

## Qualitative and Quantitative Characterization of Secondary Metabolites of Qust' Al-Hindi (*Saussurea Lappa*) Plants

### Karakterisasi Kualitatif dan Kuantitatif Metabolit Sekunder Tanaman Qust' Al-Hindi (*Saussurea Lappa*)

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#### ABSTRACT

Utilization of plants that have the potential as herbal medicines, including the Qust' Al-Hindi (*Saussurea Lappa*) plant. Identification of the secondary metabolite content of the Qust' Al-Hindi Plant (*Saussurea Lappa*) through Phytochemical analysis in the form of flavonoid, alkaloid, steroid, terpenoid, tannin, and saponin tests on the extraction results as well as the calculation of flavonoid levels which are the largest content of this plant carried out using the UV method. -Vis. The characterization of the presence of a class of secondary metabolites contained in the plant was analyzed using FTIR-ATR spectroscopy. The results of this study indicate that the plant contains secondary metabolites in the form of flavonoid compounds, alkaloids, terpenoids, and tannins. The flavonoid content obtained was  $223.33 \pm 66.5$  mgQE/g, while the results of the FTIR-ATR test showed that the extract contained the same functional groups as flavonoids, alkaloids, terpenoids, and tannins, namely the O-H, C-H, C=C functional groups. C=O and C-O contained in the structure of flavonoids, alkaloid compounds are known to have a distinctive functional group, namely N-H, for terpenoids to have a distinctive functional group, namely C-H, namely CH<sub>2</sub> and CH<sub>3</sub>, there is also CH and there is a C-O group which is a typical functional group of compounds tannins.

**Keywords:** Qust' Al-Hindi; Phytochemical Analysis; Flavonoids; UV-Vis analysis; FTIR-ATR analysis.

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#### 1. INTRODUCTION

Some herbal medicines have no known overall content in them which can have side effects on health. In the presence of various diseases caused by free radicals such as rheumatic, arthritis, respiratory disorders, and systemic lupus erythematosus. Thus, encouraging researchers to develop new alternative drugs, namely utilizing plants which are one of the largest pharmaceuticals in nature. Many plants produce a diverse group of secondary metabolites known as phytochemical compounds (Abdallah, et.al.,2017). Qust' Al-Hindi (*Saussurea Lappa*) is a traditional plant used as a stimulant, antiseptic, carminative, sedative, bronchodilator, and astringent agent (Abdallah, et.al.,2017). Based on scientific research that has been carried out, this plant has substances that help in the treatment of several diseases related to the overactivity of the complement system, such as rheumatism, arthritis, respiratory disorders, and systemic lupus erythematosus (Abdallah, et.al.,2017). This plant has become an important drug in the international market as well as medicine in India, it can be used alone or in combination with other drugs (Gwari, et.al., 2013).

Several methods can be used to extract secondary metabolites, including soxhlet, distillation, and maceration methods. The soxhlet method is more efficient when compared to distillation and

maceration because it has several advantages, namely organic solvents can repeatedly attract organic compounds in natural materials, and in time their use is more efficient (Febryanto, 2017).

Previously, research related to Qust' Al-Hindi for volatile fractions has been carried out by (Indah, in 2021). From the results of this study, it is known that there are secondary metabolites in the form of essential oils and after characterization, it is known that there is also flavonoid content<sup>4</sup>. Therefore, in this study, characterization of secondary metabolites which are expected to come from non-volatile parts was carried out. Qualitative tests were carried out with phytochemical tests and FTIR analysis to determine the content of secondary metabolites in the Qust' Al-Hindi plant, while quantitative tests were focused on detecting the presence of flavonoid compounds, using a UV-Vis spectrophotometer. In this final project research, there are several objectives to overcome the existing problem formulation. The objectives to be achieved in this final project research are, identifying the phytochemical content of the plant extract of Qust' Al-Hindi, determining the flavonoid content of the plant extract of Qust' Al-Hindi, determine the functional groups present in the extract of the Qust Al-Hindi plant.

## 2. MATERIALS AND METHODS

### 2.1. *Materials (Times New Roman 11 pts, italicized)*

Qust' Al-Hindi samples from India, 96% ethyl acetate technical, aquadest, HCl 1M, 96% ethanol p.a, Mg solids, acetic acid solution, quercetin solids (Merck), AlCl<sub>3</sub> (s) from Merck.

