2873,94	C-H aliphatic
1647,21	C=C
1458.18 dan 1367.53	C-H bending alkene
1732,08	C=O
1249,87 dan 1026,13	C-O

CONCLUSIONS

Based on the results obtained, it can be concluded that the secondary metabolite compound in the form of white powder with melting point 188-190oC which has been isolated from the n-hexane extract of M. Zapota leaves is terpenoid group compounds.

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REFERENCES

1. *Achmad, S.A. 1986. Kimia Organik Bahan Alam. Departemen Pendidikan dan Kebudayaan, Universitas Terbuka. Jakarta.*
2. *Ahmad, S.A., Hakim, E.H., Juliawaty, L.D., dkk. 2007. Ilmu Kimia Sumber Alam Hayati Indonesia : Penelitian Untuk Pengembangan Pendidikan, Ilmu Pengetahuan, Dan Sumberdaya Manusia. Seminar Nasional Kimia, Universitas Negeri Surabaya, 5 Desember 2007.*
3. *Asih, I. A. R., Gunawan, I. W. G., dan Ariani, N. M. D. 2010. Isolasi Dan Identifikasi Senyawa Golongan Terpenoid Dari Ekstrak N- Heksana Daun Kepuh (Sterculia foetida L.) Serta Uji Aktivitas Antiradikal Bebas. Jurnal Kimia, Vol 4 No.2 135-140.*
4. *Astalakshmi, A., et al. "Evaluating the potentiality of leaves of Manilkarazapota (L.) P. Royan and Mimusopselengi L. in the synthesis of silver nanoparticles." International Journal of Metallurgical & Materials Science and Engineering 4.2 (2014): 25-36.*
5. *Atun, S. 2010. Pemanfaatan Bahan Alam Bumi Indonesia Menuju Riset yang Berkualitas Internasional. Seminar Nasinal Kimia. Yogyakarta: FMIPA Universitas Negeri Yogyakarta*
6. *Caio, et al. 2013. Triterpene Esters and Biological Activities from Edible Fruits of Manilkara subsericea (Mart.) Dubard, Sapotaceae. BioMed Research International. Volume 2013 (2013), Article ID 280810.*
7. *Dalimartha, Setiawan. 2006. Atlas Tumbuhan Obat Indonesia. 4. hal.77-78. Jakarta:Puspa Swara. ISBN 979-1133-14-X.*
8. *Firdaus. 2011. Teknik dalam Laboratorium Kimia Organik. Makassar: FMIPA Universitas Hasanuddin*
9. *Gritter, Bobbitt & Schwarting, dkk. 1991. Pengantar Kromatografi. Bandung: Institut Teknologi Bandung.*
10. *Guengly, amlan et al.2013. in vivo anti inflammatory and anti pyretic activities of manilkara zapota leaves in albino wistar rats. Asian Pasific Journal of Tropical Disease 3(4):301-307.*
11. *Habib, Rezuel. 2010. Antimicrobial Investigation on Manilkara zapota (L.) P. Royen. Department of Biochemistry and Molecular Biology, Faculty of Science, Rajshahi University. -March 2011, 3(1): 185-190*
12. *Harborne, J. B. 1987. Metode Fitokimia Penentuan Cara Modern Menganalisis Tumbuhan. Bandung: Institut Teknologi Bandung.*
13. *Hendayana, S. 1994. Kimia Analitik Instrumen. Semarang: IKIP Semarang Press.*
14. *Islam, et al. 2013. Antibacterial and phytochemical screening of ethanol extracts of Manilkara zapota leaves and bark. International Journal of Pharma Sciences. Vol. 3, No. 6 (2013): 394-397*
15. *Jain, Pankaj K; et al. 2011. Evaluation of Analgesic Activity of Manilkara Zapota (Leaves). Department of Pharmacognosy, RKDF College of Pharmacy, Bhopal (M.P.), India (2011).*
16. *Khopkar, S.M. 2008. Konsep Dasar Kimia Analitik. Jakarta: UI-Press.*
17. *Kothari, V, et.al. 2010. In vitro antibacterial activity in seed extracts of Manilkara zapota, Anona squamosa, and*

Tamarindus indica. Biological Research 43: 165-168.

18. *Lenny, S. 2006. Isolasi dan Uji Bioaktifitas Kandungan Kimia Utama Puding Merah dengan Metoda Uji Brine Shrimp. Medan: Departemen Kimia FMIPA Universitas Sumatra Utar*
19. *Morton, J. F. 1987. Sapodilla. (J) Fruits of warm climates : 393–398*
20. *Nafisah, Minhatun et al. 2014. Uji Skrining Fitokimia Pada Ekstrak Heksan, Kloroform, dan Metanol dari Tanaman Patikan Kebo (Euphorbiae hirtae). Prosiding Seminar Nasional Kimia, ISBN: 978-602-0951-00-3. Universitas Negeri Surabaya.*
21. *Orwa C., Mutua A., Kindt R., Jamnadass R., dan Simons A. 2009. Agroforestree Database: tree reference and selection guide version 4.0. http://www.worldagroforestry.org/af/treedb/. 20 Oktober 2011.*
22. *Osman, et al. 2011. Antimicrobial Investigation on Manilkara zapota (L.) P. Royen. Asian Pacific Journal of Tropical Biomedicine 3 (1): 448-451*
23. *Prihatman, Kemal. 2000. Sawo (Acrhras zapota. L). Sistem Informasi Menajemen Pembanguna di Perdesaan, BAPPENAS. Jakarta.*
24. *Rao, et al. 2014. Phytoconstituents from the leaves and seeds of Manilkara zapota Linn. Scholars Research Library. Der Pharmacia Lettre, 2014, 6 (2):69-73. ISSN 0975-5071*
25. *Samini. 2008. Analisis Keanekaragman Morfologi Sawo (Manilkara zapota L.) Lokal Serang. Naskah Skripsi-S1. Fakultas Manajemen Agibisnis Universitas Mercu Buana. Jakarta. Tidak Diterbitkan.*
26. *Sastroamidjojo, A. S. 1988. Obat Asli Indonesia. Dian Rakyat. Jakarta. Hal 21-22.*
27. *Sawal, M. 1997. Isolasi dan Identifikasi Senyawa Metabolit Sekunder Ekastrak n-Heksana Daun Artocarpus dasyphylla Miq. Skripsi, Jurusan Kimia FMIPA UNM. Makassar.*
28. *Sukadana. 2007. Isolasi dan Identifikasi Senyawa Turunan Terpenoid dari Fraksi N-Heksan Batang Tinospora crispa L. Jurnal Sains dan Teknologo Kimia. UIN, Yogyakarta.*
29. *Tobo, F. 2001. Fitokimia I (Ekstraksi Komponen Kimia Bahan Alam). Makassar: Lab. Fitokimia FMIPA Universitas Hasanuddin.*
30. *Wahyuningsih, M.S.H. 2010. Deskriptif Penelitian Dasar Herbal Medicine. Yogyakarta: Universitas Gadjah Mada*
31. *Zenta, F. 1999. Teknik Laboratorium Kimia Organik. Makassar: Laboratorium Kimia Organik Jurusan Kimia FMIPA Universitas Hasanuddin.*