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Article

Effects of Solvent to Feed Ratio and Microwave Power on Extraction of Essential Oil from Red Guava Leaves (*Psidium guajava* l.) Using Microwave Hydro Distillation Method

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Abstract. Essential oils are typically hydrophobic liquid mixture of natural products containing oil, volatile, and aromatic compounds extracted from plant species. This experiment was used microwave hydro distillation as extraction method to obtain the essential oils due to its efficiency. The purpose of both treatments used to increase total yield of essential oil from red guava leaves. Completely randomized design was used as experimental design with two variables and run in triplicate. The first variable is solvent to feed ratio (4:1, 5:1, 6:1, 7:1) and the second variable is microwave power (300W and 450W). The results showed that the ratio of solvent to feed (7:1) and microwave power (450W) has the highest yield and best quality of essential oils. The best variable has some parameters, particularly yield of 1.24%, refractive index of 1.49, and density of 0.78 g/ml. Analysis of essential oil profile has some abundance compounds, namely naphtalene or β-seline at 24.45%, neointermedeol at 17.97%, and caryophyllene at 16.04%. Moreover, the IC_{50} value has an antioxidant activity of 41.95 ppm.

Keywords: essential oil, guava leaves, microwave hydro distillation, antioxidant

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1. Introduction

Indonesia has a diversity of biological natural resources that has the potential to produce essential oils. Essential oils are mixture oil products including volatile compounds and specific fragrance aroma [1]. Essential oils are secondary metabolites which are included in a large group of plant oils within viscous liquid at room temperature [2]. Indonesia produces approximately 40-50 types of essential oil which producing plants that are traded in the world. Essential oil which is producing plants are as diverse as patchouli, cloves, lemongrass, fragrant root, orange peel, agarwood, moringa leaves, and others. Chemical compounds and their derivatives have biological and physical functions such as antibacterial, insect repellent, and antioxidant ability. Essential oils are widely used throughout the world as antimicrobial agents and food additives in the aroma sector [3].

Red guava plants (*Psidium guajava* Linn.) produces two parts that are commonly consumed by the people, specifically fruit and leaves. Red guava leaves are usually be cutin agricultural cultivation aims to enlarge fruit size and yield [4]. Red guava leaves has many phytochemical compounds containing flavonoids, tannins (17.4%), phenolics (575.3 mg/g) and essential oils [5]. The main components of guava leaves essential oil are α-humulene, trans-caryophilene, and 1,8-cineol[6]. The specific aroma arising from essential oils are the volatile compounds that easily to evaporate [7].

The conventional method often used is water distillation. Distillation with water is the simplest way because the leaves to be distilled are inserted into the drum then added to water and heated, then the steam is flowed through the condenser and the extracted oil is collected in a container or bottle. This refining is began to be abandoned because the oil produced is low quality and low quantity. This method has several disadvantages, such as some types of esters will be hydrolyzed, and some compounds will undergo polymerization due to the effect of boiling water. In addition, high boiling oil components and water-soluble compounds cannot evaporate completely, so that the distilled oil components is incomplete and loss some essential compounds [8].

The latest extraction method was developed using microwaves using a distillation system known as Microwave Assisted Extraction or Microwave Hydro distillation (MHD). The advantages of MHD are utilizing the aqueous solvents (water) and using microwaves to extract the oil [9]. Previous research on extraction of essential oil from guava leaves has been carried out by [3] with the Hydro distillation method using distilled water for 3 hours with a mass of material 117.6 grams yielding 0.38% yield with iso-caryophyllene of 33.53% as the highest compound. Therefore, these experiments are focus to find out the best parameters based on solvent to feed ratio and microwaves power.