### 2.2. *Methods (Times New Roman 11 pts, italicized)*

#### 2.2.1. *Soxhlet extraction*

A total of 25 g of Qust' Al-Hindi solids were weighed using an analytical balance. The solids that have been weighed are placed in filter paper and rolled up, then tied to both ends of the filter paper using thread and put in a Soxhlet tube. Soxhlet flask filled with ethyl acetate as much as 200 mL was heated at a temperature of 60 - 80°C solvent boiling point for 6 hours. After that, the extract solution was evaporated/distilled at a temperature of 80°C to obtain a thick extract of Qust' Al-Hindi and remove the solvent that was still mixed. The results obtained are stored in a closed vial and labeled SE (Sample Evaporator) which is the result of the first soxhlet which is then evaporated using a rotary evaporator while those using distillation are labeled SD (Distillation Sample), then weighed to calculate the percent yield.

#### 2.2.2. *Phytochemical analysis*

Phytochemical analysis was carried out using standard methods referring to the research of Abdallah. 2017. The analysis carried out was in the form of analysis of Flavonoids, Alkaloids, Steroids and terpenoids, Tannins and saponins.

### 2.3. *Characterization*

#### 2.3.1. *UV-Vis spectrophotometry*

The Qust' Al-Hindi extract was then characterized using a UV-Vis spectrophotometer using a standard compound of quercetin to determine the levels of flavonoids obtained. Here are the steps of the procedure carried out.

#### 2.3.2. *FTIR spectrophotometry*

Characterization using FTIR ATR spectrophotometer instrument. The pure sample ( $\pm$  2 mL) was placed in contact with the ATR crystal, the extract was placed directly on the sampling plate above the optical window with the ZnSe crystal, then held by a micrometer controlled compression clamp to ensure good contact between the sample and the crystal. The sample is read using the FTIR ATR tool, then the resulting chromatogram is compared with the IR table.

## 3. RESULTS AND DISCUSSION

### 3.1. *Extraction of Qust' Al-Hindi (Saussurea Lappa)*

The process of extracting the Qust' Al-Hindi plant will be applied as a natural herbal medicine with the soxhlet extraction method using ethyl acetate as a solvent. According to Budiayati, et al. 2013., one of the factors that affect eaching (solids extraction) is particle size, namely the smaller the particle size, the larger the surface area, so the speed of dissolving into the particles increases. Then the extract obtained was weighed to determine the weight of the pure extract that had been

obtained, then the extract was labeled with the sample code S1E (Soxhlet 1 Evaporation), S2D (Soxhlet 2 Distillation Distillation), and S3D (Soxhlet 3 Distillation). The results of soxhlet extraction from the Qust' Al-Hindi plant are shown in **Table 1**.

**Table 1.** Percentage %Yield Extract

Code	Powder Mass (g)	Extract Mass (g)	% Yield (w/w)
S1E	25	3,398	13,6%
S2D	25	7,612	30,4%
S3D	25	3,150	12,6%

In the data calculated above, it is known that the largest percent yield is in the S2D sample of 0.304. This is because the extract obtained from Soxhlet has more volume. After all, the time required for extraction is longer than the S1E and S3D samples, which take 6 hours, so after evaporation of the extracted content in S2D, the percentage yield is higher.

### 3.2. Results of Identification of the Characterization of Qust' Al-Hindi (*Saussurea Lappa*)

#### 3.2.1. Phytochemical analysis

The phytochemical testing includes flavonoid, alkaloid, triterpenoid, or steroid tests, tannins, and saponins. Phytochemical test results on the extract of Qust' Al-Hindi showed that the extract contained flavonoids, alkaloids, terpenoids, and tannins. This can be seen from the color changes that occur, as in table 2.

**Table 2.** Phytochemical Test Analysis Results

No	Phytochemical	Observation	Result
1	Flavonoids	Embossed brick red	+
2	Alkaloids	Embossed orange	+
3	Terpenoids	Purple	+
4	Steroid	Appear purple, does not appear blue or green	-
5	Tannins	Brownish green color	+
6	Saponin	Brown color no foam	-

Note:

(+) = Contains these phytochemicals

(-) = Does not contain these phytochemicals.

