Chemical Profiling of Essential Oil Extracted from Fresh Walang Leaves (*Etlingera walang* (Blume) R. M. Sm.), an Indigenous Species in Banten, Indonesia

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Abstract *– Essential oils are a group of secondary metabolite compounds that have a distinctive aroma and are volatile. Walang leaves are one of the plants suspected of having essential oil content. This study aims to determine the constituent components of essential oil compounds from fresh walang leaves. The method used to extract essential oil from fresh walang leaves is steam distillation. The essential oil produced was analyzed using Gas Chromatography Mass Spectroscopy (GC-MS). The results showed that the essential oil of fresh walang leaves contained 15 components of essential oil compounds and 2 main components of essential oil compounds, namely: Acetic acid (CAS) Ethylic acid 24.52%, 1,2 Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl phthalate 21.09%.*

Keywords: *Walang leaves, steam distillation, GC-MS, essential oil*

INTRODUCTION

Etlingera is a genus of medicinal plants that originates from the Indo-Pacific region. Since ancient times, indigenous communities have utilized it for its taste, culinary significance, and therapeutic qualities (Ud-Daula & Basher, 2019). The indigenous walang leaf (*Etlingera walang*) from Banten has traditionally been utilized primarily as a culinary seasoning. Empirically, the walang leaf has been employed as a natural pest repellent in the Baduy community to combat pests in rice fields. Research has explored the potential of walang leaves, revealing their bio-larvicidal effects. Extract from the walang leaf exhibits noteworthy lethality against third instar larvae of *Aedes aegypti* mosquitoes, with an LC_{50} concentration of 0.88% and an LT_{50} of 56.8 hours (Putri et al., 2023).

Etlingera species yield essential oils sourced from a wide array of plant parts such as leaves, stems, flowers, peduncles, bark, rhizomes, seeds, and fruits. However, the total oil content in this genus remains notably low, scarcely exceeding 1% by mass (Ud-Daula & Basher, 2019). Based on previous research using leaves from the Etlingera genus, *E. elatior*, essential oil was obtained with a yield of 0.074% (Renaninggalih et al., 2014). Different research have demonstrated that the extraction of yellow essential oil from *E. yunnanensis* rhizomes resulted in a yield of 0.14% (Guo et al., 2015). The yellow essential oils obtained from the leaves, rhizomes, and stems of *E. sayapensis* exhibited w/w % yields of 2.37, 1.51, and 0.9, respectively (Mahdavi et al., 2017).

Essential oils and their constituents have gained significant approval from consumers due to their promising biological activities, safety, and potential for diverse practical applications (Ud-Daula & Basher, 2019). Previous studies have indicated that essential oils from the Etlingera genus possess antioxidant (Khor et al., 2017), antimicrobial (Mahdavi et al., 2017; Naksang et al., 2020), insecticidal (Guo et al., 2015), Anti-Tyrosinase (Sangthong et al., 2022), and cytotoxicity (Luo et al., 2022) activities.

Etlingera essential oils are rich in both monoterpenes and sesquiterpenes, with the presence of longer chain compounds as well. Monoterpene hydrocarbons like αpinene, β-pinene, α-phellandrene, and limonene are among those identified in Etlingera essential oils. Meanwhile, major sesquiterpene hydrocarbons in Etlingera oil include cadinene, caryophyllene, (E)-farnesene, and tetradecadiene (Ud-Daula & Basher, 2019).

As there have been no prior investigations concerning the chemical compound identification and essential oil determination from fresh Walang leaves in Indonesia, it is imperative to conduct a thorough analysis to identify the chemical constituents present in the essential oil obtained from these leaves.

MATERIALS AND METHODS

1. Materials

The research utilized the following materials: fresh Walang leaves (*E. walang*), distilled water (aquadest), n-hexane, ethyl acetate, chloroform, 96% ethanol, and 10% sulfuric acid (H_2SO_4). The study also employed various equipment, including laboratory glassware, an analytical balance, containers, scissors, a ruler, a pencil, vial bottles, Thin-layer chromatography (TLC) plates (GF254), capillary pipettes, chambers, a steam distillation apparatus, aluminum foil, UV lamps (λ 254 nm and 366 nm), and a Shimadzu QP 2010 GC-MS instrument.

