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NI P3K : 197607182021211003

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The effect of microwave power and feed to solvent ratio on antioxidant activity and volatile compounds profile of crystal seedless guava leaves essential oils extracted using microwave-assisted hydrodistillation

A D Priyanto¹, N K Erliyanti² and C Pujiastuti²

¹ Department of Food Technology, Faculty of Engineering, University of Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia

² Department of Chemical Engineering, Faculty of Engineering, University of Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia

E-mail: anugerahdany.tp@upnjatim.ac.id

Abstract. This work utilized by-product of crystal seedless guava leaves due to its cultivation for commercial fruit production persuaded to prune guava trees branches. Essential oil (EO) extracted from plant biomass is a source of natural antioxidants due to the presence of essential components. This study was outlined to explore the potential of antioxidants and identify the chemical composition of EO extracted from crystal seedless guava using Microwave-assisted hydrodistillation (MAHD). The essential oils (EOs) were extracted using various microwave powers and feed to solvent (F/S) ratios. The two independent variables showed significantly affect on IC₅₀ value of EOs, thereby there were several various IC₅₀ values in DPPH free radical scavenging assay from EOs depends on their extraction condition. The best condition resulting in IC₅₀ of EO was as low as 36.23 ± 2.07 µg/mL with a microwave power of 600 W and F/S ratio of 1:7 (w/v). The five major constituents of EO from the best extraction condition were caryophyllene (44.98%), caryophyllene oxide (14.96%), trans-nerolidol (9.16%), humulene (5.10%), and aromandendrene (5.10%). The study concluded that MAHD method possessed significant change in antioxidant activity and volatile compounds of EO extracted from crystal guava leaves.

1. Introduction

Essential Oils (EOs) are natural compounds of plant secondary metabolites in response against environmental stress factors to defense from variety of herbivores, pathogenic microorganisms, and oxidative stress [1]. EOs are mixture components including concentrated hydrophobic liquid, volatile compounds, and specific fragrance aroma. The application of EOs are widely appealing in valuable products, such as perfume, aromatherapy, food flavors, and food preservatives [2, 3]. In recent year, many popular EOs from plant sources have been explored such as, *Eucalyptus Camadulensis*, lavender, lemongrass, peppermint, and tea tree [4, 5, 6, 7, 8]. Extraction of EOs could be taken from many parts of plant, such as flowers, fruits, fruit peels, leaves, branches, and roots. Indonesia is a tropical country



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and also known as one of the most fertile land. Consequently, this country has many potential commodities to develop the EOs.

Crystal seedless guava is one of the new variety in Indonesia. This commodity has a high value due to the seedless fruit characteristic thereby presenting convenience in consumption. Pruning plays an important cultivation method in this tree to improve the flower and fruit quantity [9]. This silvicultural practice obtains some by-product, specifically branches and leaves. The guava leaves contains many compounds, such as alkaloids, glycosides, flavonoid, tannin, terpenoids, steroids, and saponins [10]. Eventually, these phytochemistry have some abilities as antioxidant, antidiabetic, anticancer, antimicrobial and another functional values [11]. Guava leaves have a lot of volatile compounds also known as Essential Oil (EO) [12]. Therefore, extraction of essential oil from crystal seedless guava leaves is attracting big research interest.

The conventional method of EO extractions are steam distillation and hydrodistillation. However, this classical technique leads to degradation of some volatile compounds in EO due to long extraction times and high temperatures. This conditions hence transformation of natural chemical structure involves reducing of functional bioactive compounds. Non-thermal methods allow the processing of natural products below temperatures used during thermal situation to avoid reduction of bioactive compounds losses. Thus, it is necessary to develop the innovative approaches method to extract the EO from plant with better quality attributes of essential components.

Microwave-Assisted Hydrodistillation (MAHD) is one of the new technology for essential oil extraction from plant biomass [13]. The advantages of this method are high quality, shorter extraction times, less amount of solvent and eco-friendly [14]. Basic principle of microwave extraction utilizes electric field ionic conduction and dipolar polarization, resulting energy transferred to surrounding medium by collisions with simultaneously fast thermal heating. Recently, many EOs extracted using MAHD from plant biomass have been published, such as oregano, black zira, *Chamaecyparis obtuse*, *Enteromorpha linza* L., and *Oliveria decumbens* Vent [15, 16, 17, 18, 19]. In-depth investigations about their EOs application as antioxidant showed good profile capacity. Thus, the objective of the present study is to evaluate the antioxidant activity of EO from crystal seedless guava leaves using MAHD with various microwave power and feed to solvent (F/S) ratio as independent variables. Furthermore, the volatile compounds of EO from crystal seedless guava leaves were analyzed using gas chromatography-mass spectrometry (GC-MS).

