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Detection of terpenoids and steroids in *Lindsaea obtusa* with thin layer chromatography

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Abstract. *Wutsqa YU, Suratman, Sari SA. 2021. Detection of terpenoids and steroids in* Lindsaea obtusa *with thin layer chromatography. Asian J Nat Prod Biochem 19: 65-69.* The diversity of ferns on this earth is very high, as well as the potential and benefits for human life, namely as ornamental plants, food material and medicines. The benefits of not all ferns are known because the lack of information on these ferns, one of ferns is *Lindsaea obtusa* J. Sm. Ex Hook. The phytochemical test is the first step in research on the search for new active compounds derived from natural ingredients. This study was conducted to determine the differences of terpenoid and steroid compounds profile in *L. obtusa* extract using Thin Layer Chromatography (TLC). Extraction of *L. obtusa* using maceration techniques with methanol and n-hexane solvents. The extraction results will be used for phytochemical screening using TLC technique with GF254 silica gel plate as a stationary phase and a mobile phase are chloroform: n-hexane (9: 1 v/v), n-hexane: ethyl acetate (4: 1 v/v), and methanol: ethyl acetate (5: 1 v/v). Detection of terpenoid and steroids using Lieberman Burchard reagent. The result shows that *L. obtusa* extract contains terpenoid and steroid compounds. Quantitatively, the secondary metabolites of *L. obtusa* extract were soluble in methanol solvent more than the compounds that were soluble in n-hexane solvent. However, qualitatively, steroid compounds that dissolve in n-hexane solvents have a more varied Rf value than terpenoids, especially in the mobile phase of chloroform: n-hexane (9: 1 v/v) and n-hexane: ethyl acetate (4: 1 v/v). *L. obtusa* contain more varied steroid compounds than terpenoid compounds.

Keywords: *Lindsaea obtusa*, phytochemistry screening, thin layer chromatography, terpenoids, steroids

INTRODUCTION

The diversity of ferns on this earth is very high. There are around 13,000 species of ferns on this earth, while the ferns in Indonesia, including the Malesiana area, are estimated to be 1250-1500 species (Machfira et al. 2016). The distribution of ferns is very large, as well as the potential and benefits that are high enough for human life, namely as ornamental plants, foodstuffs and medicines. The benefits of not all ferns are known because of the lack of information about the potentials of these ferns, one of which is the ferns *Lindsaea obtusa* J. Sm. Ex Hook .

Lindsaea obtusa is a terrestrial fern with 20-30 cm tall. These ferns have root fibers with a diameter of 1-2.5 mm, brown in color. *L. obtusa* stem is hard, stiff, and black with a length of 4-25 cm. The leaves of *L. obtusa* are folium compositum pinnatus leaves, and have black round petiolus. Leaflets are round or oval in shape, asymmetrical at the tip and base, smooth and green in color. *L. obtusa* has a round, brownish-green sorus located on the underside of the leaf. On one of leaf there are approximately 10-13 sorus. *L. obtusa* grows in forest shrubs especially in humid areas. This fern lives at an altitude of approximately 1000 meters above sea level (Dong et al. 2016). The true ferns *L. obtusa* live in temperate Asian regions such as Taiwan, and tropical climates such as Indonesia, parts of Java and Bali.

The phytochemical test is an important first step in research on the search for new active compounds derived from natural ingredients. This information can be used as initial information for the synthesis of new drugs or to become prototypes of certain active compounds (Kruk et al. 2021). Terpenoids are also called isoprenoids, this is because the carbon skeleton is the same as the isoprene compound. Synthesis and accumulation of terpenoid compounds contribute to medicinal properties for the treatment respiratory inflammation, atopic dermatitis, arthritis, and neuroinflammation among various inflammatory diseases (Kim et al. 2020).

