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Original Article

Phytochemical Screening, Thin Layer Chromatography and Fourier Transform Infra-Red Spectroscopy Analysis of Eleutherine Bulbous (Mill.) Urb Bulb Extract

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ABSTRACT

Background: Many traditional medicinal plants were studied for their phytochemical components. Dayak onions (Eleutherine bulbous (Mill.) Urb) is one of traditional medicinal plant in Indonesia. Analysis of the phytochemical compounds in the extract of the plant would help in determining various biological activities of the plant. **Objective**: This study was primarily aimed to identify the phytochemical profile of Dayak onions extract and its fractions. **Methods**: The Dayak onion bulbs were collected from Pancur Batu District, North Sumatra Province, Indonesia. Multiple solvents used to for extraction and analysis of phytochemical compound by chemical reactions, thin layer chromatography (TLC) profiling, and Fourier Transform Infrared Spectrophotometer (FTIR) profiling. **Results**: The phytochemical screening of Dayak onions ethanol extract showed it contained alkaloids, flavonoids, glycosides, tannins, and triterpenes/steroids. The n-hexane fraction contained flavonoids and triterpenes/steroids, while the ethyl acetate fraction contains flavonoids and glycosides. The water fraction contained alkaloids, flavonoids, glycosides and tannins. By TLC profiling, the bioactive compounds in ethanol extract of the Dayak onion contain steroids, tannins, flavonoids and alkaloids. The bioactive compound in ethyl acetate fraction contains steroids, tannins, triterpenes, flavonoids and glycosides. The bioactive compound in n-hexan fraction contains steroids. The bioactive compound in water fraction fraction contains steroids and tannins. The FTIR demonstrated the presence of C-H alkanes, =C-H alkenes (aliphatic), C-O alcohol, ether, esters and carboxy acid in the ethanol extract, en-hexan fractions and ethyl acetate fractions. **Conclusion**: The phytochemical qualitative profiling showed that Dayak onion bulb as a rich source of bioactive compounds and have medicinal potential as therapeutic agent. A higher content of bioactive compounds of Dayak onion bulbs found in the ethanol and ethyl acetate extracts. The FTIR demonstrated the presence of C-H alkanes, =C-H alkenes (aliphatic), C-O alcohol, ether, esters and carboxy acid in the ethanol extract, en-hexan fractions and ethyl acetate fractions.

Keywords: Dayak onion, Eleutherine bulbous, Extract, Fraction, TLC, FTIR.

BACKGROUND

Dayak onions, or sabrang onions, or tiwai onions, with the scientific name Eleutherine bulbous (Mill.) Urb or Eleutherine Americana Merr. is a traditional medicinal plant that belongs to the Iridaceae family. Several studies have shown the use of Dayak onion leaves and bulbs for medical purposes such as gastrointestinal disorders, as a traditional contraceptive, inducing abortion by the people of Haiti, Guyana and Brazil.[1] This traditional medicinal plant is used by the Dayak tribe in Kalimantan to treat various diseases such as cancer, hypertension, and diabetes mellitus.[2] Onion Dayak is known to have potential activity as an anticancer plant. Extracts from Dayak onion bulbs have been shown to have the ability to inhibit the growth of several types of cancer in vitro. Dayak bulb extract has been shown to inhibit the growth of the TD47D breast cancer cell line[3,4], colon cancer cells Widr[5], cervical cancer cells (HeLa cells)[6] and leukemia cells L1210.[7] Flavonoid found in Dayak onion extract has the activity of stopping cancer growth through inhibition of colon cancer in the cell cycle of G1/S and G2/M phases.[8,9] The phytochemical compounds found in Dayak onions have unique characteristics and properties to the plant. Therefore, the analysis of these phytochemical compounds in the extract of the plant would help in determining various biological activities of the plant.

OBJECTIVE

The objective of this study was to identify the phytochemical profile of Dayak onions (Eleutherine bulbous (Mill.) Urb) extract and its fractions and hence to evaluate the medicinal potential of the plant and justify its traditional use.