2. Materials and Methods

2.1. Material and chemicals

Crystal seedless guava leaves were collected from Prambon sub-district, Sidoarjo city, East Java Province, Indonesia in June 2019. The leaves were taken from by-products of pruning with plants of age about 2 years after planting. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and other chemicals used in this study were pro analysis grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Essential oils extraction by microwave-assisted hydrodistillation

Fresh crystal seedless guava leaves were dried using cabinet dryer for 6 hours at 55 ± 2 °C. Dried crystal seedless guava leaves were cut into leaf area per unit with scale of 1x1 cm. The dried cut-off leaves were assisted with combination variables research based on microwave power (300, 450, and 600 W) and F/S ratio (1:4, 1:5, 1:6, 1:7) (w/v). The EOs from dried cut-off leaves were further extracted using oven microwave EMM2308X with 2450 MHz frequency (Electrolux, Stockholm, Sweden). The extraction conditions were provided at atmospheric pressure for 3 h. An one liter flat-bottom extraction flask (a container for raw materials and solvents) was placed in an inside microwave oven and connected to modified condenser and cleverger extraction apparatus. The steam and essential oil vapors were condensed by indirect cooling at 30 ± 2 °C with water in the condenser and collected in narrow receiver tube. The isolation of essential oil were separated from condensed liquid mixtures by opening the tap receiver tube and collected in a vessel tube.

2.3. DPPH radical scavenging activity ^[27] IC₅₀ determination

The antioxidant activity was determined according to the method reported by Brand-Williams *et al.* with slightly modification [20]. The sample solutions containing 20 µL of EO at different concentrations (20–200 µg/mL) and methanol as control solution were mixed with 200 µL of 1mM DPPH radical solutions in methanol. After incubation for 30 min in the dark condition at ambient temperatures, the absorbance values were recorded at 517 nm, individually. The measurements of DPPH radical scavenging activity were conducted in triplicate experiments. Inhibition percent of DPPH was determined based on the following equation:

$$\text{Inhibition percent (\%)} = [(A_0 - A_s) / A_0] \times 100$$

Where A_0 and A_s were the absorbance of control and sample solutions, respectively. IC₅₀ is represented as the required concentration for 50% inhibition of DPPH radical scavenging activity. The IC₅₀ value was calculated by linear regression of inhibition percent from five samples at different concentrations.

2.4. Identification of EO compounds by GC-MS

The chemical compounds of EO were identified using GC-MS analysis and database matching their mass spectral fragmentation patterns using Wiley and US National Institute of Standards and Technology (NIST) libraries (Wiley-Blackwell, Hoboken, NJ, USA). GC-MS analysis was carried out in an ISQ 7000 mass spectrometer (Thermo Scientific, USA) with AEI (advance electron ionization) source. The samples were prepared by dissolving 50 µL in 1 mL final volume of methanol, followed by injected 1 µL of solution into a column (TG-SQC GC 3 5 m x 0.25 mm x 0.25 µm) with AS 1310 autosampler injection system (Thermo Scientific, USA). Helium gas was used as the carrier at a constant flow rate of 1 mL/min. The injector temperature and MS transferline temperature was set 230 and 250 °C, respectively. Initial temperature programming was maintained from 0–8 min at 75 °C, and then from 8–50 min linear gradient increased going from 75–250 °C, followed by constantly temperature at 250 °C from 50–60 min. The amount of bioactive compounds was illustrated by peak area as relative percentages, individually.

2.5. Statistical Analysis

Data were calculated as the mean value ± standard deviation (SD) of triplicate experiments. The one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) with the critical value at 5% significance level ($P < 0.05$) was performed to assess specific differences between mean values using the Statistical Analysis Software (SAS) (Version: SAS 9.1; SAS Institute Inc. Cary, NC).

