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IDENTIFICATION OF FLAVONOID COMPOUNDS FROM PURIFIED EXTRACT OF BEETROOT LEAF (BETA VULGARIS L)

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ABSTRACT
Flavonoids are one of the secondary metabolites that have good pharmacological activity. One of the plants that contain flavonoid compounds is beetroot leaves. Beetroot leaves are waste or crop residues from beetroots that are rarely used. The use of extracts from plant leaves in the pharmaceutical world needs to consider the presence of ballast substances that can affect the activity of the extract. However, the purification process using organic solvents has the potential to lose the content of compounds contained in the extract, one of which is flavonoids. Therefore, this study aims to examine the profile of flavonoid compounds contained in purified extracts of beetroot leaves compared to the extract content. The study began with the manufacture of beetroot leaf extract, followed by the purification process of the extract using n-hexane and ethyl acetate as solvents. Extracts and purified extracts of beetroot leaves were then analyzed using Thin Layer Chromatography (TLC), UV-Vis Spectrophotometer and Infra Red (IR) Spectrophotometer. The results showed that the profile of flavonoid compounds contained in purified extract of beetroot leaves had similarities with the profile of flavonoid compounds found in beetroot leaf extract.

Keywords: beet; isolation; partition

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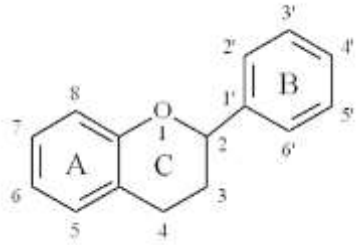
INTRODUCTION

Flavonoids are one of the polyphenol group compounds that are widespread in plants (Panche et al., 2016) Flavonoids have a characteristic structure, namely the basic framework is composed of 15 carbon atoms arranged in the form of C6-C3-C6 which is two aromatic rings connected by 3 carbon atoms (Santos et al., 2017) The rings are named rings A, B and C.



Ficture 1. Flavonoid Structure (Thilakarathna & Vasantha Rupasinghe, 2013)

The biosynthesis of flavonoids begins with the condensation of a molecule of p-coumaroylCoA (a shikimate derivative, ring B) with three molecules of malonyl-CoA (polyketide, ring A). This is to give a chalcone group (2 , 4 , 6 , 4-tetrahydroxychalcone).

Chalcone will then be isomerized by the enzyme chalcone flavanone isomerase (CHI) into flavanones. The reactions that occur in flavanones will produce various types of flavonoids (Santos et al., 2017).

There are more than 7000 types of flavonoids in nature which are divided into six classes based on their structure, namely flavonols, flavan-3-ol, isoflavones, flavanones, flavones and athocyanins. Differences in these structural components will affect the pharmacological activity produced (Thilakarathna & Vasantha Rupasinghe, 2013). One of the important pharmacological activities of flavonoids is antioxidant (Andarwulan et al., 2010) (Brunetti et al., 2013) (Yadnya Putra et al., 2020).

One of the plants that contain flavonoids is beetroot (Beta vulgaris L). The most frequently used part of the beet plant is the tuber, while the leaf part of the plant has not been widely used. The ethanol extract of beetroot leaves has been studied to contain flavonoids (Mzoughi et al., 2019) with a level of 1.5 mg QE/g FW (Gawlik-Dziki et al., 2020). This makes the ethanol extract of beetroot leaves has the potential to be developed in pharmaceutical preparations. However, in the development of pharmaceutical preparations crude extract has a weakness, namely it is still mixed with other components that are not needed so that the development of the preparation will be limited. One way to separate the components of unwanted compounds in the extract is to perform extract purification. Based on this background, this research was conducted to qualitatively identify the flavonoid compounds contained in the purified extract of beetroot leaves compared with the crude extract.

METHOD

This research is an experimental research. This study looked at the effect of the purification process on the extract on the profile of its flavonoid content. The research was carried out using tools including oven (Memmert®), rotary evaporator (IKA®), water bath (Memmert®), UV-Vis spectrophotometer (Shimidzu®), cuvette (helmet), IR spectrophotometer, UV lamp 254 and 366 nm. The materials used in this study were beetroot leaves (Beta vulgaris var Rubra (L) Moq.), 70% ethanol, n-hexane, ethyl acetate, AlCl3 (Merck®), Silica GF 254 Thin Layer Chromatography (TLC) plate. , and standard quercetin (Sigma Aldrich ®). The research population is beetroot leaves harvested from beetroot cultivation in Selo District, Boyolali Regency, Central Java Province. The samples used were beetroot leaves which were harvested from the cultivation of beetroot plants in the Selo District, Boyolali Regency, Central Java Province.