Steroids are lipids that do not have fatty acid groups and are not ester derivatives. Steroids are complex, fat-soluble organic molecules. This compound acts as a major component of cell tissue (Rashidinejad et al. 2021). Synthetic steroids that are often used are glucocortico-steroids, estrogen, methylprednisolone, corticosteroids, androgens, squalamine and hydrocortisone. These steroid compounds are commonly used for the treatment of diseases due to neurodegenerative dissorder (Bansal and Singh, 2018). Ferns from the Equisetaceae, Osmundaceae, Lygodiaceae, Dennstaedtiaceae, Woodsidaceae, Thelypteridaceae and Dryopteridaceae families also contain steroid compounds (Yokota et al. 2017).

The method that can be used for phytochemical screening is chromatography. Chromatography is the separation of a mixture of compounds in a sample based on differences in the interaction of the sample with the stationary and mobile phases. One of the chromatography methods that can be used for phytochemical screening is Thin Layer Chromatography (TLC). Separation in Thin

Layer Chromatography is based on differences in polarity between the sample and the solvent. The stationary phase is a solid that is applied flat in the form of glass or aluminum as a support. The mobile phase is a mixture of several liquids of different polarity. Research on the content of terpenoids and steroid in *L. obtusa* was very limited. Therefore this study was conducted to determine the difference of terpenoids and steroid profile in *L. obtusa* J. Sm. Ex Hook.

MATERIALS AND METHODS

Sample preparation

Sampling of *L. obtusa* was carried out using the cruise method, namely exploring the area in the KGPAA Mangkunagoro I Botanical Forest Park, Ngargoyoso, Karanganyar, Central Java, Indonesia which generally contained these ferns. Extraction process and phytochemistry screening was conducted in the Biology Laboratory, Faculty of Mathemathics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia.

The samples of *L. obtusa* ferns were separated from dirt or foreign materials and then cleaned with running water until clean. These sample dried until the fern has reduced its moisture content. After drying, the sample is mashed using blender until it becomes a powder and then weighed which is then used for the extraction process (Seremet et al. 2020).

Secondary metabolites extraction

The extraction of secondary metabolites was carried out by maceration method using methanol and n-hexane as solvents. 100 g of *L. obtusa* powder was macerated using methanol as a solvent with a ratio of 1 : 5 which was carried out for overnight. The same is done with the n-hexane solvent. The extraction process is carried out by soaking in a solvent and then filtered using filter paper. The solvent is evaporated with a rotary evaporator at 400C, a speed of 40 rpm and a pressure of 0.06-0.08 MPa until a thick extract is obtained for approximately 1 hour (Sati et al. 2019). The viscous extract is weighed and the yield is calculated against the weight of the initial simplicia. The formula for the percentage of extract yield according to Wahyuni and Widjanarko (2015) is :

$$\text{Yield Extract} = \left| \frac{\text{extract weight obtained (g)}}{\text{extracted weight (g)}} \times 100\% \right.$$

Thin layer chromatography (TLC) profile analysis

Chemical compound profile analysis was performed on each extract using the Thin Layer Chromotagraphy (TLC) method. The n-hexane and methanol extracts were eluted using the same stationary and mobile phases. This was done to determine the results indicated by the presence of different color spots on TLC for each extract (Rubiyanto, 2013). The n-hexane and methanol extracts were spotted on the TLC plate with the silica gel GF254 as stationary phase and eluted using the mobile phase in the form of chloroform: n-hexane (9:1 v/v), n-hexane: ethyl acetate (4:1 v/v) and methanol: ethyl acetate (5:1 v/v) in the developer vessel. GF254 silica gel plate was made with a width of 2 cm and a length of 9 cm and given an initial limit of 1 cm and an end limit of 0.5 cm. The limit of the solvent is below the line where the spots are. After the eluent reaches the finish line of elution, the plates are removed and dried.

