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RESEARCH ARTICLE

GC-MS ANALYSIS OF ETHYL ACETATE FRACTIONS OF QUST AL HINDI (*Saussurea lappa*) ROOT

Sukmawati*, A. Muflihunna
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia.

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***Address for Correspondence:**
Sukmawati, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia. Tel: + 0821-8733-3914;
E-mail: *sukmawati.syarif@umi.ac.id*

Abstract

Background: Qutsh al Hindi (*Saussurea lappa*), commonly known as Indian wood, is a traditional medicine that had been used empirically as anti-inflammatory and pneumonia. Its potential as anti-inflammatory activity is proven by many researches *in vitro* and *in vivo*.
Aim and objective: This study aims to identifying active compounds from the ethyl acetate fraction of Qutsh al Hindi (*S. lappa*) using GC-MS.
Method: The extract was obtained by powder of *S. lappa* roots using maceration method and evaporated to obtained thick extract. Then, the isolate of extract would be fractionated using n-hexane and ethyl acetate. The compounds that carried out by fractionation would be identified using GC-MS.
Result: Based on this research, the analysis of *S. lappa* ethyl acetate fraction showed 119 compounds using GC-MS. Other supporting factors using Mass Chromatography. The result was one of target compound identified, i.e. β-cyclocostunolide, a derivate compound from costunolide that has an anti-inflammatory activity.
Conclusion: It can be concluded that a fraction of *S. lappa* roots ethyl acetate contain β-cyclocostunolide as anti-inflammatory compounds.
Keywords: Anti-inflammatory, β-cyclocostunolide, Indian wood, *Saussurea lappa* fraction, GC-MS.

INTRODUCTION

Qutsh al Hindi (*Saussurea lappa*), commonly known as Indian wood, is a traditional medicine that had been used empirically as anti-inflammatory and pneumonia. Its potential as anti-inflammatory activity is proven by many researches. Based on phytochemical studies, Qutsh al Hindi has been reported to contain carbohydrate, steroid, alkaloid, cardiac glycoside, terpenoid, tannin, and flavonoid compunds. Sukmawati *et al.*[1], study showed that *S. lappa* has a stronger affinity than sodium diclofenac to inhibit the activity of cyclooxygenase receptors (COX-2) and iNOS. It can be predicted that *S. lappa* has large potential as ant-inflammatory agent[2].

S. lappa also contains another active compounds for an anti-inflammatory activity i.e alphacyclocostunolide, β-cyclocostunolide, and costus acid. While tannins have the ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules[3]. *S. lappa* activity as an anti-inflammatory agent frommethanolic extraction was also found in several studies. It showed an inhibitory effect (50%) on cytokine-induced neutrophil chemotactic factor[4]. Another maximum anti-inflammatory effect was shown in the sesquiterpene lactone fraction of *S. lappa* in 0.05-0.2 g/kg concentration level[5]. Another study of *S. lappa* ethanolic extract showed a significant value for anti-inflammatory activity through leg edema *in vivo* after administered with 50-200 mg/kg extract orally. *S. lappa* has several biomarker compounds i.e. alantolactone, caryophyllene, costic acid, costunolide, and dehydrocostuslactone and cynaropicrin. Anti-inflammatory effects *in vitro* study of cynaropicrin, a sesquiterpene lactone from *S. lappa*, showed from the release of TNF-α, NO, and lymphocyte proliferation[6,7].

In this study, a series of tests will be carried out on the fractionation of n-hexane and ethyl acetate from the Quts al Hindi root (*S. lappa*) ethanolic extract. This study was obtained to identified, characterized, isolated and traced the active compounds mechanism that responsible as anti-inflammatory *in vitro*.

MATERIALS AND METHODS

Qust al Hindi (*S. lappa*) roots sample were cleaned from dirt using flowing water. After that, they were sorted and chopped. Then dried by airing for several days and blended into powder.

Extraction

A total of 50 g of Qusth al Hindi (*S. lappa*) root powder was put into a cellulose thimble 300 ml of 96% ethanol was added into the Soxhlet. Soxhletation was carried out at a temperature of 700°C. The extract obtained was filtered using Whatman filter paper and then concentrated using a rotary evaporator at a temperature of 45°C for 30 minutes until a thick extract was obtained[3].

Fractionation

Fractions were carried out successively using the liquid-liquid extraction method using n-hexane and ethyl acetate solvents with increasing polarity. A total of 5 g of extract was dissolved in 50 ml of distilled water. The solution was then partitioned by adding 50 ml of ethyl acetate, shaken in a separating funnel and left to stand until there were two layers (distilled water in the lower layer and n-hexane in the upper layer). Take the ethyl acetate layer. This was done 3 times until the ethyl acetate layer became clear. The distilled water layer was then fractionated again in the same way using ethyl acetate solvent. The results of the ethyl acetate fractionation were evaporated using a rotary evaporator[4].