MATERIALS AND METHODS

Materials

The fresh Dayak onion bulbs for the extract in this study were collected from Pancur Batu District, Deli Serdang Regency, North Sumatra Province, Indonesia. Identification and authentication of the plant species was carried out at the Medanense Herbarium, Universitas Sumatera Utara, Kampus USU Medan by external morphological character. For extraction process, characteristic identification and phytochemical screening process, we used ethanol 96% solution, n-hexan solution, ethyl acetate solution, Dragendroff reagent, Bouchardat, Meyer, Powder Mg^{+} amyl alcohol, HCl^{-}, H_2SO_4, $FeCl_3$, and Lieberman-Bourchat.

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Extraction Process

The fresh Dayak onion bulbs that have been collected then wet sorted to separate dirt or foreign materials, then washed thoroughly with running water, and dried using a drying oven with a temperature of not more than 60^0 C. The dried Dayak onion bulbs were then weighed as dry weight, and continued to grind the Dayak onion bulb to make the simplicia, then the powder is sieved until a fine simplicia powder is obtained. (10). Extract preparation is carried out by multilevel maceration, the simplicia was soaked with successive filter fluids n-hexane, ethyl acetate and ethanol. One part of fine dry simplicia powder is placed into a closed vessel, then 10 parts of solvent are added, and let soaked for 72 hours. After that, the macerate is separated by filtering. Then all the macerate is collected and evaporated using a vacuum evaporator or low pressures evaporator until finally a thick extract is obtained for each solution.[10] For crude ethanol extract we obtained the product yield as much as 11%, n-hexan fraction with product yield 10%, and ethyl acetate fraction with product yield 16.7%.

Characteristic identification

Analysis of the characteristics of Dayak onion bulb simplicia and the extract solution was carried out to determine water content, determine water soluble essence content, ethanol soluble essence content, total ash content and acid insoluble ash content.[10]

Phytochemical Screening

The phytochemical compounds in the extract and its fractions identified by a series of chemical reactions. The Dragendroff, Bouchardat and Meyer reagents were used to identify alkaloids. The alkaloids in the extract formed a reddish (Dragendorff reagent), brown (Bouchardat reagent) and white to yellowish (Meyer reagent) color in the test tube.[11,12] To identify the glycosides, we started by addition of 25 ml of H_2SO_4 to 5 ml of extract then boiled for 15 minutes. After the mixture cooled off , NaOH and 5 ml Fehling solution added and red brick color formation indicated the presence of glycosides.[11]

A simpler method was used to identify saponins by boiled the extract and then shaken. The presence of saponins would create a foam layer.[12] To identify the flavonoids, we used 0.1 mg of magnesium powder, 1 ml of amyl alcohol and hydrogen chloride to the test tube and yellow or orange to reddish color was the sign of flavonoids.[12] The greenish to black color indicated the presence of tannins after adding two drops of 1% $FeCl_3$ to the extract and its fractions.[12] The Liebermen-Bourchar test were used to identify the steroids and triterpene and after chloroform, acetic anhydride, and H_2SO_4 the extract would showed a dark pink or red color or reddish brown in the lower layer if it contained steroids and green color in the upper layer for triterpene.[12,13]

Thin Layer Chromatography (TLC)

The isolation of the fundamental components in Dayak onion bulbs extract and its fractions were performed using a thin layer chromatography (E. MERCK KGaA). The stationary phase adsorbent was a 10x10 cm High Performance Thin-Layer Chromatography (HPLTC) plates precoated with silica gel 60 F 254, the mobile phase solvent was a mixture of n-hexan:ethyl acetate (7:3), and visualization with vanillin – sulphate acid with ultraviolet lamp 254 nm and 366 nm. To activate the TLC plate, it must be dried in an oven followed by application of the extract and allowed to dry. Next the plate was put in the TLC tank, and the spots were migrated.

The Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) was used to identify the types of chemical bonds (functional groups) contained in Dayak onion bulb ethanol extract, n-hexan, ethyl acetate and water fractions. To determine the profile, the extract is mixed with ground powder, then homogenized and put into a sample container. The sample for each extract is put into the FTIR Spectrophotometer.[14]

RESULTS

Extract Characteristics

The Dayak onion bulb simplicia and its extract characteristics can be seen in table 1. The total ash content was 2.32% (simplicia) and 4.25% (extract). The content insoluble ash in acid was 0.37% (simplicia) and 1.04% (extract). The water content in simplicia was 8.65% and the extract contained 15.99%.