2. Methods

a. Sample Preparation

Fresh walang leaves (*E. walang*) samples were obtained and subjected to a wet sorting process to remove any impurities or foreign materials. Afterward, the leaves were washed with running water to eliminate soil and other contaminants. The prepared material was then cut into approximately 2 cm pieces, weighed, and subjected to steam distillation for the extraction of chemical compounds constituting the essential oil.

3. Chemical Compound Separation Testing

b. Steam Distillation

Fresh walang leaves were collected and chopped into approximately 2 cm pieces. A total of 200 g of the leaves was weighed and placed into a distillation flask, together with 300 mL of distilled water as a solvent. Due to the limited capacity of the distillation apparatus for processing the entire amount of leaves (400 g) and solvent (600 mL of distilled water), the testing was conducted in two separate runs. The heating process was carried out using either a heating mantle or a microwave heater, with the temperature set at 100°C. The distillation process took approximately ≤ 6 days to obtain the essential oil. Afterward, a decantation process was performed to separate the mixture of water and essential oil. The collected essential oil was then stored in vial bottles, packed with aluminum foil, and kept in a designated sample storage area. Finally, the essential oil was analyzed by identifying its chemical compounds using the GC-MS method.

c. Gas Chromatography Mass Spectrometry (GC-MS)

The essential oil components were analyzed using GC-MS with an injector temperature of 250°C and an oven temperature of 100°C. The pressure was set at 8.8 Psi, and a capillary column was used for the analysis. Helium gas was used as the carrier gas at a flow rate of 1 mL/minute. A 1 μL sample was injected and analyzed using gas chromatography, followed by mass spectrum analysis to determine the component structures. The MS conditions included 70 eV ionization energy, 230°C ion temperature, and a mass range between 30-550 amu. Several major components of the essential oil from the walang leaves were identified by considering the components with the highest percentage values, expressed as >90%.

RESULT AND DISCUSSION

1. Sampel Walang Leaves

Fresh walang leaves (*E. walang*) were collected from Mekarwangi Village, Saketi District, Pandeglang Regency, Banten Province in July 2022. The leaves were cleaned with running water and sorted to remove impurities and damaged parts (Boor & Lefebvre, 2021). Afterward, the leaves were chopped into approximately 2 cm pieces to expedite the drying process and preserve the essential oil content. This approach aimed to preserve the essential oil present in the plant and prevent any damage to the secondary metabolites that are released shortly after cutting the leaves ((Ud-Daula & Basher, 2019)). The chopped leaves, totaling 1000 g, were then weighed, and a 400 g sample was taken for the steam distillation process with 600 mL of solvent. The results of the preparation of fresh Walang leaves (*E. walang*) samples can be observed in Table 1.

2. Chemical Profile from Walang Leaves

a. Essential oil from Walang Leaves

Essential oil, being a volatile compound that is insoluble in water, can be extracted from plant tissues using the distillation process. In this method, the plant tissues are heated with water or steam, causing the essential oil to vaporize along with the steam or water vapor formed. The mixture of water vapor and essential oil is then condensed at a relatively low temperature. The resulting condensate comprises a mixture of water and essential oil, which can be easily separated due to their inability to dissolve in each other (Božović et al., 2017).