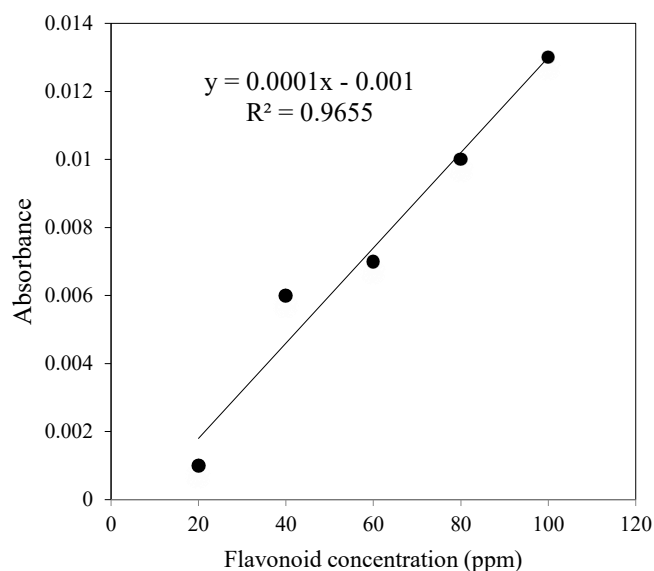
From the results of the above observations, it can be concluded that there are flavonoids, alkaloids, terpenoids, and tannins in the obtained Qust' Al-Hindi extract. This is because based on the phytochemical testing literature by Rozalina *et al.*, 2017. In this research, we just focused on flavonoids. Flavonoids are compounds containing two aromatic rings with more than one hydroxyl group. The presence of phenolic compounds that are highly soluble in polar solvents (Ergina, *et al.*, 2014), so that the extract obtained from semi-polar solvents is also quite high. Flavonoid testing using concentrated Mg and HCl powder, the purpose of adding Mg and HCl powder is to reduce the benzopyran core contained in the flavonoid structure to form red or orange flavilium salts. In this flavonoid test, it was proven that the extract contained flavonoids, this was seen by the red or orange color change after the addition of HCl and magnesium powder. With the flavonoid content in the Qusta 'Al-Hindi plant, the plant has the potential as an antioxidant.

### 3.3. Characterization

#### 3.3.1. UV-Vis spectrophotometry

This UV-Vis spectrophotometric test is intended to analyze the levels of flavonoid compounds present in the sample extract of Qust' Al-Hindi quantitatively. Quercetin standards were used to determine the total flavonoid content. The use of standard quercetin in UV-Vis spectroscopic tests is because quercetin is a flavonol compound that belongs to the most flavonoid plant group (Amara *et al.*, 2017). Before testing the UV-Vis spectrophotometer, first perform steps including

sample and blank preparation. Then the absorbance measurement was carried out to determine the maximum wavelength of the Qus' Al-Hindi extract with a wavelength range of 300-500 nm. Based on the measurement results, the maximum wavelength is 410 nm. Furthermore, sample measurements were taken and concentration data were obtained, so that the standard curve and regression equation was obtained, namely  $Y=0.0001x-0.001$  and the correlation coefficient ( $R^2$ ) was 0.9655 (**Figure 1**)



**Figure 1** Flavonoid Standard Curve

From the linear regression equation, calculations were then performed and the total flavonoid content was  $223.33 \pm 66.5$  (mgQE/g) (Table 3).

**Table 3.** Calculation Results of Total Flavonoid Levels

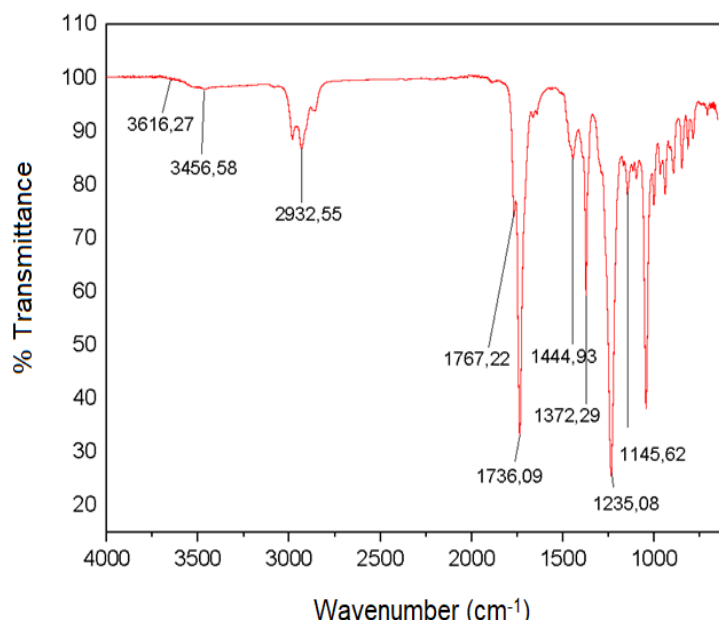
Absorbance (410 nm)	Konsentrasi (mg/mL)	KTF (mgQE/g)	Rata-rata KTF (mgQE/g)	SD	KTF  SD (mgQE/g)
0,025	0,26	260			
0,027	0,28	280	223,33	66,5	223,33 ± 66,5
0,012	0,13	130			