2. Material and Method

2.1. Materials and Chemicals

The main material was dark red guava leaves within the dark green color which had been trimmed and separated from their branches. Those leaves were obtained from Plantation of Kebaron Village, Tulangan District, Sidoarjo Regency. All others chemicals used for analytical protocol are pro analysis grade purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Instruments

Some of devices to extract the essential oil from red guava leaves were handgloves, mask, cabinet dryer, knife, scissors, microwave Electrolux EMM2308X, clevenger, pump, condenser, hose, and reflux. The apparatus to analyse the physical properties were pipette, analytical scale, pycnometer, refractometer, vial bottle, and beaker glass. The Instruments to identify the profile of essential oil were GC-MS Thermo Scientific ISQ-LT 1310, measuring cup, funnel, test tube, and Milton Roy Spectronix 21 Spectrophotometer.

2.3 Experimental Design

The research design used was a factorial Complete Randomized Design (CRD). Thera were2 variables, specifically for the first variable wassolvent to feed ratio consisting 4 levels, particulary4:1, 5:1, 6:1, and 7:1 (v/w) and the second variable was microwave power consisting 2 levels, particulary 300W and 450W.Each unit experiment was conducted in triplicate. The dataswere analyzed by statistically using analysis of variance (ANOVA), if there are significantly data, it was continued with the Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Physical Properties of Essential Oil from Red Guava Leaves

3.1.1. Yield

Based on analysis of variance, it can be seen that there was significantly effect ($p \leq 0.05$) from both variables to the yield. Figure 1 showed that the higher solvent to feed ratio and microwave power lead the higher yield produced. This was influenced by differences value in solvent to feed ratio, so the higher solvent volume was able to soak the whole leaves are a caused evaporate the essential oils from red guava leaves in maximum level.

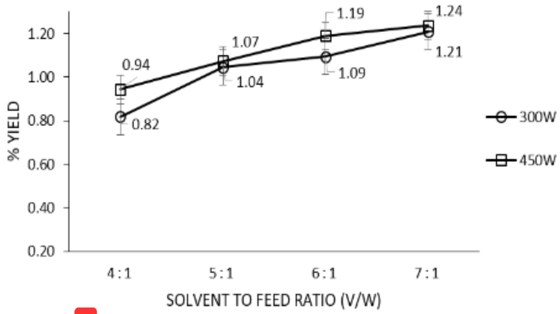

Fig. 1. Effects of Solvent to Feed Ratio and Microwave Power on Yield of Essential Oil from Red Guava Leaves

Otherwise the treatment of microwave power also affected the yield produced. High power delivers large amounts of energy to the sample material and solvent lead the faster elevating of temperature extraction. Therefore, the higher power made the energy received by the material to be converted into heat and the refining rate was also faster lead the yield produced was higher number. The volume of the solvent must be sufficient to ensure that all the sample material is submerged so that it is affected during the radiation process from the microwave [10].Microwave power acts as a driving force to break the structure of plant cell wall due to the oil can diffuse out and dissolve in solvents [11].

3.1.2. Refractive Index

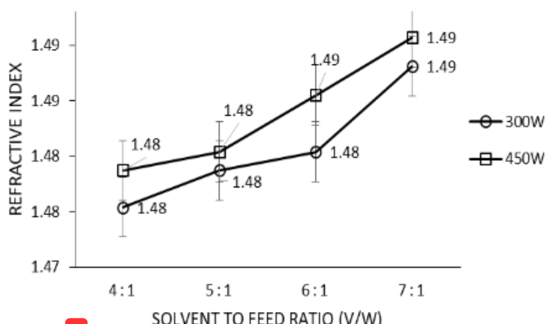

Fig. 2. Effects of Solvent to Feed Ratio and Microwave Power on Refractive Index of Essential Oil from Red Guava Leaves

Based on variance analysis, it can be seen that there was a real interaction (p≤0.05) between the treatment of solvent to feed ratio and microwave power. Figure 2 showed that the higher of solvent to feed ratio and microwave power lead the higher refractive index value produced. The higher the ratio of solvent and material lead the higher refractive index number. The higher volume solvents are used will cause more components are extracted so that the refractive index value was higher. Otherwise, the second treatment of microwave power showed a significant increase in the refractive index numbers due to the ability of the microwave power to carry out friction and random movements in pushing the oil to diffuse from the guava leaves cell wall.