The Phytochemical Screening

Table 2 shows the phytochemical compounds in the Dayak onion bulbs extract and its fractions. The ethanol extract contained alkaloids, flavonoids, glycosides, tannins, and triterpenes/steroids. The n-hexane fraction contained flavonoids and triterpenes/steroids, while the ethyl acetate fraction contains flavonoids and glycosides. The water fraction of the Dayak onion bulbs contained alkaloids, flavonoids, glycosides and tannins.

The Thin Layer Chromatography (TLC) Profiling

The thin layer chromatography showed that Dayak onion bulb ethanol extract contained three (254 nm) and four (366 nm) color spots, n-hexan fraction contained one color spot (both 254 nm and 366 nm), and ethyl acetate fraction contained four (254 nm) and six (366 nm) color spots and water fraction contained one (254 nm) and two (366 nm) color spot. The Retention factor (Rf) value, color and identification of bioactive compounds can be seen in table 3.

Table 1. The characteristics of Dayak onion bulbs simplisia and extract.

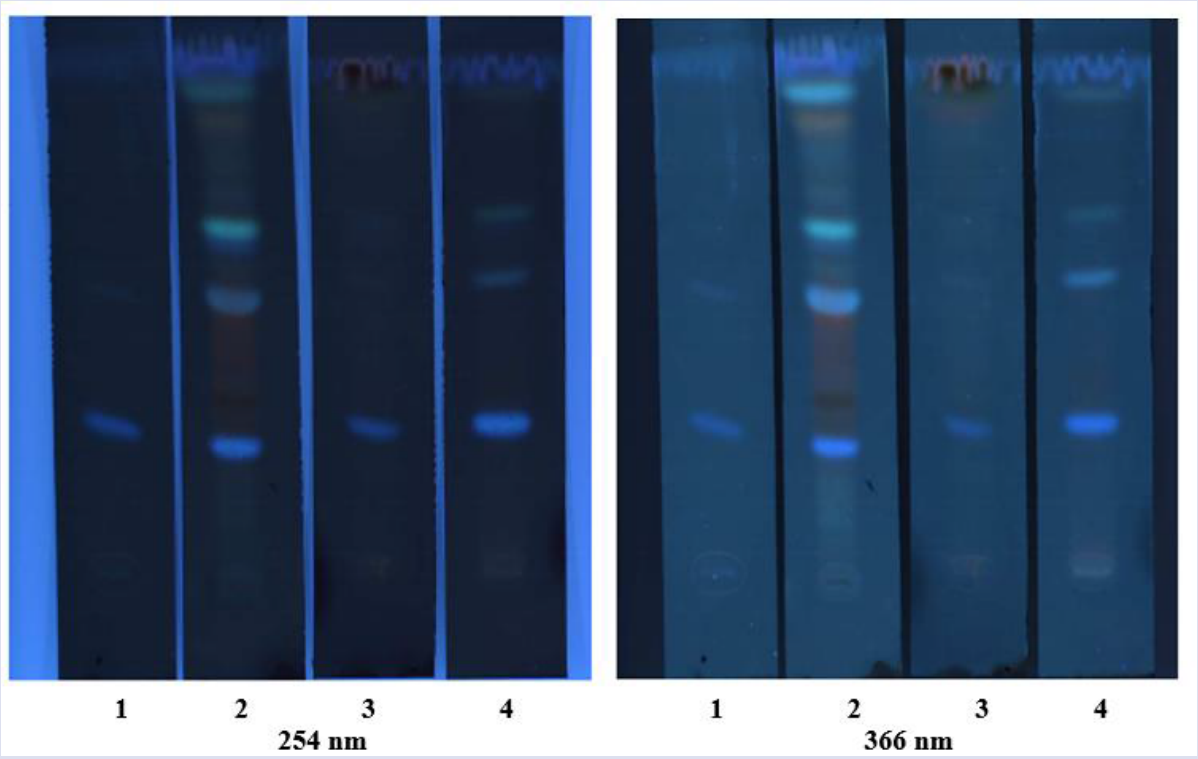
Characteristics	Simplicia (Percentage)	Extract (Percentage)
Ash content	2.32	4.25
Insoluble ash in acid	0.37	1.04
Water content	8.65	15.99
Soluble substance in water	13.80	
Insoluble substance in ethanol	7.60	

