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Phytochemical Screening and Chemical Analysis of Ethanol Extract of Kari Leaves (Murayya koeginii) Using GC-MS Method

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Abstract. Kari leaves (Murraya koeginii) uses as seasoning material for many kinds of food in Aceh and it can be used for health treatment because of it consists of flavonoid, steroid and phenolic that has activity as antioxidant and antibacterial. The aim of this research is to determine the phytochemistry component of ethanol extract of Kari leaves. The extraction process was conducted using maceration technique in ethanol 96% (1:3 w/v). The determination of extract content of Kari leaves was performed using GC-MS. The yield of extract was about 17,68% and the phytochemical screening showed the presence of phenolic, terpenoid/ steroid, flavonoid, tannin and saponin. The GC-MS analysis revealed that the ethanol extract of Kari leaves has 47 compounds, which the major content is vitamin E (12,18%), beta-caryophyllene (7,67%), pyrazine, tetrakis(1-methylethyl) (10,58%), N, N-Dimethyl-Tridecylamine (6,81%) andd-Nerolidol (7,32%). This result indicated the ethanol extract of Kari leaves has activity as antioxidant and antibacterial.

1. Introduction

Many kinds of plant can be used as traditional medicine and seasoning material for food. That is caused by the chemical content that presence in the plant. The result of chemical reaction between many compounds and water in the plant metabolism process produced essential oil. At the room temperature, the essential oil is easily to evaporate without the decomposition process, has distinctive fragrance, soluble in organic solvent and not soluble in water. The essential oil can be obtained from some part of plant, such as leaves, flowers, seed and stem. One kind of plant that can produce essential oil is Kari leaves (Murraya koeginii).

Kari leaves (Murraya koeginii) is a family of Rutaceae that can be found easily in India [1]. In Indonesia, Kari leaves can be found easily in Aceh. Usually, Kari leaves use as seasoning material to improve the flavour and fragrance of food. Also, it uses as herbal medicine to cure health problem such as, external wounds, snake bites and stomach aches. This is caused by the presence of P-gurjunene, P- caryophyllene, P- elemene and phellandrene in Kari leaves. These chemicals, β-pinene, β-caryophyllene, β-phellandrene and α-pinenehave ability to control food spoilage [2] [3].

Based on the research, Kari leaves showed the presence of steroid, flavonoid, phenolic, alkaloid and saponin. This is suitable with the other previous works [4]–[7]. Polyphenol group caused the Kari leaves has an ability as antioxidant. [5] and [6] revealed Kari leaves has some metabolite secondary, such as alkaloid, flavonoid, terpenoid and steroid, that active as antibacterial. Other hand, [7] found the Kari leaves has activity as antibacterial and antiinflammation, and the saponin compound showed the activity as cytotoxic and antiulcer. The other metabolite, such as steroid and triterpene can act as antibiotic and antifungal. The presence of those metabolite caused the Kari leaves has the activity as antibacterial and antioxidant. For those reasons, the focused of this study was to determine the chemical content in the Kari leaves extract through phytochemical screening and GC-MS technique.

2. Methododology

Kari leaves (Murraya Koeginii) was obtained from Kampung Jawa Belakang village, Langsa, Aceh. The equipment that used during this research was rotary evaporator, GC-MS, and water bath.The phytochemical screening was conducted in the laboratory of Graduate School of Chemistry, USU-Medan. The determination of extract component using GC-MS was performed in LPPT UGM.

2.1.The extraction of Kari leaves, the extraction process of Kari leaves followed the maceration technique of [8]. This process was begun with the drying process of leaves for 3 days. The dried leaves were grounded to be powder. 1000 g of Kari leave powder was extracted using ethanol 96% with ratio of 1:3 (w/v) for 48 h. The obtained residue was removed, and the filtrate was concentrated using vacuum rotary evaporator and followed with the evaporation process in water bath. The concentrated extract then analysed for the phytochemical screening and GC-MS technique.