The refractive index showed the number of components contained in oil [12]. The higher the refractive index value of substance was thought to be the more components contained in the substance because substances with many components would be difficult to refract. The higher the ratio, the contact between water and material becomes greater because more water is used to lead the penetration of solvents into the material is greater and the extracted compound also increases [13]. The alteration in power simply affects the value of the oil refractive index, there is a possibility caused by differences in component content and composition of oil produced [14].

3.1.3. Density

Based on analysis of variance, it can be seen that there was interaction (p≤0.05) between two treatments. Figure 3 showed that the higher numbers of solvent to feed ratio and microwave power resulted the increasing of density. This was influenced by solvent to feed ratio due to the higher solvent to feed ratio. The material would be completely submerged so that the essential oil component in the material could be extracted optimally.

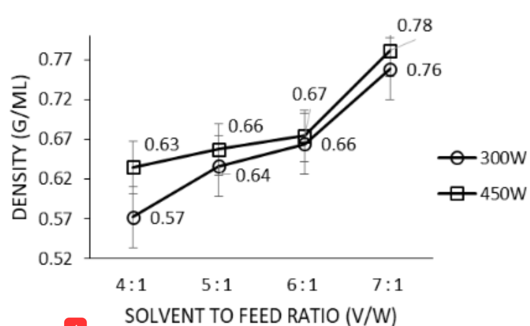

Fig. 3. Effects of Solvent to Feed Ratio and Microwave Power on Density of Essential Oil from Red Guava Leaf

Treatment of microwave power also affected the density of the essential oils produced. This was caused by the ability of the microwave power to produce friction and random movements so as to be able to push oil to diffuse from the guava leaves cell wall. The ratio of solvent to feed was large, it will also increase the amount of dissolved compounds [13].

The differences of solvent to feed ratio and extraction method might affect the extracted components so that it indirectly affected the density of the oil [14]. The extraction method of microwaves from this research was different from conventional methods, because heating used microwaves by generating heat from inside of material so that it is more evenly distributed with the direction of heating from inside the material to the outside of the material so that it is more effective and faster.

3.2 Identification of Mixture Compounds of Essential Oil from Red Guava Leaves

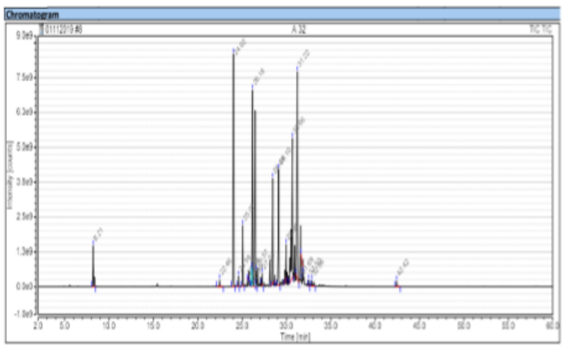

Fig. 4. Profile of GC-MS Chromatogram of Essential Oils from Red Guava Leaves

Based on Figure 4 showed the content of essential oil compounds from red guava leaves which was performed by the Gas Chromatography-Mass Spectrometry (GC-MS) method composed of 19 compounds. The chromatogram of GC-MS analysis resultedin Table 1 showed that the most dominant compounds are Caryophyllene, Naphtalene (β-Selinene), Caryophyllene Oxide, and Neointermedeol with the highest peak compared to other compounds. Caryophyllene, Naphtalene (β-Selinene), Caryophyllene Oxide, Caryophylla-4 (12),8(13)-dien-5a-ol, and Neointermedeol compounds are the main active compounds found in this study, amounting to 16.04%, 24.45%, 7.40%, 13.51%, and 17.97%. These five compounds are sesquiterpenes which form a special aroma in the essential oils from red guava leaves.

There are some variation numbers compares to previous studies. The composition of essential oils of red guava leaves using conventional water distillation for 3 hours are d-limonene, Iso-Caryophyllene, α-humulene, Farnesene, and Veridiflorene with a percentage of 9.85%, 33.54%, 3.48%, 11.65%, 13% [3]. The composition of guava leaf essential oil compound is caryophyllene, β-Selinene, and Caryophyllene Oxide with the percentage of 13.44%, 11.65%, and 2.16% [6].