Analysis of secondary metabolite compounds

The detection of terpenoid and steroid content from n-hexane extract and methanol extract was used by Lieberman Burchard spray reagent. The parameters were observed by the terpenoid and steroid compounds from *L. obtusa* and the Rf (Retardation Factor) value (Yin et al. 2017). In thin layer chromatography, the degree of retention is expressed as Rf which can be formulated:

$$\text{Retardation Factor (Rf)} = \left| \frac{\text{Distance of movement of the solute}}{\text{Distance of movement of the solvent}} \right.$$

Lieberman Burchard is a spotting reagent for detecting steroids and terpenoids. The Lieberman burchard spray reagent was prepared by mixing 5 ml of acetic acid anhydride with 5 ml of concentrated sulfuric acid, then adding this mixture to 50 ml of absolute ethanol. Each substance mixing was carried out by cooling. The application of this method to the TLC plate was sprayed with Lieberman Burchard reagent and then heated for 10 minutes at a temperature of 100^0C. The presence of terpenoids is indicated by the appearance of a red violet color, while the presence of steroids is indicated by the appearance of a reddish brown color (Gummadi et al. 2021).

RESULTS AND DISCUSSION

Secondary metabolite extraction

Extraction of secondary metabolites from *L. obtusa* was carried out by maceration method using methanol and n-hexane as solvents. The results showed that the extraction with methanol as a solvent produced a higher yield than n-hexane (Table 1).

Extraction with methanol solvent resulted of 5.8 g so that the yield percentage was 5.8%. The extraction result with n-hexane solvent resulted of 0.7 g so that the yield percentage was 0.7%. The difference in yield percentage is due to differences in the solubility of the extracted compound in each solvent used, so that it can affect the yield and characteristics of the extracted chemical compound. The n-hexane solvent is intended to dissolve compounds that are semi-polar to nonpolar, while methanol is used to dissolve compounds that are more polar. Compounds can be said to be nonpolar compounds if they have bonds between atoms that have attractive ability to gain electrons together, resulting in a distant state where the electrons are shared equally. Meanwhile, if a compound has one atom capable of attracting electrons stronger than other atoms and the electrons from the bond will not be

used together equally, then the compound is called a polar compound (Tunega et al. 2020). These results are similar to those of (Azka and Abdullah, 2012) who showed that the secondary metabolites of water clover ferns *Marsilea crenata* most of them also dissolved in polar solvents with the yield of methanol extract of 11.98%, ethyl acetate extract of 1.37%, and chloroform extract of 0.31%. Methanol is a polar solvent while ethyl acetate and chloroform are semi polar and nonpolar solvents.

Thin layer chromatography profile analysis

Research shows that *L. obtusa* extract contains terpenoid and steroid compounds. This is indicated by a positive reaction to Liebermann-Burchard reagent (Table 2). The Liebermann-Burchard reaction was first described by Liebermann and then extensively developed by Burchard (Xiong et al. 2007). The Liebermann-Burchard reaction has been studied to determine the sterol content. Libermann Burchard reagent produces a varies color response depending on the double bond system, other functional groups, and the presence of non-polar bonds (Xiong et al. 2002). Liebermann-Burchard is a spotting reagent for detecting steroids and terpenoids. This analysis was based on the ability of terpenoid and steroid compounds forming colour with concentrated H_2SO_4 in anhydride acetic acid (Parbuntari et al. 2018). Liebermann-Burchard reagent will show a red-violet stain which indicates the presence of terpenoid compounds, while the presence of steroids is indicated by the appearance of a reddish brown stain.