Table 2. Phytochemical compounds of Dayak onion bulbs extract and its fractions**.**

Phytochemical compounds	Reagents	Ethanol extract	n-hexan fraction	Ethyl acetate fraction	Water fraction
Alkaloids	Dragendroff	+	-	-	+
	Bouchardat	+	-	-	+
	Meyer	+	-	-	+
Flavonoids	Powder Mg+ amyl Alcohol + HCl^-	+	+	+	+
Glycosides	Molish+H_2SO_4	+	-	+	+
Saponins	Hot water/ shaken	+	-	-	+
Tannins	$FeCl_3$	+	-	+	+
Triterpene	Lieberman-Bourchat	+	-	-	-
Steroids	Lieberman-Bourchat	+	+	-	-

Table 3. Identification of compounds based on Rf values and color from TLC profiling.

Extract/ Fractions	Rf Value	Color	Colour from Reference(15)	Identification
Ethanol extract	0.225	Purple	Purple	Steroids
	0.5	Blue	Blue	Tannins
	0.5875 0.975	Yellow	Yellow	Flavonoids, alkaloids
n-hexan fraction	0.225	Purple	Purple	Steroids
Ethyl acetate fraction	0.2 0,5625	Purple	Purple	Steroids
	0.475	Blue	Blue	Tannins
	0.575	Green	Green	Tannins, triterpene
	0.125	Yellow	Yellow	Flavonoids, alkaloids
	0.25 0.4375	Red	Red	Glycosides
Water fraction	0.225	Purple	Purple	Steroids
	0.475	Blue	Blue	Tannins



UV Lamp	1 Water Fraction	2 Ethyl Acetate Fraction	3 n-hexan Fraction	4 Ethanol Extract
254 nm	Rf 1 (Purple): 0.225	Rf 1(Purple): 0.2 Rf 2 (Blue): 0.475 Rf 3 (Purple): 0.5625 Rf 4 (green): 0.575	Rf 1(Purple): 0.225	Rf 1 (Purple): 0.225 Rf 2 (Blue): 0.5 Rf 3 (Yellow): 0.5875
366 nm	Rf 1 (Purple): 0.225 Rf 2 (Blue): 0.475	Rf 1 (Yellow): 0.125 Rf 2 (Purple): 0.2 Rf 3 (Red): 0.25 Rf 4 (Red): 0.4375 Rf 5 (Blue): 0.475 Rf 6 (Green): 0,575	Rf 1(Purple): 0.225	Rf 1 (Purple): 0.225 Rf 2 (Blue): 0.5 Rf 3 (Yellow): 0.5875 Rf 4 (Yellow): 0.975

Figure 1. TLC Profiling of Dayak onion bulbs extract and its fractions.