Table 1. Mixture Compound of Essential Oil from Red Guava Leaves

No	Senyawa	*R.Time (min)*	% Area
1	*D-Limonene*	8.214	2.89
2	*Copaene*	22.465	0.34
3	*Caryophyllene*	24.023	16.04
4	*Alloaromadendrene*	24.578	0.47
5	*Humulene*	25.074	2.88
6	*Aristolochene*	25.577	1.02
7	*Cedren-13-ol, 8-*	25.985	0.24
8	*Naphtalane, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aa,7a,8aβ)]-*	26.176	24.45
9	*β-Longipinene*	26.573	0.80
10	*Tau-Cadinolacetrate*	27.240	0.30
11	*Nerolidol*	28.444	4.92
12	*Caryophyllene Oxide*	29.104	7.40
13	*Neointermedeol*	29.947	1.62
14	*Caryophylla-4(12),8(13)-dien-5a-ol*	30.665	13.51
15	*Neointermedeol*	31.223	17.97
16	*Alloaromadendrene oxide-(2)*	31.689	3.97
17	*Corymbolone*	32.502	0.32
18	*1,6,10,14-Hexadecatetraen-3ol, 3,7,11,15-tetramethyl-, (E,E)-*	32.859	0.59
19	*Neophytadiene*	42.422	0.26

3.3 Antioxidant Activity (IC_{50})

The best treatment was tested its antioxidant activity to find out how high the inhibition as much as 50% concentration to inhibit the oxidation. The experiments was using variation of concentration, particularly 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm. Figure 5 showed an increasing of antioxidant activity with increasing concentrations of essential oil used from red guava leaves.

The higher concentration of essential oils leads the higher antioxidant activity. In this study the instrument used was a UV-Vis spectrophotometer with the principle of its work absorbing visible light by a colored solution. The line equation also known as the regression formula was y = 1.8614x - 28.059. The equation was used as calculation of inhibition concentration (IC_{50}) value of essential oil from red guava leaves. The data was obtained by finding the value of x (abscissa). The calculation was conducted by replacing the value of y (ordinate) with the number of

50 where the value of x was the concentration sought and the y value was the desired inhibition concentration of 50%. The result of IC_{50} value is 41.95 ppm. This value was lower than 50 ppm which means it has very strong antioxidant properties.

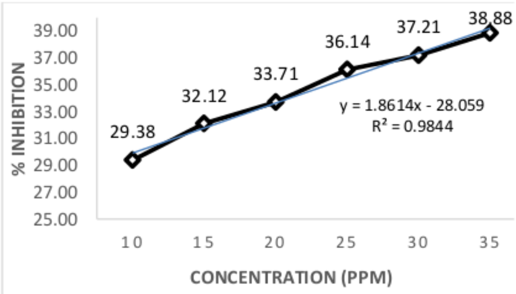

Fig. 5. Antioxidant Activity Expressed as IC_{50} of Essential Oils from Red Guava Leaves

IC_{50} values below 50 ppm have very strong antioxidant properties [15]. The study of essential oil from guava leaves antioxidants using distilled water for 4 hours produced antioxidant activity that varied with each concentration [16]. The concentration of 50 ppm it produces 28.12%; The concentration of 100 ppm produces 51.51%; the concentration of 250 ppm produces 89%; the concentration of 500 ppm produces 92.79%; and the concentration of 1000 ppm produces 93.86%.

4. Conclusions

The results showed that the ratio of solvent with 7: 1 material and 450 W microwave power was the best treatment with a yield percentage of 1.2357%, refractive index 1.4907, specific gravity 0.7813, and IC50 antioxidant activity value of red guava leaf essential oil which is 41.95 ppm. The most dominant compound components in essential oils of red guava leaves are naphtalene or β-seline with a percentage of 24.45%, Neointermedeol with a percentage of 17.97%, Caryophyllene with a percentage of 16.04%, Caryophyllene oxide with a percentage of 7.40%, and Caryophylla-4 (12), 8 (13) -dien-5a-ol with a percentage of 13.51%.

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