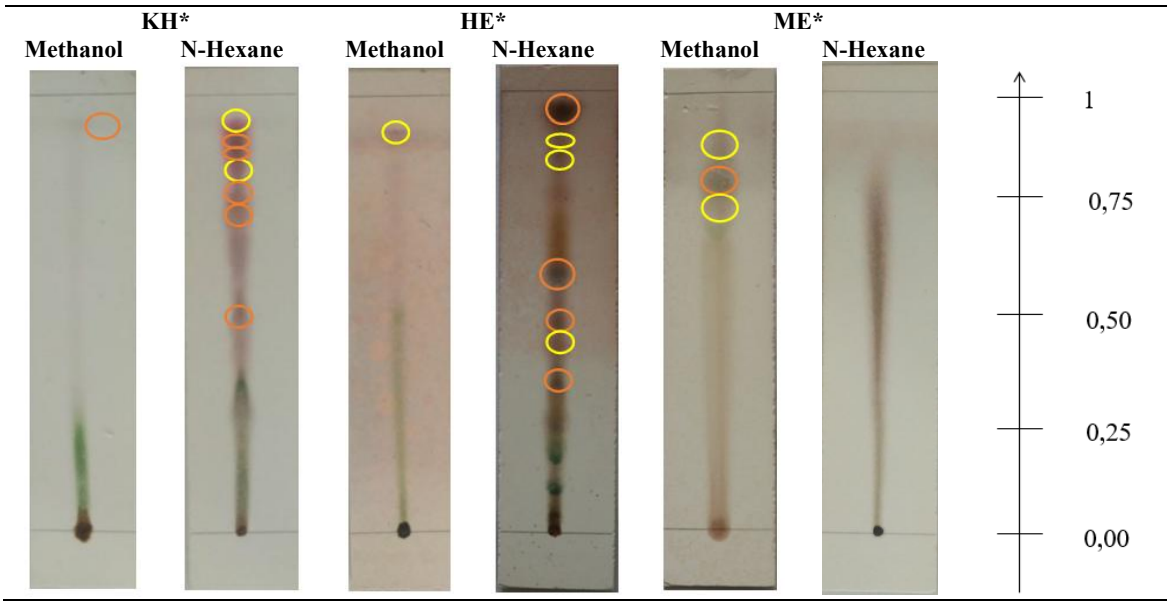
There are two fractions that give negative results on the detection of terpenoids with no visible red violet spots, namely in the mobile phase methanol extract of chloroform: n-hexane (9: 1 v/v) and n-hexane extract in the mobile phase of methanol: ethyl acetate (5 : 1 v/v) (Table 2). In the detection of steroids, there were also two fractions that gave negative results with no visible reddish brown spots, namely the mobile phase methanol extract n-hexane: ethyl acetate (4: 1 v/v) and n-hexane extract in the mobile phase of methanol: ethyl. acetate (5: 1 v/v).

Table 3 shows that the Rf values for detection of terpenoid compounds in the mobile phase of chloroform: n-hexane (9: 1 v/v) n-hexane extract are found at Rf 0.82 and 0.93. The methanol extract in the mobile phase of n-hexane: ethyl acetate (4: 1 v/v) was found at Rf 0.90 and for n-hexane extract 0.42; 0.76; and 0.85. Methanol extract in the mobile phase of methanol: ethyl acetate (5: 1 v/v) was found at Rf 0.73 and 0.88. Meanwhile, the Rf value for the detection of steroid compounds in the mobile phase of chloroform: n-hexane (9: 1 v/v) methanol extract was found at Rf 0.93 and for n-hexane extract 0.49; 0.64; 0.73; 0.86; and 0.90. The n-hexane extract in the mobile phase of n-hexane: ethyl acetate (4: 1 v/v) was found at Rf 0.34; 0.48; 0.58; and 0.96. Methanol extract in the mobile phase of methanol: ethyl acetate (5: 1 v/v) was found at Rf 0.78. These results indicate that the n-hexane extract gave clearer results than the methanol extract, especially in the mobile phase of n-hexane: ethyl acetate (4: 1 v/v).

Tabel 1. Maceration result of *Lindsaea obtusa* extract

Solvent	Powder mass (g)	Extract mass (g)	Yield (%)
Methanol	100	5.8	5.8
N-hexana	100	0.7	0.7

Table 2. Thin Layer Chromatography Profile (TLC) of *Lindsaea obtusa* extract with Liebermann-Burchard reagent



Note: *KH = chloroform: n-hexane (9: 1 v/v); HE = n-hexane: ethyl acetate (4: 1 v/v); ME = methanol: ethyl acetate (5: 1 v/v). The yellow sign indicates the presence of a terpenoid compound and the orange sign indicates the presence of a steroid compound