Table 1. Fresh Walang Leaves

Sample	Initial sample weight required	Fresh sample weight required	Sample description
Walang leaves	1000 g	400 g	Fresh Walang leaves

Distillation techniques can be achieved using various methods, including the boiling method, in which the material is boiled in boiling water. This causes essential oils to vaporize alongside the steam, which is then passed through a condenser for the purpose of condensation. The equipment employed for this approach is referred to as a boiling still. Another technique is the steaming method, involving the steaming of the substance within a vessel constructed similarly to a steamer. This steaming causes essential oils to vaporize and be conveyed by the steam flow, directed into a condenser for condensation. The apparatus used in this method is known as a steaming still. Additionally, the direct steam method entails subjecting the substance to steam derived from a steam-generating kettle. This leads to the vaporization of essential oils, carried along by the steam flow, which is then channeled into a condenser to undergo condensation. The equipment utilized for this technique is termed a direct steam still (Machado et al., 2022).

Distillation serves the purpose of isolating essential oil from walang leaves. Steam distillation is chosen for this research, as the inherent essential oil in walang leaves is susceptible to high temperatures and readily volatile. Moreover, this technique is well-suited for essential oils with lower vapor pressure and boiling points, facilitating efficient distillation and reducing the potential for oil degradation through hydrolysis. As a result, the anticipated outcome of this distillation process is a superior quality essential oil (Ud-Daula & Basher, 2019).

To prevent steam and essential oil leakage, a precaution involves applying vaseline to glass pipe joints during distillation, sealing them with aluminum foil due to the volatile nature of oils. The process entails steam carrying essential oil upward, which vaporizes and condenses in the condenser, then collects in a calibrated tube. The separation of oil and water phases occurs based on their different densities, with water settling at the bottom and the lighter oil phase above (Machado et al., 2022).

In this study, the material utilized was freshly harvested walang leaves, which underwent initial chopping (whole material was diced to approximately 2 cm). Following this, 200 g of leaves were weighed and placed into a distillation flask, along with an addition of 300 mL of distilled water (used as a solvent). Due to the limited capacity of the distillation apparatus, which was insufficient for processing the entirety of 400 g of leaves and 600 mL of distilled water as a solvent, the procedure was conducted twice. Achieving essential oil distillation results required approximately ≤ 6 days for each trial. The distilled oil was separated through decantation, effectively isolating the remaining oil-water mixture.

The essential oil yielded was a clear light yellow with a strong aromatic fragrance. In this research, a total of 3 mL of distillate was obtained through two replicates, using different sample weights and producing non-reproducible distillate volumes (Sastrohamidjojo, 2021). This discrepancy arose due to inconsistent sample cutting sizes, leading to insufficiently uniform pieces that did not fully fit the distillation flask. The research outcome of steam distillation testing on walang leaf essential oil demonstrated successful results, indicating the presence of oil in the walang leaves with a determined density of 0.003268 g/mL. The yield from fresh walang leaf essential oil was calculated as 0.75%. The vapor distillation results from fresh walang leaves (*E. walang*) are presented in Table 2.

Table 2. Distillate of Walang Leaf Essential Oil

Sample	Sample Weight	Solvent Volume	Distillate	Density	Yield	Description
Fresh Walang Leaves	400 g	600 mL	3 mL	0,003268 g/mL	0.75 %	(+) essential oil

Table 3. Results of GC-MS Fresh Walang Leaf Essential Oil

Peak	R.Time	Area	Area%	Name (Similarity index)	Formula
1	2.892	421454	2.74	Hydrazine, methyl-(CAS) Methylhydrazine	CH_6N_2
2	3.083	3771533	24.52	Acetic acid (CAS) Ethylic acid	$C_2H_4O_2$
3	5.883	400146	2.60	Benzene, 1-methyl-3-(1-methylethyl)-(CAS) m-Cymene	$C_{10}H_{14}$
4	7.502	1145443	7.45	Benzene, 1-methyl-3-(1-methylethyl)-(CAS) m-Cymene	$C_{10}H_{14}$
5	8.050	388225	2.52	Heptanoic acid (CAS) Heptoic acid	$C_7H_{14}O_2$
6	8.442	415635	2.70	Benzene, 1-Isopropenyl-2 Methyl	$C_{10}H_{12}$
7	9.093	799742	5.20	2,5-Furandione, 3-(1,1-dimethylethyl)	$C_8H_{10}O_3$
8	9.217	285593	1.86	2,5-Furandione, 3-methyl-4-propyl	$C_8H_{10}O_3$
9	9.363	654392	4.25	Octanoic acid (CAS) Caprylic acid	$C_8H_{16}O_2$

