

CHEMICAL PROFILE AND BIOLOGICAL ACTIVITY OF ESSENTIAL OILS FROM *Psidium guajava* GROWN IN TIMOR ISLAND-EASTERN INDONESIA

Antonius R. B. Ola^{1,2,✉}, Yosefa Cysilia Bheku Dje^{1,2}, Agustina E Nahas³,
Petronela Nenotek³, Theo Da Cunha¹, Dodi Darmakusuma^{1,2}, Henderiana L.
L. Belli² and Herianus J. D. Lalel^{2,3}

¹Department of Chemistry, Faculty of Science and Engineering, University of Nusa Cendana, Kupang-NTT 85118, Indonesia

²Biosains Research Center Laboratory, University of Nusa Cendana, Kupang-NTT 85118, Indonesia

³Agrotechnology Department, Faculty of Agriculture, Nusa Cendana University, Kupang-NTT 85118, Indonesia

✉Corresponding Author: antonius.ola@staf.undana.ac.id

ABSTRACT

Psidium guajava was abundantly found in arid land of Timor and traditionally used for treatment against diarrhea. Research had been performed to analyze the chemical profiles together with antibacterial and fruit flies attractant activity of the leaf essential oil from *P. guajava*. The powder of dried leaves was subjected to hydro-distillation using Clevenger apparatus to obtain essential oil with a yield of 0.5%. Analysis of GC-MS spectra revealed that limone and β -caryophyllene were the main components of the essential oil. The essential oil showed strong antibacterial activity with the zone inhibition was observed at 16.3 for *Staphylococcus aureus* and 15.9 mm for *Escherichia coli*. On the other hand, no fruit flies were trapped during the field test in guava fruit.

Keywords: *Psidium guajava*, Timor Island, Essential Oil, Fruit Fly, Antibacterial.

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INTRODUCTION

Timor Island is in Eastern Indonesia composed of mostly warm semi-arid lands due to the exceptionally high solar radiation and water shortage.¹ Therefore, medicinal plants are required to adapt with higher ecological stress² as for optimum growth, the medicinal plants need enough water.³ *Psidium guajava* locally known as *kujawas* was one of the medicinal plants that grow widely in semi-arid lands of Timor. The leaves and fruit of *kujawas* (*P. guajava*) have been used as one of the alternative treatments against diarrhea.

The medicinal properties of plants might be due to the presence of the chemical metabolites inside the plants including leaves and fruits.^{4,5} Moreover, chemical components are known to contribute the plant adaptation against pathogens, herbivores, and drought. For example, higher production of β -caryophyllene from *Arabidopsis thaliana* was caused by the invasion of pathogenic *Pseudomonas syringae* to the flower of the plant.⁶ β -caryophyllene had been also reported to attract specifically the guava fruit fly *Batrocera correcta*⁷ and one of the main metabolites of the essential oil from *P. guajava*.⁸ As chemical metabolites were responsible for the medicinal and ecological properties of the plant, this study was aimed to investigate the profile of chemical components together with antibacterial activity and fruit fly attractant potency of guava leaf oil from Timor Island and Indonesia for the first time.

EXPERIMENTAL

Material

Fresh leaves of *kujawas* (*Psidium guajava*) were taken from Timor Island (Kefamenanu) in the Province of East Nusa Tenggara, Indonesia. The leaves were then brought to the Laboratory of Bioscience of Universitas Nusa Cendana.

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Distillation of Essential Oils

The essential oil of *P. guajava* was obtained from hydro-steam distillation. The fresh leaves of *P. guajava* were dried at room temperature and ground into powder. The powder (150 g) was immersed with distilled water for two hours. The mixture was poured into the Clevenger apparatus and distilled for 4 hours. The distillate containing essential oil was separated from water and dried again with anhydrous sodium sulfate. The leaf oil was kept at 4^o C for the next chemical analysis. The calculation of oil yield was based on the formula:

$$\text{The yield of EO (w/w)} = \text{EOs obtained (g)/crude leaves (g)} \times 100 \%$$

GC-MS Analysis

Gas chromatography-mass spectrometry for analysis of the essential oil was performed using a GCMS-QP2010 (Shimadzu, Kyoto, Japan). Separation was carried out using Rtx 5 MS column (5% diphenyl/95% dimethyl polysiloxane, 30 m, 0.25 mm i.d. and film thickness of 0.25 μ m) with helium as the carrier gas at a flow rate of 0.66 mL/min with an injection of 0.2 μ L of 10% HPLC hexane solution. The injection temperature was 300^o C and the GC oven initial temperature was 40^o C which was held for 2 min and then heated to 310^o C at 5^o C/min and held for 10 min again with a total analysis time of 35 min. The transfer line temperature was 250^o C and the MS ion source was 200^o C.

