



Analysis of Total Terpenoids from *Maniltoa Grandiflora* (A. Gray) Scheff Leaves Using TLC and HPLC Methods

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ABSTRACT

Terpenoids screening were carried out using Liebermann Burchard and Salkowski reagent on the extract of Saputangan leaves. It showed that the leaves contained terpenoid compounds with appeared of a reddish brown ring in the extract and a reddish brown stain appeared on the TLC plate tested with 1% CeSO₄ reagent in 10% H₂SO₄. The macerate of saputangan leaves processed separation using the partition method (Liquid-liquid Extraction). Extracts dissolved with methanol were partitioned with n-Hexane and then partitioned between aquadest and ethyl acetate in a ratio of 1: 1 to obtain 50 g of total terpenoids. Furthermore, TLC analysis was performed on total terpenoids using n-hexane: ethyl acetate (80:20 v/v) solvent to obtain 11 separate stains on the TLC plate with different R_f each. Analysis was enhanced in HPLC using 100% acetonitrile and 0.1% phosphoric acid at a wavelength of 210 nm, a flow rate of 0.500 mL/min and eluted for 30 minutes. Based on the HPLC results, there were 25 peaks which indicated the presence of total terpenoid compounds with the highest peak being peak no. 8 (ret.time's 6.234, area's 8503532 and height's 276032), peak no. 9 (ret.time's 6.674, area's 3322572 and height's 141859) and peak no. 10 (ret.time's 7.288, area's 2758231 and height's 103927).

Keywords: *Terpenoids, HPLC, TLC and R_f*

INTRODUCTION

Terpenes are included the biggest class of secondary metabolites and basically consist of five carbon as isoprene units which are connected to each other by thousands of ways. Terpenes are simple hydrocarbons, Most of linear terpenes and cyclized terpenes are exclusively composed of hydrocarbons, so these molecules are non-polar. While terpenoids are modified class of terpenes with different functional groups and oxidized methyl group moved or removed at various positions (Lingala & Ghany, 2016). Terpenoids are divided into monoterpenes, sesquiterpenes, diterpenes, sesquiterpenes, and triterpenes (Perveen, 2018).

Terpenoids have great uses as flavors, fragrances, high grade lubricants, biofuels, agricultural chemicals, and medicines. Most of the terpenoids with the variation in their structures are biologically active and are used worldwide for the treatment of many diseases. Some sesquiterpenoids are antifungals, carminatives, and insecticides. Many aroma components of essential oil, such as terpenes and terpenoids, were contributed to antioxidant activity; that is include β -terpene and β -terpinolene in *Melaleuca alternifolia*, 1,8-cineole in *Mentha aquatic*, and linalool in black cumin. Some terpenes are potent against diseases such as heart disease, malaria, and cancer (Rassem et al., 2016).

Saputangan plants (*Maniltoa grandiflora* (A. Gray) Scheff) are one type of plant that belongs to the genus *Maniltoa* and *Fabaceae* family. Saputangan leaves according to the screening test was positively contained phenolics and terpenoids. Saputangan plants are usually used as ornamental plants that can reduce pollution by absorbing pollutants such as carbon monoxide. The *Fabaceae* family was mostly useful as medicinal plants. Parts used as medicine include leaves, flowers, root bark, and bark (Scheff & Sinurat, 2018).

Terpenoids can extract by maceration method and separated by the liquid-liquid extraction. The solvent of partition involves two immiscible solvents in a separating funnel and the compounds are distributed in two solvents according to their different partition coefficients (Otsuka, 2006).

Maceration is immersing powder plants in a closed container with the solvent at room temperature minimum 3 days, which is allowed to soak for a certain period with several times stirring. In maceration method, the choice of solvents will determine the type of compound extracted from the samples. The purpose of maceration is to support plant metabolite compounds that dissolve and leave their residues (Nn, 2015). The principle of maceration is the interaction between chemical compounds in plants and solvents based on polarity so that chemical compounds can be extracted. One of the advantages of the maceration method is simple to work it. It could be used to extract the thermolabile components (Zhang et al., 2018).

Analysis of total terpenoid is performed on Thin Layer Chromatography (TLC). TLC is useful for separating non-volatile mixtures. Thin layer chromatography can be used to monitor the progress of reactions, separating the compounds present in a mixture, and determining the purity of a substance. TLC is performed on an aluminum plate coated with a thin layer of adsorbent material, usually silica gel, alumina or cellulose which is known as the stationary phase (Namir et al., 2019).