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Extract Making

Simplicia powder of beetroot leaves was macerated using 70% ethanol as a solvent with a ratio of ingredients: total solvent was 1:10. Maceration was divided into 2 stages, the first stage was beetroot leaf material soaked in 70% ethanol with a ratio of 1:7.5 for 3 days. After 3 days the filtrate and dregs were separated. The dregs are then soaked again with the remaining solvent, namely 1:2.5 for 2 days. The filtrate obtained in steps 1 and 2 were combined and evaporated using a rotary evaporator and a water bath to obtain a thick extract.

Purified Extract Manufacturing

The thick extract of beetroot leaves obtained in the next step was suspended using 30% ethanol, then fractionated using n-hexane and ethyl acetate in the same proportion as 30% ethanol. Furthermore, the ethyl acetate fraction is concentrated to become a thick extract called purified extract of beetroot leaves (Puspitasari and Pramono., 2015)

Identification of Thin Layer Chromatography
Extracts and purified extracts of beetroot leaves, and quercetin were dissolved in ethanol. The purified extracts and extracts as well as quercetin standards were then spotted on a 254 nm GF silica plate, and eluted using various mobile phase systems. The mobile phase used was butanol:acetic acid:water (4:2:1), methanol: acetic acid (7:6), and methanol: acetic acid (8:6).

UV-Vis Spectroscopy Identification
Extracts and purified extracts of beetroot leaves were dissolved in ethanol. The solution was then scanned at a wavelength of 200-400 nm on a UV-Vis spectrophotometer.

IR Spectroscopic Identification
The extract and purified extract of beetroot leaf were each compressed into a thin and transparent pellet. The pellet was then read its absorption on an IR . spectrophotometer

RESULTS
The TLC results from each optimization are shown in Figure 2.

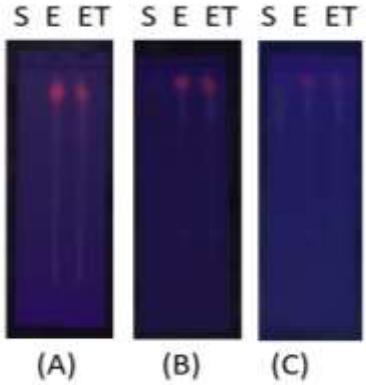
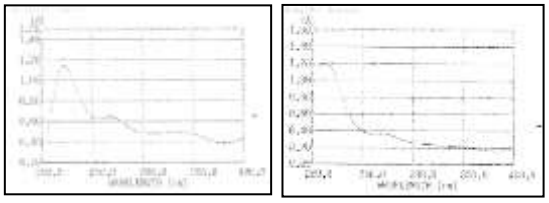


Figure 2. TLC Profile of Extract (E), Purified Extract (ET) and Quercetin Standard (S) at 366 nm UV. (A) Optimization of 1 mobile phase Butanol:Acetic acid:Water (4:2:1); (B) Optimization of 2 mobile phases methanol:acetic acid (7:6); (C) Optimization of 3 mobile phases methanol:acetic acid (8:6)

TLC identification was carried out at UV 366 nm, because flavonoid compounds will show yellowish green fluorescence at UV 366 nm (). Figure 2 shows the results that optimization of TLC 3 using 8:6 methanol mobile phase showed the clearest spot of quercetin standard with an Rf value of 0.88. The quercetin spot looks parallel to the spot in the extract and purified extract. This shows that based on the results of TLC, one of the flavonoid compounds contained in the extract and purified extract is quercetin. The TLC results produced in optimization 1, 2 and 3 showed the presence of red fluorescence spots in the extract and purified extract. This is thought to be the chlorophyll compounds present in the extracts and purified extracts. This shows that the purification process has not been able to remove chlorophyll optimally.