2.2. Yield percentage, yield percentage was calculated to obtain the information about the quantity of extract that obtained from extraction process.
% Yield = (weight after extraction / weight before extraction) x 100%

2.3. Phytochemical screening, the phytochemical screening was conducted to obtain the characteristic data of active compound that presence in the Kari leaves extract.The conducted test was specific to alkaloid, tannin, flavonoid, saponin, steroid and triterpenoid. The procedure of each test was explained below:

1. Alkaloid test. Zero point five g of Kari leaves extract was dissolved in 5 mL of ethanol, the solution was mixed until it dispersed well. After that, the Mayer, Bouchardat and Dragendorf reagent was added into the mixture. The positive result for alkaloid was shown with the presence of these colour yellow (precipitate), chocolate (precipitate) and no precipitate, respectively.
2. Terpenoid and steroid test. Zero point fiveg of Kari leaves extract was dissolved in 5 mL of ethanol, the solution was mixed until it dispersed well. Into this solution was added some drops of anhydride acid and one drop of concentrated H_2SO_4 (Lieberman-Burchard reagent). The red or violet colour indicated the positive result of terpenoid and green or blue for steroid.
3. Saponin test. Zero point fiveg of Kari leaves extract was dissolved in 5 mL of ethanol, the solution was mixed until it dispersed well. The obtained filtrate was shaken for 10 minutes. The presence of saponin was indicated with the formed of stable foam
4. Phenolic test. Zero point five g of Kari leaves extract was dissolved in 5 mL of ethanol, the solution was mixed until it dispersed well. The $FeCl_3$ 1% (w/v) was added into filtrate of Kari leaves extract. The presence of blue to black-greenish colour indicated the positive result of phenolic test.
5. Tannin and Flavonoid test. one g of Kari leaves extract was dissolved in 10 mL of ethanol, the precipitate was formed after heating process. The addition of ethyl acetate can indicate the presence of tannin and flavonoid based on the solubility of that precipitate, if it soluble it indicates the presence of flavonoid.

2.4.The component of Kari leaves extract (GC-MS), The analysis of the component that presence in that extract was performed through qualitative method using GC-MS. 0,5 g of extract of Kari leaves was dissolved in 5 mL of methanol. The obtained essential oil of Kari leaves was analysed using GC-MS. The obtained mass spectrum was compared to the database of that instrument.

3. Result and Discussion

3.1.Yield

The extraction of Kari leaves extract was performed by following method (i) drying process of Kari leaves for 3 days and (ii) maceration process. The drying process was conducted to reduce the water content of leaves, it will influence the storage time and minimalize the potency of fungal growth. After the maceration process using vacuum rotary evaporator the yield was obtained about 17,68% (Figure 1). This result is higher than the obtained result of [8], 12%. This was caused by the influence of the modification that applied on this maceration technique, such as extraction time and solvent quantity.

Figure 1. Ethanol Extract of Kari Leaves (Murayya koeginii)

3.2 Phytochemistry

The result of phytochemical screening of Kari leaves extract was shown in Table 1.

Table 1. The result of phytochemical screening of Kari leaves extract

Secondary metabolite	Result
Phenolic	++++
Steroid/ terpenoid	+++
Alkaloid	----
Saponin	+
Flavonoid	+++
Tannin	+

Based on Table 1, Kari leaves extract consisted of steroid, flavonoid, phenolic, tannin and saponin. This result was confirmed by others previous works [4]–[7]. The in-vitro test that performed by [8] showed Kari leaves extract using ethanol/water (1:1) has antioxidant compound that has polyphenol structure. [9] explained that phytochemical compound is a chemical compound that presence in the plant and has an ability for health treatment also can prevent any degenerative health problem. Mostly phytochemical compound can act as antioxidant, such as carotenoid, phytosterol, saponin, glicocynolate, polyphenol, inhibitor protease, monoterpene, phytoestrogen and sulphide. Alkaloid was confirmed can be found in the extract of Kari root an leave [5] [6] [10]. Kari leaves extract also can act as antibacterial and antiinflammation, the saponin content in that extract has a potency as cytotoxic

and antiulcer. The antibiotic and antifungal potencies were shown by the steroid/ triterpenoid content in that extract [7].

3.3. The composition of Kari leaves extract based on GC-MS

Determination of compound in the Kari leaves extracts based on GC-MS analysis showed in Figure 2.