From the results of the calculation of the extract using a standard quercetin curve, it is known that the flavonoid content in the extract obtained is 223.33 mgQE/g with ethyl acetate solvent where this level is greater than that of previous studies, which is 16.20 mgQE/g with hexane solvent (Singh, et.al., 2018). So it can be concluded that the difference in solvent affects the levels of compounds extracted, this is proven by using ethyl acetate solvent which is semipolar, the flavonoid content is greater than the flavonoid content of the non-polar hexane extract.

In addition, it is known based on (Kemit et.al., 2016) the nature of flavonoids is polar compounds. so that by using ethyl acetate solvent which is semi-polar, the total flavonoid content is greater than the non-polar solvent. With the acquisition of greater levels, the potential of this Qust' Al-Hindi plant as an antioxidant is also greater. Thus, these plants can be used for health because these antioxidants can ward off free radicals that cause various diseases such as cancer, heart disease, cataracts, premature aging, and other degenerative diseases (Oka, 2015).

### 3.3.2. Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR test to determine the functional groups contained in the extract obtained qualitatively. The following are the results of the FTIR Extract of Qust' Al-Hindi:



**Figure 2.** FTIR results of Qus' Al-Hindi extract

From the spectrum results above, it can be made table data of the results of the FTIR test of the extract of Qust' Al-Hindi, presented in Table 4 as follows:

**Table 4.** FTIR Test Results for Qus' Al-Hindi Extract

Wavelength (nm)	Vibration	Functional Group	Characteristic
3616,27	Stretching O-H	Alcohol	Medium
3456,58	Stretching N-H	Amina	Medium
2932,55	Stretching C-H	Alkil	Strong
1767,22 and 1736,09	Stretching C=O	Carbonyl	Strong
1444,93	Stretching C=C	Alkene	Medium
1372,29	Bending C-H	Alkil	Strong
1235,08 and 1145,62	Stretching C-O	Ester and Carboxylic Acid	Strong

In the FTIR test of the Qust' Al-Hindi extract, it was found that there were stretching vibrations of functional groups such as aliphatic C-H, C=O carbonyl bonds, C=C bonds, C-O alcohol bonds, and found a wide valley of stretching vibrations of O-H bonds which are phenolic hydroxy groups and N-H. (Mohamed, et.al., 2015). It is known that flavonoid compounds have distinctive functional groups, namely O-H, C-H, C=C, C=O, and C-O groups, so it can be said that the extract contains flavonoids. Then for alkaloid compounds known to have a distinctive functional group, namely N-H, it can be said that the extract also contains alkaloids. Furthermore, terpenoids have a distinctive functional group, namely, C-H which is CH<sub>2</sub> and CH<sub>3</sub> and in the FTIR results there is a C-H functional group or dimethyl functional group which is typical of the terpenoid functional group. So, it can also be said that the extract contains terpenoid compounds. In addition, the results of this FTIR also contain CH (sp<sub>3</sub>) at 2932.00 cm<sup>-1</sup> and there is also a C-O group which is a typical functional group of tannin compounds.

#### 4. CONCLUSIONS

Acquisition of flavonoid levels from the plant extract of Qust' Al-Hindi after the calculations were carried out, which was obtained at  $223.33 \pm 66.5$  mgQE/g. 3) From the results of the analysis using FTIR – ATR on plant extracts of Qust' Al-Hindi, it is known to have the same functional group with the characteristics of flavonoid compounds, alkaloids, terpenoids, and tannins. The functional groups include O-H, N-H, C-H, C=O, C-O, and C=C bonds.

#### ACKNOWLEDGMENT

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