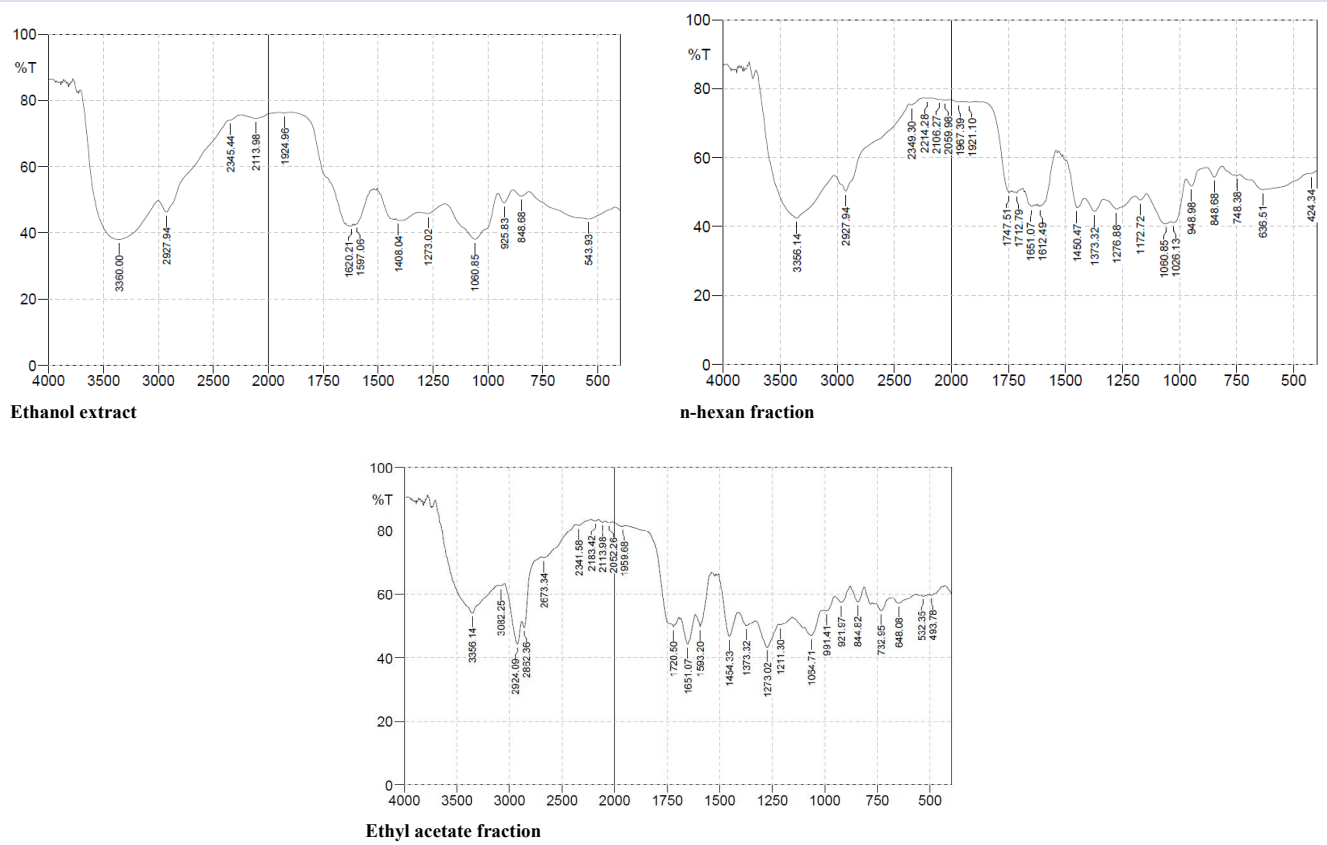


Figure 2. The absorbance of Dayak onion bulb ethanol extract and its fractions.

The Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR results confirmed the biopolymer functional groups found in Dayak onion bulb extract and its fraction. The Dayak onion bulb ethanol extract showed absorbance of C-H alkanes at wavenumber 2927.94 and 1408.04 cm^{-1}, =C-H alkenes (aliphatic) at wavenumber 848.68 and 925.83 cm^{-1}, O-H at wavenumber 3360 cm^{-1}, C=C alkenes at wavenumber 1620,21 cm^{-1}, C=C aromatics at wavenumber 1597.06 cm^{-1}, C≡C alkynes at wavenumber 2113.98 cm^{-1}, C-O alcohol, ether, ester, carboxy acid at wavenumber 1060.85 and 1273.02 cm^{-1}.

The n-hexane fraction showed absorbance of C-H alkanes at wavenumber 2862.36, 2924.09, 1373.32 and 1454,33 cm^{-1}, =C-H alkenes (aliphatic) at wavenumber 3082.25, 732.95, 844.82, 921.97 and 991.41 cm^{-1}, O-H phenol, alcohol monomer, hydrogen bond alcohol phenol at wavenumber 3356.14 cm^{-1}, O-H hydrogen bond carboxylate acid at wavenumber 1593.2 cm^{1}, C=C alkenes at wavenumber peak value 1651.07 cm^{-1}, C=C aromatic at wavenumber 1593.2 cm^{-1}, C≡C alkyne at wavenumber 2113.98 and 2183.42 cm^{-1}, C-O alcohol, ether, ester, carboxy acid at wavenumber 1064.71, 1211.3 and 1273.02 cm^{-1}, C-O at wavenumber 1720.5 cm^{-1}.