Peak	R.Time	Area	Area%	Name (Similarity index)	Formula
10	9.645	1406850	9.15	2,5-Furandione, 3-methyl-4-propyl	C8H10O3
11	10.678	657444	4.27	2-Cyclohexen-1-one, 6-methyl-3-(methylrthyl)- (CAS) Carvenone	$C_{10}H_{16}O$
12	12.790	711568	4.63	2-Decenoic acid $$ (2E)-2-Decenoic acid	$C_{10}H_{18}O$
13	19.467	85909	0.56	Cyclopropanamine, 2-pheyl-, trans	$C_9H_{11}N$
14	19.752	3243399	21.09	1,2 Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl phthalate	$C_{16}H_{22}O_4$
15	20.550	994587	6.47	1,2 Benzenedicarboxylic acid, bis(2-methxyethyl) ester (CAS) Kesscoflex MCP	$C_{14}H_{18}O$

Table 4. Main Constituents of Fresh Walang Leaf Essential Oil

Peak	Compound	Area (%)
1	Acetic acid (CAS) Ethylic acid $$ Vinegar acid $$ Ethanoic acid $$ Glacial aceticacid $$ Methanecarboxylic acid	24.52
2	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl phthalate $$ Isobutyl O-Phthalate	21.09

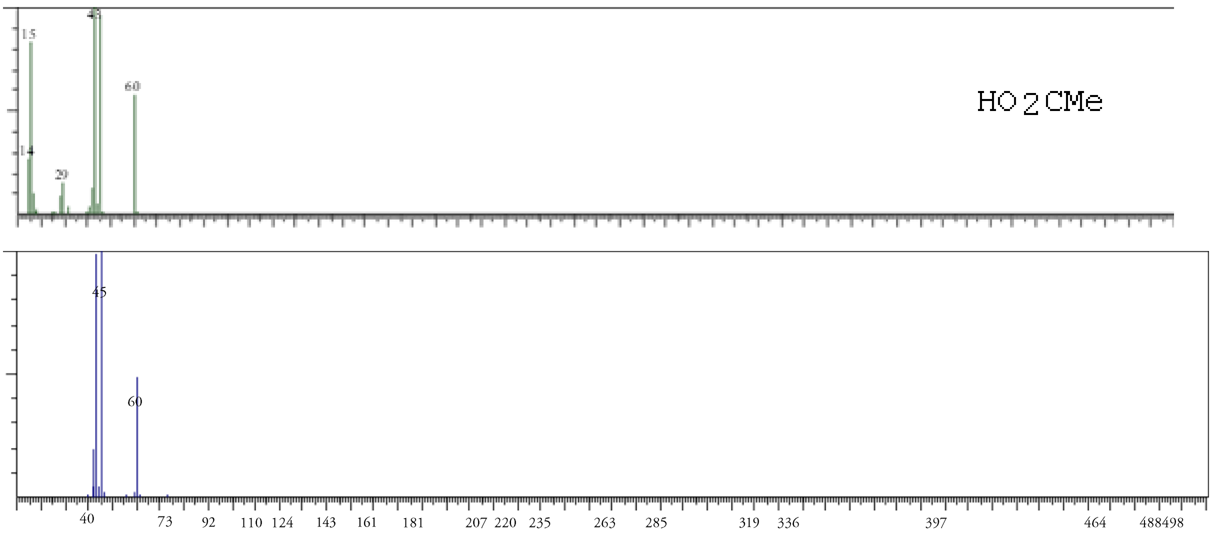
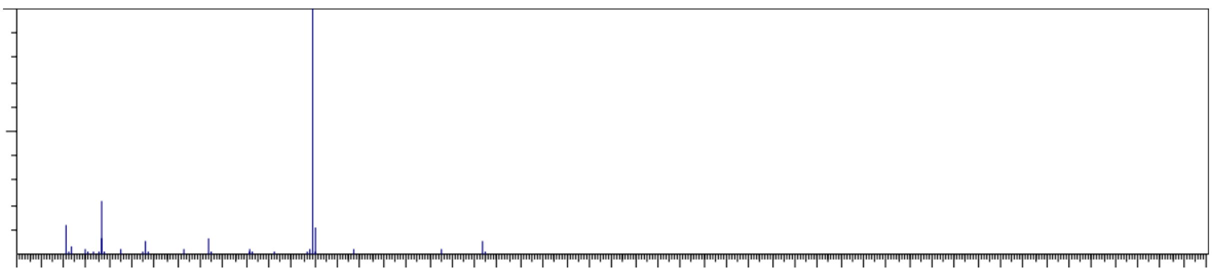