Table 3. Retardation Factor (Rf) value of *Lindsaea obtusa* extract with Liebermann-Burchard reagent

Senyawa	KH		HE		ME	
	Methanol	N-Hexane	Methanol	N- Hexane	Methanol	N- Hexane
Terpenoid	-	0.82	0.9	0.42	0.73	-
		0.92		0.76	0.88	
				0.85		
Steroid	0.93	0.49	-	0.34	0.78	-
		0.64		0.48		
		0.73		0.58		
		0.86		0.96		
		0.9				

Note: *KH = chloroform: n-hexane (9: 1 v/v); HE = n-hexane: ethyl acetate (4: 1 v/v); ME = methanol: ethyl acetate (5: 1 v/v).

The Rf value of 0.90 indicates the existence of a terpenoid compound. This is also found in medicinal Herb *Hypochaeris radiata* using High Performance Thin Layer Chromatography (HPTLC) technique (Senguttuvan and Subramaniam, 2016). The results of the thin layer chromatography profile showed that red-violet spots were more visible in the n-hexane extract than in the methanol extract. This is consistent with the nonpolar secondary metabolites of terpenoids and steroids. Most of the terpenoid compounds also contain an –OH group so that the presence of a hydroxyl group substituent attached to the hydrocarbon chain can be attracted by semi-polar and even polar solvents (Stachowiak et al. 2020). So it can be concluded that *L. obtusa* positively contains secondary metabolites in the form of terpenoids and steroids. Other ferns such as *Azolla microphylla* also contains terpenoid and steroid compounds (Rashad, 2021).

In conclusion, *Lindsaea obtusa* J. Sm. ex Hook extract contains terpenoid and steroid compounds. Quantitatively, the secondary metabolites of *L. obtusa* extract that were soluble in methanol solvent were more than the compounds that were soluble in n-hexane solvent. However, qualitatively, steroid compounds that dissolve in n-hexane solvents have a more varied Rf value than terpenoids, especially in the mobile phase of chloroform: n-hexane (9: 1 v/v) and n-hexane: ethyl acetate (4: 1 v/v).

REFERENCES

Azka A, Abdullah A. 2012. Aktivitas antioksidan dan komponen bioaktif semanggi air (*Marsilea crenata*). Jurnal Inovasi dan Kewirausahaan 1 (3): 152-158. [Indonesian]

Bansal R, Singh R. 2018. Exploring the potential of natural and synthetic neuroprotective steroids against neurodegenerative disorders: A literature review. Med Res Rev 38 (4) : 1126-1158. DOI: 10.1002/med.21458

Dong SY, Zuo ZY, Chao YS, Damas K, Sule B. 2016. New species of the fern genus *Lindsaea* (Lindsaeaceae) from New Guinea with notes on the phylogeny of *L.* Sect. *Synaphlebium*. PLoS One 11 (10): e0163686. DOI:10.1371/journal.pone.0163686

Gummadi SB, Belvotagi VAR, Kommidi DR, Bobbala RK, Achanta VNAR, Sterner, O. 2021. Steroidal glycosides from *Caralluma pauciflora*. Phytochem Lett 41: 43-48. DOI: 10.1016/j.phytol.2020.10.016

Kim T, Song B, Cho KS, Lee IS. 2020. Therapeutic potential of volatile terpenes and terpenoids from forests for inflammatory diseases. Int J Mol Sci 21 (6): 2187. DOI: 10.3390/ijms21062187

Kruk A, Piwowarski JP, Pawłowska KA, Popowski D, Granica S. 2021. High molecular pyrogens present in plant extracts interfere with examinations of their immunomodulatory properties in vitro. Sci Rep 11 (1): 799. DOI: 10.1038/s41598-020-79579-2

Machfira Y, Nery S, Fitmawati. 2016. Keanekaragaman Pteridaceae berdasarkan karakter morfologi dan fitokimia di Hutan PT. Chevron Pacific Indonesia (PT. CPI) Rumbai. Jurnal Riau Biologia1 (2): 165-172. [Indonesian]