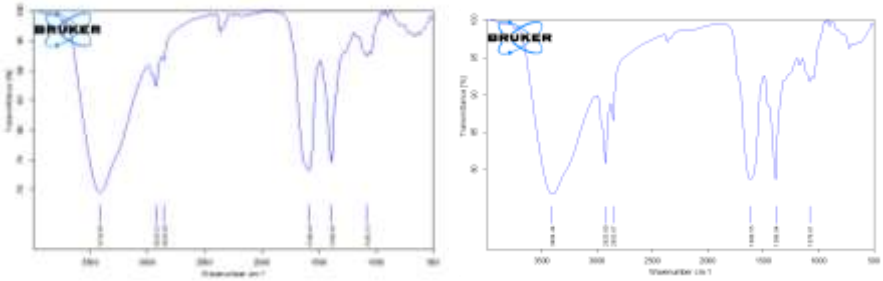
Identification of flavonoid compounds was also carried out using the UV-Vis spectrophotometer method. The results of the UV-Vis spectrophotometer on the extract, purified extract and quercetin standard in this study are shown in Figure 3.



(a) (b)

Figure 3. Spectra of identification using UV-Vis Spectrophotometer (a). Spectra of Beetroot Leaf Extract (b) Spectra of Beetroot Purified Extract (c) Quercetin Standard Spectra

Figure 3 shows that purified extract of beetroot leaves has fewer peaks than beetroot extract at the same concentration. This indicates that there are compounds that were previously present in the beetroot leaf extract, but not in the peak scan results of the purified extract of the beetroot leaf. This could be because the compound was dissolved in n-hexane which was used to purify the extract.Qualitative identification of the content contained in extracts and purified extracts was also carried out using an IR spectrophotomer. The spectra of the results of the IR spectrophotomer along with the interpretation of the wave numbers of the extracts and purified extracts are shown in Figure 4 and Table 1**.**



(a) (b)

Figure 4. Spectrophotometer results of IR spectrophotometer (a) Extract of beetroot leaf (b) purified extract of beetroot leaf.

Table 1.
Interpretation of IR . Spectra

No	Bilangan Gelombang (cm^{-1})		Bentuk Pita	Intensitas	Dugaan Gugus Fungsi
	E	ET			
1	3416,86	3404,44	Lebar	Medium	OH
2	2925,23 2855,06	2923,8 2853,67	Tajam	Lemah	C-H alifatik
3	15864,8 1389,55	1608,55 1386.94	Tajam	Medium	C=C aromatik
4	1080,70	1078,61	Lebar	Lemah	C-O

Information :
E = Beetroot leaf extract
ET = Purified extract of beetroot leaves

DISCUSSION

Purified extract is an extract that has been separated from ballast substances that can interfere with the pharmacological activity of the active compound of the extract (Widyaningtias et al., 2014). The purification process carried out in this study used n-hexane which is non-polar. The use of non-polar n-hexane can attract non-polar compounds, both ballast substances that

can affect pharmacological activity, but also has the potential to attract other non-polar active compounds. Flavonoids are compounds that have polarity from polar ones such as glycosides to non-polar ones such as isoflavones, flavanones, flavone alcohols and flavanols (Hendryani et al., 2015). So in this study, we wanted to see the effect of the purification process on the profile of the main flavonoids in the beetroot leaf extract.

Identification of flavonoid compounds in extracts and purified extracts was carried out using Thin Layer Chromatography (TLC), UV-Vis spectrophotomer and IR Spectrophotometer. TLC identification was carried out using the stationary phase of GF 254 silica plate. The GF 254 nm silica plate was a polar stationary phase. The mobile phase used in this research is 3 mobile phase optimization system. The optimization mobile phase 1 is butanol:acetic acid:water (4:2:1). Optimization 2 uses methanol as mobile phase: acetic acid (7:6), and the third optimization uses methanol as mobile phase: acetic acid (8:6). The mobile phase selected in optimization 1 was based on research related to the identification of flavonoids by TLC previously conducted by Haeria (2013) (Haeria, 2013). However, in the research conducted using the optimization of the mobile phase, the spots of the quercetin compound could not be seen properly. Therefore, optimization was carried out using 2 types of mobile phase, namely methanol: acetic acid (7:6) and methanol: acetic acid (7:6).

CONCLUSION

The results showed that there were similarities between the profiles of flavonoid content contained in the extract and purified extract of beetroot leaves.

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