The ethyl acetate fraction showed absorbance of C-H alkanes at wavenumber 2927.94, 1373.32 and 1450.47 cm^{-1}, =C-H alkenes (aliphatic) at wavenumber 748.38, 848.68 and 948.98 cm^{-1}, O-H phenol, alcohol monomer, hydrogen bond alcohol phenol at wavenumber 3356.14 cm^{-1}, C=C alkenes at wavenumber peak value 1612.49 and 1651.07 cm^{-1}, C≡C alkyne at wavenumber 2106.27 and 2214.28 cm^{-1}, C-O alcohol, ether, ester, carboxy acid at wavenumber 1060.85, 1172.72 and 1276.88 cm^{-1}, C-O at wavenumber 1712.79 and 1747.51 cm^{-1}.

DISCUSSIONS

Quality control of simplicia and extracts is one of the requirements that must be carried out to ensure their quality and pharmacological effectiveness. The quality of simplicia and extracts is influenced by many aspects, such as processes before the plant is harvested, for example genetic aspects of the plant, planting area, planting conditions, and harvest time, as well as post-harvest processes, such as drying, extraction and storage.[16] The total ashes value of extract raw materials is determined to evaluate the quality and purity of raw materials containing inorganic radicals such as phosphate, carbohydrates, potassium, magnesium, ferrous calcium and lead as well as to prove that simplicia does not contain dangerous heavy metals.[17,18] Preethy et al found that the total ash content of Eleutherine bulbous (Mill.) Urb tubers originating from the Botanical garden of Vaidyaratnam P.S.Varier Ayurveda College, Kottakkal, Kerala, India was 3.12%[18], while the total ash content of our simplicia was only 2.32%.

There are several standards that are used as a reference for good simplicia, including water content which must be less than 10%.[19] The water content on simplicia that will be used as raw material for extracts should not exceed 14% to avoid bacterial growth. Water content also crucial because it may influence the stability and affecting the pharmacological effectiveness.[20] Our simplicia meet the standard by Indonesian Ministry of Health and by Chanda S 2014 because its water content only 8.65% although the specific standard profile for Dayak onion bulb extract simplicia and extract has not been listed in the Monografi Farmakope Herbal Indonesia.[10]

Analysis of the bioactive compounds in the extract and simplicia is used to estimate the bioactivity and mechanism of action which will provide an overview of the pharmacological activity of the extract.[16]

Based on the phytochemical screening of the ethanol extract of the Dayak onions bulb and its fractions in this study contain alkaloids, flavonoids, glycosides, tannins and triterpenes/steroids. Research on the phytopharmaceutical content of Dayak onions mostly comes from analysis of the tubers and shows the presence of secondary metabolites such as napthalene, napthaquinone and anthraquinone.[1,21] Putri and Haryoto in 2018, who analyzed the ethanol extract content of Dayak onions, found that it contained alkaloids, polyphenols, steroid saponins and flavonoids[4] Preethy et al, found that Eleutherine bulbous (Mill.) Urb bulbs originating from the Botanical Garden of Vaidyaratnam P.S.Varier Ayurveda College, Kottakkal, Kerala, India in hot temperature water and alcohol fractions of Dayak onion extract contained carbohydrates, steroids and tannins. Meanwhile, the cold alcohol fraction contains flavonoids, alkaloids, carbohydrates, steroids and tannins.[18] Lubis et al in found that Dayak onion ethyl acetate extract originating from plantations in Simalingkar B Village, Medan Tuntungan District, North Sumatra also contained alkaloids, flavonoids, glycosides, saponins, triterpenoids and steroids.[5]

TLC profiling of the Dayak onions extract and its fractions reveals an important information about the presence of various numbers of phytochemicals with variation of Rf values in different solvent system. This different Rf values of the phytochemicals adds a very important data of their polarity and also provide a guidance for the selection of proper solvent system. The ethanol extract showed 3 bands having Rf values of 0.225, 0.5, 0.5875 (254 nm UV lamp) and 4 bands having Rf values of 0.225, 0.5, 0.5875, 0.975 (366 nm UV lamp). With n-hexan fraction showed one band having Rf value of 0.225. With ethyl acetate showed 4 bands having Rf values of 0.2, 0.475, 0.5625, 0.575 (254 nm UV lamp) and 6 bands having Rf values of 0.125, 0.2, 0.25, 0.4375, 0.475 (366 nm UV lamp). With water extract showed one band having Rf value of 0.225 (254 nm UV lamp) and 2 bands having Rf values of 0.225, 0.475 (366 nm UV lamp). This result will aid in choosing the convenient solvent system for further separation of bioactive compound from the Dayak onions plant.