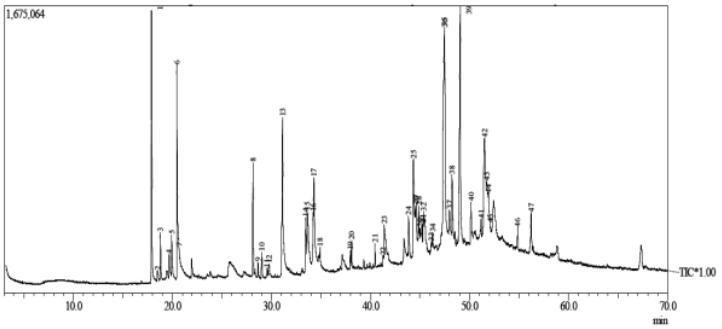

Figure 2. Chromatogram of Kari leaves extract

Chromatogram in Fig 2 showed signals with different percentage of area. There are 47 signal that detected, and the highest signal was found in the 39th signal with 49.058 of retention time. That signal was confirmedas Vit. E, the second highest signal was followed by the 1st signal with 17.982 of retention time and confirmed as beta caryophyllene. The 35th signal with 47.458 of RT was confirmed as pyrazine, tetrakis(1-methylethyl). The composition of Vit E in Kari leaves extract was found to be 12.18%and this result explain the antioxidant activity of Kari leaves extract. The of Kari leaves extract can be seen in Table 2.

Table 2. The composition of Kari leaves extract based on GC-MS

No	Compounds	% Area
1.	Beta-Caryophyllene	7.67
2.	Beta-Caryophyllene	0.35
3.	Beta-selinene Naphtalene	1.35
4.	Germacene	0.77
5.	Alfa-Selinene	1.32
6.	N,N-Dimethyl-Tridecylamine	6.81
7.	5-Nonanone	0.39
8.	Oktadekanal	2.74
9.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.36
10.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	0.58
11.	Farnesol	0.13
12.	Farnesol	0.23
13.	Tetradecanoic Acid	6.02
14.	N-methyl-N-benzyldodecanamine	1.96
15.	Phytol	3.48
16.	9,12-Hexadecadienoic acid	1.30
17.	9,12,15-Octadecatrienoic acid	3.35
18.	Octadecanoic acid	0.20
19.	Trans-Farnesol	0.42
20.	Cis-Farnesol	0.60
21.	1,2-Benzenedicarboxylic acid	0.58

22.	Glycomaurin	0.22
23.	Isoglycomaurin	0.86
24.	Pyrazine, tetrakis(1-methylethyl)	1.08
25.	10-methoxy-3,3,8-trimethyl-3H,11H-pyrano [3,2-a]carbazole	4.39
26.	Geranyl Linalool Isomer	1.55
27.	11-Methyl Squalene	1.43
28.	Squalene	1.65
29.	Pyrazine, tetrakis(1-methylethyl)	0.55
30.	Ellipticine-7,9-D2	0.73
31.	5,5,8a-trimethyl-4a,5,6,7,8,8a-hexahydro-N-(2'-phenylcyclopropyl)pyrrolo[3,4-a]naphthalene	0.21
32.	Methyl 2-(4-methoxyphenyl)-3-quinolinecarboxylate	0.17
33.	Nonadecane	0.18
34.	Geranyl Linalool Isomer	0.25
35.	Pyrazine, tetrakis(1-methylethyl)	10.58
36.	Pyrazine, tetrakis(1-methylethyl)	4.73
37.	Gamma.-Tocopherol	0.92
38.	Pyrazine, tetrakis(1-methylethyl)	2.24
39.	Vitamin E	12.18
40.	Squalene	1.16
41.	Farnesyl Acetate	0.34
42.	d-Nerolidol	7.32
43.	Lycopersen	2.41
44.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.85
45.	Alpha.-Levantenolide	0.28
46.	Neophytadiene	0.63
47.	Geranyl Linalool Isomer	1.47

Based on GC-MS result showed Kari leaves extract has multivariate secondary metabolites. Other than vit. E, the content of pyrazine in the Kari leaves extract was 19.18%. This compound has a function as fragrance sources of Kari Leaves.

Caryophyllene is one compound that found in Kari leaves extract, it was sesquiterpene group. Beta caryophyllene in the extract was found to be 8.02%. This compound has an ability as antiinflammation and antiarthritic [11] . The other sesquiterpene compound that found in the extract was farnesol and nerolidol. Farnesol act as antiinflammation and antiallergic [12]. Other hand, nerolidol act as antibacterial [13]. The monoterpene (geranyl linalool) and triterpene (squalene) also found in this extract. Squalene can act as antiaging and anticancer [14].

4. Conclusion

The ethanol extract of Kari leaves revealed the presence of steroid, flavonoid, phenolic, tannin and saponin. Kari extract leaves has 47 compounds with 5 major content, such asvitamin E (12,18%), beta-caryophyllene (7,67%), pyrazine, tetrakis(1-methylethyl) (10,58%), N,N-Dimethyl-Tridecylamine (6,81%) dan d-Nerolidol (7,32%). This result indicated Kari leaves extract can be used as antioxidant and antibacterial.

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