Identification of biochemical compounds using Gas Chromatography-Mass Spectrophotometry (GC-MS)

Preparation.

Sample of 0.1 ml was added with a mixture of chloroform and methanol (1:1) as much as 5 ml. Extraction using a sonicator for 20 mintes at a temperature of 40°C. The upper layer formed was pipetted into a vial and tested for GC-MS.

Operating GC-MS Ultra QP 2010 Shimadzu.

Pipette 0.5 ml of isolate into a 50 ml volumetric flask and dilute with acetone then squeeze to the limit mark. Pipette 3 ml and put into a vial. GC-MS instrument conditions Injector temperature 250°C with Splitless mode, pressure 76.9 kPa and flow rate 14 ml/min and ratio 1:10. Ion source and interface temperature 200°C and 280°C, solvent cut time 3 minutes, 400-700 m/z. Column type SH-Rxi-5Sil MS column length 30.

Percentage of yield extract

Percentage of yield extract was measured to determine amount of dried powder of *S. lappa* roots that used and chemical compounds were obtained by extraction process, following this formula:

$$\% \text{ yield} = \frac{\text{Extract weight (g)}}{\text{Dried powder weight (g)}} \times 100\%$$

Then, the qualitative and quantitative data of this research were obtained using GC-MS based on chromatography and spectrophotometry methods.

Statistical analysis

Experimental results were expressed as mean±SD. Student's *t*-test and one-way analysis of variance (ANOVA) were applied to check significant differences in different evaluation parameters. Differences were considered to be statistically significant at $p<0.05$.

RESULT AND DISCUSSION

Qutsh al Hindi (*S. lappa*) is one of the traditional medicines that had been used emipirically as anti-inflammatory. The main part used for treatment is the dried root[8]. This study was begun with plant extraction using the maceration method. Maceration is a method of separating compounds by soaking using organic solvents at a certain temperature[9]. This method is very beneficial for isolating natural compounds because in addition to being cheap and easy to do, soaking plant samples will result in the breakdown of cell walls and membranes due to the difference in pressure between inside and outside the cell, so that secondary metabolites in the cytoplasm will dissolve in the solvent. The roots of the Qusth al Hindi plant (*S. lappa*) that have been obtained are ground to expand the contact between the sample and the solvent used in the extraction process[8].

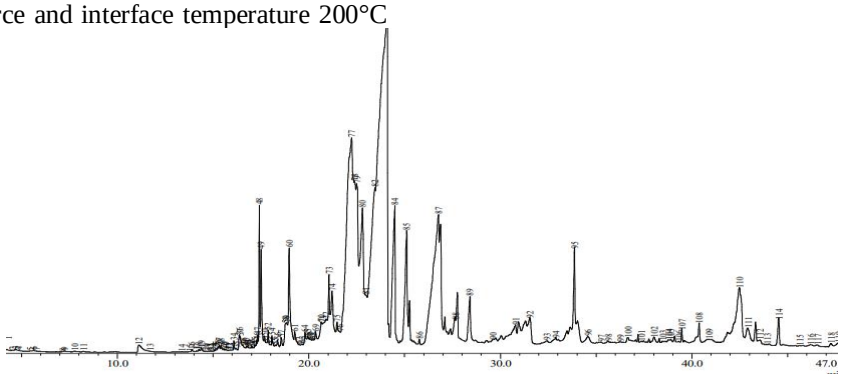

Figure 1: Result of GCMS analysis.

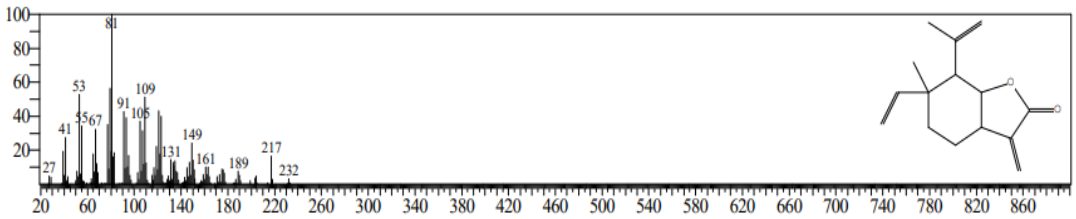

Figure 2: 2(3H)-Benzofuranone fragmentation.