We used n-hexan:ethyl acetate (7:3) mixture as mobile phase solvent for TLC analysis. This solvent mixture known to be nonpolar so they can attract compounds like alkaloid, flavonoids and steroids. The ethanol extract of the Dayak onions showed purple (Rf 0.225) for steroids, blue (Rf 0.5) for tannins, and yellow (Rf 0.5875;0.975) for flavonoids and alkaloids. The n-hexan fraction showed purple (Rf 0.225) for steroids. The ethyl acetate fraction showed purple (Rf 0.2;0,5625) for steroids, blue (Rf 0.475) for tannins, green (Rf 0.575) for tannins and triterpene, yellow (Rf 0.125) for flavonoids and red (Rf 0.25; 0.4375) for glycosides. The water fraction showed purple (Rf 0.225) for steroids and blue (Rf 0.475) for tannins.[15]

The FTIR were used to predict the presence of the chemical bonds or functional groups in the Dayak onions extracts by interpreting the infrared absorption spectra. In the ethanol extracts, strong bonds were found at 2927.94 cm^{-1}, 1408.04 cm^{-1}, 848.68 cm^{-1}, 925.83 cm^{-1}, 1060.85 cm^{-1}, 1273.02 cm^{-1}, while the others varied from weak to medium. These results demonstrated the presence of C-H alkanes, =C-H alkenes (aliphatic), C-O alcohol, ether, ester and carboxy acid in the ethanol extracts.

In the n-hexan fractions, strong bonds were found at 2862,36 cm^{-1}, 2924,09 cm^{-1}, 1373,32 cm^{-1}, 1454,33 cm^{-1},732.95 cm^{-1}, 844.82 cm^{-1}, 921.97 cm^{-1}, 991.41 cm^{-1}, 1064.71 cm^{-1}, 1211.3 cm^{-1}, 1273.02 cm^{-1}, 1720.5 cm^{-1}, while the others varied from weak to medium. These results demonstrated the presence of C-H alkanes, =C-H alkenes (aliphatic), C-O alcohol, ether, esters and carboxy acid in the n-hexan fractions.

In the ethyl acetate fractions, strong bonds were found at 1373.32 cm^{-1}, 1450.47 cm^{-1}, 748.38 cm^{-1}, 848.68 cm^{-1}, 948.98 cm^{-1}, 1060.85 cm^{-1}, 1172.72 cm^{-1}, 1276.88 cm^{-1}, 1712.79 cm^{-1}, 1747.51 cm^{-1}, while the others varied from weak to medium. These results demonstrated the presence of C-H alkanes, =C-H alkenes (aliphatic), C-O alcohol, ether, esters and carboxy acid in the n-hexan fractions.

CONCLUSION

The phytochemical qualitative profiling showed that Dayak onion bulb as a rich source of bioactive compounds and have medicinal potential as therapeutic agent. A higher content of bioactive compounds Dayak onion bulbs found in the ethanol and ethyl acetate extracts, which can be used as proper solvent system for further researches on this plant. From TLC profiling we know that the bioactive compounds in ethanol extract of the Dayak onion contain steroids, tannins, flavonoids and alkaloids. The bioactive compound in ethyl acetate fraction contains steroids, tannins, triterpenes, flavonoids and glycosides. The FTIR demonstrated the presence of C-H alkanes, =C-H alkenes (aliphatic), C-O alcohol, ether, esters and carboxy acid in the ethanol extract, n-hexan fractions and ethyl acetate fractions. These finding opens up possibilities for the development of natural products from Dayak onions in drug discovery.

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- **Author's contribution:** LDL contributed in study conception and design, data acquisition, analysis and interpretation, and drafting article. MFGS and F contributed in study conception and design, data analysis and interpretation, and drafting article. IPAN contributed in data analysis and interpretation, and drafting article. DS, KBS, PCE, IA contributed in study conception and design, data analysis and interpretation, drafting article. MR and MMA contributed in drafting article. All authors: manuscript critically revised and final approval.
- **Declaration of interest**: All authors declare no conflicts of interest.

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