3. Results and Discussion

3.1. Antioxidant activity of EOs from crystal seedless guava leaves

DPPH assay was used method to evaluate the antioxidant activity in this study and expressed as IC₅₀ value to consider the potency of essential oil based on its concentration in inhibiting 50% activity of DPPH free radicals. It was evaluated that the antioxidant activity of EOs extracted from crystal seedless guava leaves using MAHD with different conditions showed variously IC₅₀ values, as shown in Table 1. The IC₅₀ values of EOs were obtained within the range of 36.23 ± 2.07 to 111.64 ± 1.07 µg/mL, and presented significantly different ($P < 0.05$) among them. DPPH is stable organic radical compound 2,2-diphenyl-1-picrylhydrazyl (C₁₈H₁₂N₅O₆) with the odd electron of nitrogen atom. Basic principle of this method is delocalized electrons from superiority of antioxidant substances by donating their hydrogen atom to the unpaired electrons in the outermost orbital of free radicals [21]. Interpretation of this assessment is based on absorbance of deep violet solutions by reaction of the antioxidant scavenging capacity towards DPPH free radicals [22].

Earlier study about three conventional methods were used to extract the EOs from *Psidium guajava* leaves, particularly focused on hot percolation, steam-distillation, and hydrodistillation techniques,

followed by their IC_{50} values of DPPH radical scavenging assay were 950 $\mu\text{g/mL}$, 460.37 ± 1.33 , and 18.52 ± 0.25 mg/mL using aqueous solvent, respectively [23, 24, 25]. However, the IC_{50} values in this study is superior to those used method to extract the EOs due to the lowest IC_{50} value indicates that high potential antioxidant activity. Supportive study on EO extracted using MAHD from red flesh fruit of *P. guajava* leaves that the antioxidant activity showed the similar IC_{50} value (41.95 ppm) to this study [26]. In addition, it has been reported that EO from *Callistemon citrinus* skeels using MAHD (7.48 $\mu\text{g/mL}$) has better antioxidant activity than HD extract (8.08 $\mu\text{g/mL}$) [27]. Thus, MAHD technique contribute to extract EO from crystal seedless guava leaves with significant high antioxidant activity.

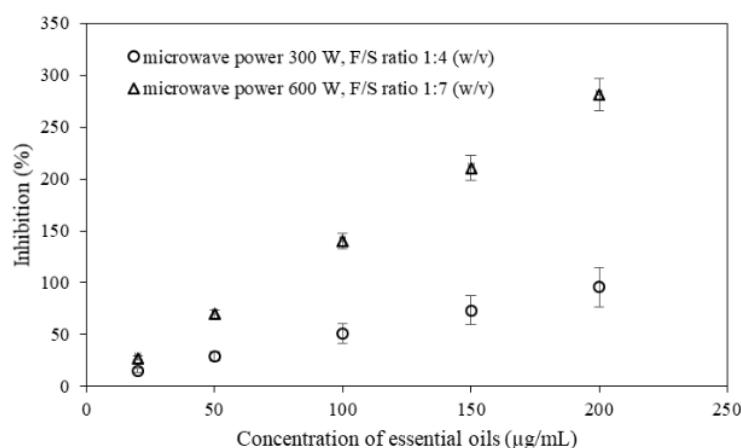


Figure 1. IC_{50} values determination for antioxidant activity from Eos.

Table 1. Effects of microwave power and F/S ratio on IC_{50} values of EOs.

Microwave power (W)	F/S ratio (w/v)	IC_{50} ($\mu\text{g/mL}$)
300	1:4	111.64 ± 1.07^a
	1:5	77.94 ± 3.98^b
	1:6	74.47 ± 3.58^c
	1:7	52.06 ± 2.84^d
	1:4	66.38 ± 6.65^f
450	1:5	55.65 ± 7.10^g
	1:6	50.02 ± 0.30^h
	1:7	38.23 ± 1.21^i
	1:4	55.60 ± 2.63^j
600	1:5	43.88 ± 0.93^k
	1:6	41.19 ± 3.99^l
	1:7	36.23 ± 2.07^m

Means followed by different letters in the same column are significantly different ($P < 0.05$)

Comparison IC_{50} values based on their extraction conditions were assumed that high number of microwave power and F/S ratio resulting favourable antioxidant activities. A comprehensively IC_{50} values were illustrated from two different extraction condition, as shown in Figure 1. These increased numeral activities were comparable case with the results of antioxidant activities of essential oil from black zira using MAHD, where in the manner of enlarging microwave power possess increasingly DPPH scavenging capacities [16]. Elevating the microwave power enhances the solvent power as driving force

to destroy the plant matrix in rapid extraction times [28]. The volume of solvent regulates as media to contact with surface area of plant, and consequently earn extraction efficiency. Shorter time extraction provides to prevent thermally sensitive compounds. In this study, extraction using microwave power of 600 W and F/S ratio of 1:7 (w/v) may lead to simplify the EO comes out from inside cell and it recovers from degradation by high temperature.