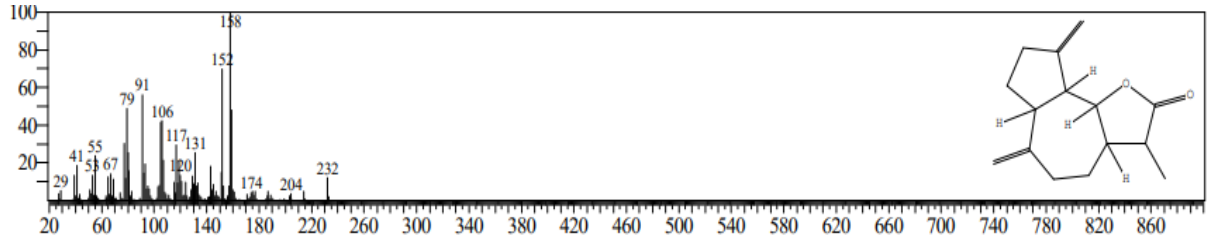

Figure 3: Dihydrodehydrocostus lactone fragmentation.

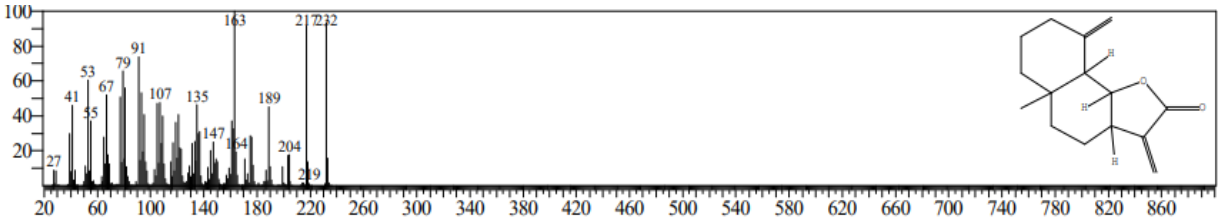

Figure 4: β -Cyclocostunolide fragmentation.

In the extraction process, 96% ethanol solvent is used because of its selective properties, good absorption and high solvent capacity so that it can extract non-polar, semi-polar and polar compounds[10]. The results of Qutsh al Hindi extraction obtained extracts from 96% ethanol solvent. The thick extract from the maceration of ethanol solvent obtained a yield of 29.45%. The yield percentage calculation aims to determine the amount of dried powder of *S. lappa* root used during the extraction process and related to the active compounds contained in a sample, where the more yields produced, the more active compounds contained in the sample[11].

The thick extract of *S. lappa* roots obtained through maceration process using 96% ethanol solvent as much as 88.35 g with % yield of 29.45%, then fractionated using liquid-liquid partition method using ethyl acetate and n-hexane solvents. The purpose of fractionation is to separate the components of active compounds from the extract that has been produced. The principle of the fractionation process is based on the difference in polarity level and the difference in specific gravity between the two fractions.

Based on the data, the fraction of Quts al Hindi that obtained using ethyl acetate is 7.11 g from the thick extract. The fraction percentage of yield is 23.7%.

Identification of chemical compounds from the ethyl acetate fraction using GC-MS spectroscopy was carried out with the following results. The hydrodistilled ethyl acetate fraction was analyzed using Gas Chromatography-Mass Spectrometer (GC-MS). Chromatography of the GC-MS analysis results showed were 119 compounds from the isolate. Other supporting factors that can be used for separation evaluation are the MS (Mass spectrometry) identity of each peak that appears in each chromatogram. The MS (Mass spectrometry) identity is marked as the first highest peak. The second peak is able to provide one peak with the identity of the detected compound. The reading of these peaks as the same compound is due to the similarity of the MS spectrum of these peaks. Based on the results of the analysis above, in the fragmentation results, there are 4 compounds that are detected the same as the target compound.

Based on Dae Yong Kim[12] research, the compound with a molucule weight 232.1 g/mol is a costunolide compound derived from dehydrodehydrocostus lactone with molecule formula $C_{15}H_{20}O_2$, then based on previous research it was said that the methanolic extract of the roots of *S. lappa* contained 11.29±0.34 mg/100 g costunolide compounds. In the chromatogram of extract compared to several solvents, it was proven that the highest peak was in ethyl acetate. Based on the GC-MS results, Costunolide compound was identified based on chromatogram and fragmentation pattern. Costunolide is one of compounds that have been identified in *S. lappa* roots. Costunolide is a naturally occurring sesquiterpene lactone and is reported to have several activities such as antioxidant, anti-inflammatory, anti-allergic, bone remodeling, neuroprotective, hair growth promoter, anticancer, and antidiabetic[13].

Limitation of this study

The current research is a basic stage using ethyl acetate fraction to find the anti-inflammatory potential of secondary metabolites analyzed by GC-MS.

CONCLUSIONS

Based on the results, it was concluded that the secondary metabolites from ethyl acetate fraction of Qust al Hindi (*S. lappa*) roots were found to contain 119 compounds. There was Costunolide detected as the target compound, a derivative of Dehydro-dehydrocostus lactone.

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AUTHOR'S CONTRIBUTIONS

Sukmawati: methodology, investigation. **Muflihunna A:** critical review, data processing. Both authors reviewed and approved the final version of the article.

DATA AVAILABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

None to declare.

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