Antibacterial Activity

The paper disk method was applied to investigate the antibacterial property of the essential oil from *P. guajava*. The oil was tested against *Staphylococcus aureus* camp and *Escherichia coli* 0175H7 following the protocol described early⁹ using tetracycline with the concentration 30 mg/mL and aquades as positive and negative controls, respectively. The paper disk was fully soaked into the guava essential oil (30 μ g) and put into the surface of the media containing the bacteria. The plates were then kept in the incubator for about 24 hours at 37^o C. The observed clear zone was then measured as the diameter of inhibition.

Fruit Fly Trapping on *Psidium guajava*

Trapping fruit flies in the field was undertaken using a modified clear trap made from aqua mineral water bottle baited with essential oil of *P. guajava*. The trap was baited with 0.2 mL essential oil of *P. guajava*. The experiment was performed on guava (*Psidium guajava*) fruits. Only one trap was used for the experiment. The trap was hung at 1.50 m above the ground level and was observed for twelve (12) days.

RESULTS AND DISCUSSION

The oil from the leaves of kujawas (*P. guajava*) was yellowish white with guava aromatic scent. The yield of the guava essential oil obtained from hydrodistillation was 0.5% (v/w). The oil content from the mature leaves of guava collected in Timor was comparable with early reports. The small difference in the oil yield obtained here was probably due to the geographic location and seasonal variation.¹⁰ The essential oil was further analyzed its chemical profiles using GC-MS. The chemical profile from the analysis of GC-MS is depicted in Table-1, according to their order of elution from HP-5 column.

A total of 11 chemical metabolites (Fig.-1) were identified as the constituents of essential oil. The major constituents of the oils (Table-1) were limonene (35.4%), β -caryophyllene (29.7%), α -muurolene (6.03%), caryophyllene oxide (5.7%) and nerolidol B (5.2%). Other chemical components found in the EO were α -copaene (4.46%), germacrene (4.01%), alloaromadendrene (2.24%), α -humulene (2.32%), β -bisobolene (2.05%) and δ -cadinene (2.78%).

The EO components of *P. guajava* from Timor-Eastern Indonesia were similar to those reported from Nigeria¹¹ with limonene (42.1%) and β -caryophyllene (21.3%) and also from Lucknow-India with limonene (29.1%) and β -caryophyllene (15.7%) as the major chemical components.¹² It is interesting to note that β -caryophyllene of the EO from Timor is found higher (29.7%) than those reported from Nigeria, Lucknow-India, Pakistan, Brazil, Cuba, Tunisia and from Northeast India. The EO of *P. guajava* from Pakistan¹⁰ contained the concentration of β -caryophyllene about 20.34% while from Cuba¹³ was 21.6%. On the other hand, the EO major chemical component of *P. guajava* from Brazil, Northeast India and Tunisia were reported to different. The EO of *P. guajava* from Brazil¹⁴ was composed mainly of α -

pinene (23.9%) and 1,8-cineole (21.4%) with β -caryophyllene content only 5.2% while EO of *P. guajava* from Tunisia¹⁵ had a higher content of veridiflorol (36.4%) and trans-caryophyllene (5.9%). α -Terpinyl acetate (23.57%) was observed as the main chemical component of the *P. guajava* essential oil from northeast India¹⁶ together with β -caryophyllene (17.65%) and nerolidol (12.16%). The variation in the chemical profile of guava (*P. guajava*) leaf oil from different studies might be due to the difference in genetic diversity, geographic location and also seasonal variation.