3.2. Chemical compositions of EOs from crystal seedless guava leaves

Table 2. Composition of volatile compounds in EO from crystal seedless guava leaves extracted by MAHD based on GC-MS from two different extraction conditions.

RT	Compound	Relative peak area (%)	
		300 W, 1:4 (w/v)	600 W, 1:7 (w/v)
5.464	Benzaldehyde	0.04	-
8.205	Eucalyptol	0.39	0.64
8.460	Cyperene	0.05	-
8.490	α -Ocimene	-	0.09
8.939	cis-Ocimene	0.04	-
15.385	α -Terpineol	0.04	-
22.146	Ylangene	0.04	-
22.472	Copaene	5.55	3.83
23.591	Neoclovene	-	0.10
23.615	(-)- α -Gurjunene	0.15	-
24.081	Caryophyllene	36.40	44.98
24.206	Methyl 4,7,10,13-hexadecatetraenoate	-	0.12
24.598	Aromandendrene	5.84	5.10
24.696	α -Selinene	-	0.54
25.074	Humulene	6.97	5.10
25.295	9-epi-(E)-caryophyllene	1.26	-
25.659	δ -Cadinene	5.91	4.16
25.771	?-Murolene	1.75	0.63
26.091	β -Selinene	0.82	-
26.145	ω -3 Arachidonic acid methyl ester	-	0.12
26.349	α -Guajene	-	0.62
26.513	α -Murolene	0.95	-
26.529	cis- α -Bisabolene	-	0.73
26.737	β -Bisabolene	-	0.39
26.846	Cadine-1,4-diene	0.18	2.01
26.937	β -Copaene-4 α -ol	-	0.28
27.520	Cubenene	1.88	-
27.815	α -Calacorene	0.32	0.13
28.111	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	0.77	0.32
28.475	trans-Nerolidol	11.76	9.16
28.556	Alloaromadendrene	-	0.64
28.631	Retinal	-	0.29
28.856	Spathulenol	-	0.82
29.039	Caryophyllene oxide	-	15.58
29.135	(-)-Globulol	14.06	-
29.628	Pogostol	-	3.62
29.683	Ledol	4.83	-

EOs compounds profile were listed in Table 2 with comparing constituents from two different extraction conditions. It was intended to understand the chemical compositions from the highest and lowest antioxidant activity, which compounds played role to contribute in precious sources of antioxidant agent. Overall, 23–25 divergent compounds from EOs extract were identified using GC-MS analysis. In this study, the EO extracted by MAHD with a microwave power 600 W and F/S ratio 1:7 (w/v) was apparently characterized by the main fractions of caryophyllene (44.98%), caryophyllene oxide (15.58%), trans-nerolidol (9.16%), humulene (5.10%), aromandendrene (5.10%), δ -cadinene (4.16%), copaene (3.83%), pogostol (3.62%), cadine-1,4-diene (2.01%) and other compounds (6.46%). Similar results were reported the EO extracted by steam-distillation techniques from its genesis geographic region that caryophyllene was the most abundant compound in endemic Taiwan *P. guajava* leaves species [29]. Caryophyllene is primary compound corresponding in EO with highest IC₅₀ value. In this work, tree cultivation farm were grown in Prambon sub-district, Sidoarjo city, East Java Province, Indonesia. Their origin plants were imported from Taiwan and it is analogous compositions to be compared with EO from its native commodity.

4. Conclusions

In this study, EO from crystal seedless guava leaves were successfully extracted using MAHD technique and selected from a microwave power of 600 W with F/S ratio of 1:7 (w/v) and it showed the best IC₅₀ value of DPPH radical scavenging assay ($36.23 \pm 2.07 \mu\text{g/mL}$) compared to other EO based on extraction conditions. Its five major chemical compositions were caryophyllene (44.98%), caryophyllene oxide (14.96%), trans-nerolidol (9.16%), humulene (5.10%), and aromandendrene (5.10%). EO extracted from crystal seedless guava leaves using MAHD technique is the absolutely suitable technology applied in extraction of volatile compounds well-designated as high antioxidant activity. Obviously data showed by appearance of caryophyllene as relatively high abundance substance compared to other method, which was responsible to obtain the EO with highest activity of antioxidant. Thus, this proprietorship technology should be applied to scheming in the industrial design.

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