Figure 1. Mass Spectrum of Compound Acetic Acid (CAS) Ethylic acid at 45 m/z



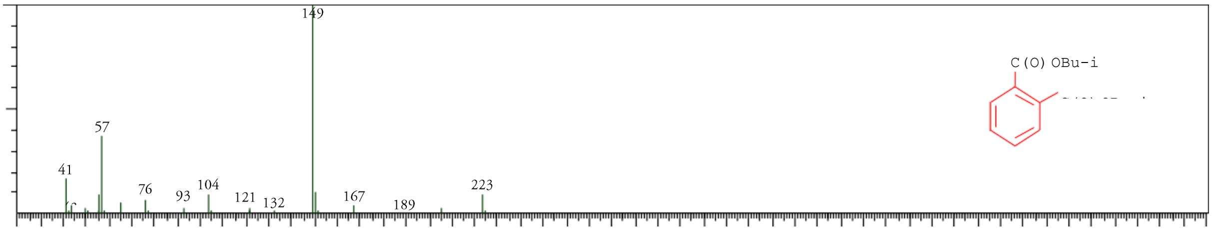


Figure 2. Mass Spectrum of Compound 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl phthalate at 149 m/z

b. Chemical Profile From Fresh Walang Leaf Essential Oil

The determination of chemical compound identification of the essential oil from fresh walang leaves (*E. walang*) in this study was conducted to ascertain the constituents present within, along with their respective molecular formulas and structural compositions. Through identification using GC-MS equipment, a total of 15 compounds were detected in the chromatogram, corresponding to 15 peaks, each with its molecular formula and structural configuration. The results of the GC-MS analysis of the essential oil are presented in the Table 3.

In the analysis of the main components present in the essential oil derived from fresh walang leaves, two major constituents stand out due to their highest percentage areas. The first significant compound at retention time 3.083 is observed in the second peak, identified as Acetic acid (CAS) Ethylic acid accounting for 24.52% of the oil composition. Acetic acid holds importance as a chemical reagent and a crucial industrial raw material, playing a role in polymer production like polyethylene terephthalate, cellulose acetate, and polyvinyl acetate, as well as diverse fibers and fabrics. It is believed to possess antibiotic attributes, potentially alleviating bacterial infections in fungi. The second noteworthy compound emerges in the fourteenth peak at retention time 19.752 making up 21.09% of the composition, and is recognized as 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl phthalate. This specific ester is categorized among organic compounds formed by substituting one hydrogen atom and a carboxyl group with an organic group. The geraniol ester compound is hypothesized to exhibit antitumor properties.

CONCLUSION

Fresh walang leaf essential oil is composed of 15 constituent compounds. The two predominant primary constituents are Acetic acid (CAS) Ethylic acid; 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl phthalate. These two compounds are recognized for their potential antibacterial properties. Based on the outcomes of steam distillation for compound separation, the presence of essential oil in fresh walang leaves is affirmed, with a density of 0.003268 g/mL and a yield of 0.75%.

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