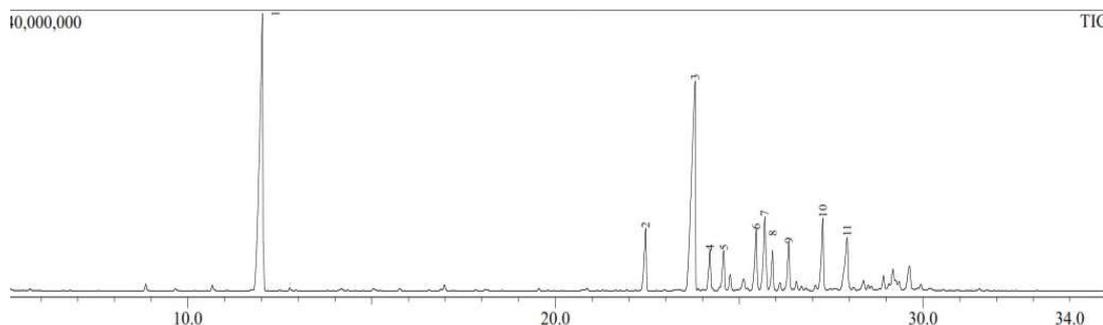


Fig.-1: GC Chromatogram of Essential Oil from *Psidium guajava*

Table-1: Chemical Components of Guava Essential Oil analyzed from GC-MS

No.	Retention Time (min)	Compound	Composition (%)	Similarity Index (SI)
1	12.02	Limonene	35.40	94
2	22.45	α -Copaene	4.46	97
3	23.79	β -Caryophyllene	29.70	97
4	24.2	Alloaromadendrene	2.24	93
5	24.57	α -Humulene	2.32	96
6	25.46	Germacrene	4.01	91
7	25.7	α -muurolene	6.03	91
8	25.9	β -Bisobolene	2.05	94
9	26.34	delta-Cadinene	2.78	82
10	27.27	Nerolidol B	5.14	95
11	27.93	Caryophyllene oxide	5.70	92

The essential oil was evaluated for its antibacterial property against *S. aureus camp.* and *E. coli 0175H7* using tetracycline and aquades as a positive and negative control. Thirty (30) μ g of essential oil was transferred to a paper disc. The EO of *Psidium guajava* inhibited the growth of the tested bacteria with zone inhibition was observed at 16.3 mm for *S. aureus* and 15.9 mm for *E. coli* (Table-2). As only 30 μ g of essential oil was used, it is interesting to note that the antibacterial activity of the essential oil was stronger compared to the tetracycline as a positive control. This found may explain that the essential oil components were responsible for the antibacterial activity of the leaves from *P. guajava* that traditionally have been used for the treatment of stomachache, diarrhea and ulcers. β -caryophyllene was recently reported to possess antibacterial activity by altering membrane permeability that causes membrane damage leading to cell death.¹⁷ This may confirm that β -caryophyllene was also responsible for the antibacterial property of *P. guajava* essential oil.

Table -2: Antibacterial Activity of Guava Leaf Essential Oil

No.	Bacteria	Diameter of Zone Inhibition (mm)		Aquades
		Essential Oil (30 μ g)	Tetracycline (30 mg/mL)	
1	<i>Staphylococcus aureus</i>	16.3	17.9	0
2	<i>Escherichia coli</i>	15.9	17.4	0

As β -caryophyllene was one of the main chemical constituents, the guava leaf essential oil was also tested for its activity to attract fruit fly in guava fruits. Although β -caryophyllene was reported to specifically

attract the guava fruit fly *Batrocera correcta*, no *B. correcta* was trapped in our experiment. This might be due to the absence of *B. correcta* in Timor Island. This confirmed the previous finding that *B. correcta* has not been found in Indonesia but have spread to several regions such as Vietnam, Thailand, China, Burma, India, Nepal, Pakistan and Srilanka.⁷

CONCLUSION

Hydrodistillation of the leaves of *P. guajava* collected in Timor Island yielded the essential oil with high content of β -caryophyllene and strong antibacterial activity. Therefore, the essential oil of *P. guajava* from Timor Island may be developed as a potential source of β -caryophyllene and as an antibacterial agent.

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