Parbuntari H, Prestica Y, Gunawan R, Nurman MN, Adella F. 2018. Preliminary phytochemical screening (qualitative analysis) of cacao leaves (*Theobroma Cacao* L.). Eksakta 19 (2): 1-8. DOI: 10.24036/eksakta/vol19-iss2/142

Rashad S. 2021. An overview on the aquatic fern *Azolla* spp. as a sustainable source of nutrients and bioactive compounds with resourceful applications. Egyptian J Aquat Biol Fish 25 (1): 775-782. DOI: 10.21608/ejabf.2021.150205

Rashidinejad A, Marze S, Singh H. 2021. Lipid digestion and bioaccessibility of lipid-soluble compounds. In: Grundy MML, Wilde PJ (eds) Bioaccessibility and Digestibility of Lipids from Food. Springer, Cham. DOI: 10.1007/978-3-030-56909-9_10

Sati P, Dhyani P, Bhatt ID, Pandey A. 2019. *Ginkgo biloba* flavonoid glycosides in antimicrobial perspective with reference to extraction method. J Trad Complem Med 9 (1): 15-23. DOI: 10.1016/j.jtcme.2017.10.003

Senguttuvan J, Subramaniam P. 2016. HPTLC fingerprints of various secondary metabolites in the traditional medicinal herb *Hypochaeris radicata* L. J Bot 2016: 5429625. DOI: 10.1155/2016/5429625

Seremet L, Nistor OV, Andronoiu DG, Mocanu GD, Barbu VV, Maidan A, Rudi L, Botez E. 2020. Development of several hybrid drying methods used to obtain red beetroot powder. Food Chem 310: 125637. DOI: 10.1016/j.foodchem.2019.125637

Stachowiak W, Rzemieniecki T, Klejdysz T, Pernak J, Niemczak M. 2020. "Sweet" ionic liquids comprising the acesulfame anion - synthesis, physicochemical properties and antifeedant activity towards stored product insects. New J Chem 44 (17): 7017-7028.

Tunega D, Gerzabek MH, Haberhauer G, Lischka H, Solc R, Aquino AJA. 2020. Adsorption process of polar and nonpolar compounds in a nanopore model of humic substances. Europe J Soil Sci 71 (5): 845-855. DOI: 10.1039/C9NJ06005G

Wahyuni DT, Widjanarko SB. 2015. Pengaruh jenis pelarut dan lama ekstraksi terhadap ekstrak karotenoid labu kuning dengan metode gelombang ultrasonik. Jurnal Pangan dan Agroindustri 3 (2): 390-401. [Indonesia]

Xiong Q, Ruan B, Whitby FG, Tuohy RP, Belanger TL, Kelley RI, Wilson WK, Schroepfer GJ Jr. 2002. A colorimetric assay for 7-dehydrocholesterol with potential application to screening for Smith-Lemli-Opitz syndrome. Chem Phys Lipids 115 (1-2):1 -15. DOI: 10.1016/s0009-3084(01)00205-5

Xiong Q, Wilson WK, Pang J. 2007. The Liebermann-Burchard Reaction: Sulfonation, desaturation, and rearrangment of cholesterol in acid. Lipids 42: 87-96. DOI: 10.1007/s11745-006-3013-5

Yin D, Guan Y, Gu H, Jia Y, Zhang Q. 2017. Polymerized high internal phase emulsion monolithic material: A novel stationary phase of thin layer chromatography. RSC Adv 7 (12): 7303-7309. DOI: 10.1039/C6RA27609A

Yokota T, Ohnishi T, Shibata K, Asahina M, Nomura T, Fujita T, Ishizaki K, Kohchi T. 2017. Occurrence of brassinosteroids in non-flowering land plants, liverwort, moss, lycophyte and fern. Phytochem 136: 46-55. DOI: 10.1016/j.phytochem.2016.12.020.