The compounds are separated on the basis of its interaction with the solid particles of a tightly packed column and the solvent of the mobile. HPLC is useful for compounds that cannot be vaporized under high temperatures. This provides both qualitative and quantitative measurements in a single operation. HPLC coupled with a UV photodiode array detector provides more information about terpenoids

through chromatogram appeared (De Silva et al., 2017).

EXPERIMENTAL SECTION

Materials

Saputangan leaves powder, Ethanol, Methanol, Ethyl Acetate, n-Hexane, Methanol, Acetonitrile, Phosphoric Acid, Reagent of Ceric Sulfate, Reagent of Liebermann Burchard, Reagent of Salkowski and Aquadest. All the solvents and reagents used in this study as pro analysis and high grade.

Equipments

Macerator and Separatory Funnel Schoot Duran merck, Waterbath Memert merck, Rotary Evaporator Heidolph Merck, TLC plate was performed using silica gel 60 F₂₅₄ (E.Merck) and Chamber and HPLC-UV instrument with Shimadzu merck and Degasser with Branson merck.

Procedure

Preparation Sample and Maceration

Saputangan leaves as sample were washed clean with water and cut into small pieces and dried the leaves. Saputangan was blended became leaves powder as 1000 g is taken from the environment of around the Universitas Sumatera Utara (USU). Macerated for ± 24 hours with 5 liters of ethanol at room temperature. Maceration was carried out repeatedly using ethanol solvent until the ethanol extract obtained all extract and gave a negative test result on the terpenoid reagents. The ethanol extract obtained was concentrated using a rotary evaporator at a temperature of 80°C with a rotation of 80 rpm.

Screening of Terpenoids

Determining the presence of terpenoid in saputangan leaves, a qualitative test was carried out. 2 g of the sample powder was macerated with ethanol and then filtered. the filtrate was tested using reagents. a) The filtrate is added with 3 drops of CeSO₄ 1% reagent in 10% H₂SO₄ to form a brown solution as appearance of terpenoids. c) Liebermann Burchard Test: The filtrate is dissolved with chloroform and then filtered. The filtrate is added a few drops of acetic anhydride, boiled and then cooled. The appearance of a brown ring indicates terpenoids were present (Linn et al., 2017). d) Salkowski test: The filtrate is dissolved with chloroform and then filtered. The filtrate is added with a few drops of concentrated sulfuric

acid, stirred and allowed to react until a red color as terpenoid or golden yellow color as tri terpenoid appeared (Abebayehu et al., 2016).

Partition (Liquid-liquid Extraction)

The extract was dissolved with methanol and partitioned in separatory funnel using n-hexane as a solvent to obtain a lower layer in the form of methanol and an upper layer in the form of n-hexane. Then the n-hexane layer was evaporated at 70°C with rotation of 40 rpm. After sample was dried, extract dissolved with methanol and continued partition with ethyl acetate. The ethyl acetate extract obtained was concentrated using a rotary evaporator at a temperature of 80°C with a rotation of 60 rpm (Abu et al., 2017).

Thin Layer Chromatography

Thin layer chromatography analysis was carried out on dry extract using a stationary phase of Merck 60 F₂₅₄ silica gel which was found on the surface of the TLC plate. Measure of TLC plat is 10 x 2 cm. The mobile phase was used n-hexane: acetone with a solvents ratio varying 90:10, 80:20, 70:30 and 60:40 v/v.

Total Terpenoids by High Performance Liquid Chromatography Analyses

The sample was weighed 0.25 g and dissolved with 25 mL methanol. The sample was put into the vial and then injected as much as 25.0 µL into the HPLC-UV device with the mobile phase acetonitrile (100%) and 0.1% phosphoric acid at a wavelength of 210 nm, and a flow rate of 0.500 mL/minutes and then recorded the peak area (Verma et al., 2016).

RESULTS AND DISCUSSION

Terpenoids screening

Terpenoid screening test was carried out to ensure terpenoids in extract of Saputangan leaves. The terpenoids in sample were analysed use Cerium Sulfat, Lieberman Burchard and Salkowski Reagents. The results showed in Figure 1 that *Maniltoa grandiflora* indicated the presence of monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids and tetraterpenoids with the appearance of a reddish brown ring on the sample (Malik et al., 2017).

Maceration and Partition

Macerate was filtered and evaporated using a rotary evaporator to obtain a solid extract of 350 g. Then proceed with the liquid-liquid

extraction process (partition) using n-hexane and ethyl acetate solvents which are carried out in stages so that the extract of the saputangan leaves is contained a total terpenoid as 50 g.

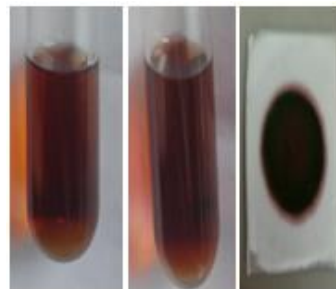


Figure 1. Result of terpenoid screening

Analysis of Total Terpenoid Using TLC

Total terpenoid analysis was performed in TLC using the best eluent n-Hexane: Acetone (80:20 v/v). After the elution was carried out, the TLC plate was sprayed with Cerric Sulfate and heating due to all terpenoids and steroids (Ludwiczuk et al., 2017) will produce red stains which were separated into 11 stains, each of which had a different R_f, including R_{f1} (0.27), R_{f2} (0.36), R_{f3} (0.47), R_{f4} (0.61), R_{f5} (0.68), R_{f6} (0.85), R_{f7} (0.87), R_{f8} (0.88), R_{f9} (0.90), R_{f10} (0.93) dan R_{f11} (0.98) which are shown in figure 2.

Analysis of Total Terpenoid Using HPLC-UV

Analysis of total terpenoid on HPLC-UV was using 100% acetonitrile and 0.1% phosphoric acid at a wavelength of 210 nm, a flow rate of 0.500 mL/min and eluted for 30 minutes. (Lü et al., 2020)



Figure 2. Total Terpenoid on TLC analyses

HPLC-UV results, there were 25 peaks which indicated the presence of terpenoid compounds with the highest peak being peak no. 8 (ret.time's 6.234, area's 8503532 and height's 276032), peak no. 9 (ret.time's 6.674, area's 3322572 and height's 141859) and peak no. 10 (ret.time's 7.288, area's 2758231 and height's 103927). The compound analyzed in HPLC-UV is terpenoids characterized by the appearance

of all peaks read at a wavelength of 210 nm, relevant with analysis terpenoids (Giese et al., 2015) measured on 214 nm. Chromatogram of HPLC-UV is shown on figure 3.

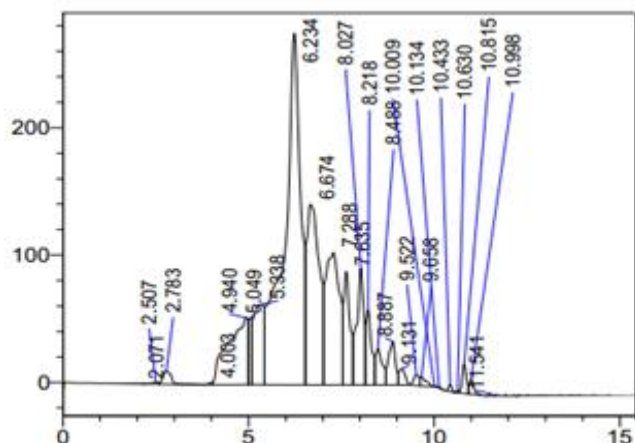


Figure 3. Chromatogram of total terpenoids on HPLC-UV

The result of all peak chromatograms was showing the total terpenoids were obtained based on the HPLC-UV instrument analyses. In table 1, Total terpenoids are arranged according to the order of the peaks that appeared, retention time, area and height.

Table 1. Result analysis of total terpenoids on HPLC-UV

Peak	Ret. Time	Area	Height
1	2.071	4733	365
2	2.507	19273	1412
3	2.783	154376	10151
4	4.003	12424	1797
5	4.94	1957596	52670
6	5.049	311129	52098
7	5.338	1195774	63166
8	6.234	8503532	276032
9	6.674	3322572	141859
10	7.288	2758231	103927
11	7.635	1039984	89221
12	8.027	1203315	91263
13	8.218	594930	59119
14	8.488	368723	28343
15	8.887	444862	34108
16	9.131	145049	12803
17	9.522	80716	8115
18	9.658	73457	6870
19	10.009	3899	953
20	10.134	4281	816
21	10.433	33313	5337
22	10.63	8485	1640
23	10.815	184647	23347
24	10.998	98361	11776
25	11.541	12201	1299

CONCLUSION

Saputangan leaves contained terpenoids which appeared reddish brown rings when tested using Liebermann Burchard and Salkowski reagents and appeared reddish brown stain on TLC plate when dropped with ceric sulfate. Saputangan leaves were macerated and partitioned to obtain total terpenoids using a separatory funnel. The total terpenoids were analyzed with TLC Methods and proved that Terpenoids have 11 terpenoids as red stain based on their Rf. Analyses of total terpenoids were eluted on HPLC-UV Method and found 25 peak as terpenoids with the highest peak subsequently as peak no. 8, peak no. 9 and peak no. 10. In the future, this research will be able to improve the isolation process and bioactivity test towards terpenoid in saputangan leaves.

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