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CHARACTERIZATION AND BIOACTIVITY OF ORANGE PEEL ESSENTIAL OILS: ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY POTENTIAL

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ABSTRACT

This study explores the potential of essential oils from citrus peels (lime, pomelo, and lemon) as antioxidants and xanthine oxidase inhibitors. The essential oils were extracted using steam distillation and analyzed using GC-MS. Major components identified include limonene, ß-pinene, and ß-phellandrene. Antioxidant activity was measured using the DPPH free radical scavenging method, while inhibition of the xanthine oxidase enzyme was measured using a microplate reader. Lemon peel essential oil exhibited the highest antioxidant activity with an IC50 value of 68.865 µg/mL, while lime peel oil showed the strongest xanthine oxidase inhibition with an IC50 of 112.283 mg/mL. The essential oils met standard physicochemical properties, including solubility in alcohol, specific gravity, and refractive index. These findings suggest that citrus peels have the potential to be a source of essential oils useful in various pharmaceutical and aromatherapy applications.

Keywords: Citrus Peels, Characterization, Essential Oil, Xanthine Oxidase, Antioxidant, GC-MS.
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INTRODUCTION

People in many nations are turning to natural ingredients and herbal medicines as an option for illness prevention and treatment. Among these resources, citrus plants from the Rutaceae family stand out due to their abundant fruit production, with significant amounts of peel waste generated annually. While orange peels are typically discarded, they are rich in bioactive compounds, including essential oils with potential therapeutic applications.[1] Essential oils are plant-produced secondary metabolites that are often liquid in nature and have unique scents depending on the plant from which they originate. The fragrant and medicinal qualities of citrus oils are attributed to the presence of several molecules such as limonene, alpha-pinene, alpha terpineol, beta-myrcene, linalool, citronellal, geranial, and sabinene.[2] The fruit skin of the Pamelo (*Citrus maxima* (Burm). Merr) contains a multitude of nutritional components, the most prevalent of which are pectin and tannin compounds. Other components found include alkaloids, flavonoids, lycopene, and ascorbic acid. It also includes hesperidin and naringin, which are classified as flavonoid molecules. The components in question had modest antioxidant activity, as indicated by their IC_{50} value of 111.69 ppm.[3,4,5] The characteristic scent of Pamelo peels is generated by limonene chemicals and other essential oils. Limonene (95%) myrcene (2%), nocturnal (1%), pinene (0.4%), linalool (0.3%), decanal (0.3%), sabinene (0.2%), geraniol (0.1%), neral (0.1%), dodecanal (0.1%), and citronella (0.5%) are the essential oils found in pamelo peels.[6,7] Lemon (*Citrus limon* (L) Osbeck) contains citric acid, essential oils, bioflavonoids, polyphenols, coumarins, and flavonoids which have potential as antioxidants. And limes also known as chili oranges (*Citrus amblycarpa* (Hask.) Ochse) contain ascorbic acid, flavonoids, essential oils, coumarin, and rosmarinic acid derivatives, which are useful for aromatherapy[10] and have antioxidant activity. The free radicals constantly created by the body, can deactivate many enzymes, oxidize lipids, disrupt DNA, and cause cell mutations. Free radicals produced in the metabolism of xanthine oxidase, such as hydrogen peroxide (H_2O_2), can lead to increased production of uric acid in the body.[11] Although the benefits of essential oils are well-known, there has been limited research on the antioxidant and xanthine oxidase inhibitory activities of essential oils derived from different citrus peel varieties. This study seeks to fill that gap by characterizing the essential oils from lime, pomelo, and lemon peels, and evaluating their bioactivity, particularly their antioxidant capacity and enzyme inhibition potential. Recent studies have emphasized the potential of citrus peels as a source of essential oils rich in bioactive compounds like limonene and pinene,

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which exhibit significant antioxidant and enzyme inhibitory effects.[2,5] However, a comparative analysis of essential oils from various citrus species remains largely unexplored.

EXPERIMENTAL

Sample Collection and Distillation

The samples were collected from the Manoko plantation, Lembang, West Java, Indonesia. Each fresh orange peel was chopped and weighed, then steam distillation was carried out to obtain essential oils at atmospheric pressure until all the essential oils had evaporated. The essential oil obtained is separated with anhydrous Na_2SO_4 until all the water in the crude essential oil is absorbed.[12]

Analysis GC-MS

The essential oils were analyzed using a GC-MS system (Shimadzu, GCMS-QP2010) under the following conditions: oven temperature at 70°C, helium as the carrier gas, and a column flow rate of 1.11 mL/min.

Characterization Essential Oil

Characterization of orange peel essential oil was carried out using organoleptic observations by observing color, smell, and taste, determining the refractive index, specific gravity, solubility parameters in alcohol, and determining the acid number and ester number.[14,15]

Antioxidant Activity Assay

The antioxidant activity of each essential oil and vitamin E was evaluated using the DPPH method by measuring absorbance at 516 nm with a microplate reader. Various concentrations of each essential oil sample were mixed with methanol and DPPH in a 1:1 ratio, following the same procedure for the vitamin E standard. Blank wells containing only methanol and DPPH were used as references. After a 30-minute incubation at room temperature to allow for a complete reaction, absorbance was recorded using a Thermo Scientific microplate reader. The percentage of inhibition was calculated for each sample and vitamin E, with analyses performed in triplicate.[16]

Determination of Xanthine Oxidase Inhibition

A spectrophotometer was used to measure the inhibitory activity of xanthine oxidase on 96-well plates. To guarantee a final DMSO concentration of no more than 0.5%, 50 µL of the sample was first dissolved in DMSO and then diluted with buffer. Allopurinol was used as the reference. The composition of the test solution was as follows: 50 µL of the sample, 35 µL of pH 7.5 phosphate buffer, 30 µL of recently made enzyme solution (0.2 units/mL xanthine oxidase in pH 7.5 phosphate buffer), and 60 µL of substrate solution (0.15 mM xanthine in pH 7.5 phosphate buffer). Every sample and allopurinol were examined three times each. The inhibitory activity of xanthine oxidase was determined. I% is equal to [(A-B)/A] x 100, where A is the absorbance of the xanthine oxidase enzyme without sample, or blank A (absorbance without XO and sample), and B is the sample absorbance, or blank B (absorbance without XO). A calibration curve between concentration and percent inhibition is used to calculate the IC50 value.[17,18]

RESULTS AND DISCUSSION

For every 2 kg of orange peel, the essential oil was extracted by distilling it for a total of ± 2 hours for each sample. Peels from lemons, pomeloes, and limes produced essential oils yielding 0.35, 0.45, and 0.85% v/w, respectively. The steam distillation process has a high distillation efficiency because of its short distillation duration and large product output. The oil is not combined with water, which yields high-yield results.[19] The sample of lime peel in this study had the highest yield, coming in at 0.85%. The amount of oil that is extracted during the distillation process is referred to as yield. A higher yield indicates that more essential oil was produced. However, it's frequently noted that the essential oil's quality tends to decline as the yield increases. The higher the yield, the lower the quality obtained.[20,21] Numerous factors, including genetics, environment, extraction technique, soil height and fertility, plant age, refining procedure, and disease infestations, can affect the yield of high- or low-quality essential oils.[20,22,23] Orange peel essential oil composition is examined using GC-MS. It is designed to perform dynamic separation and identification of all volatile organic compounds using a mass spectrometer that can select charged gas molecules based on their mass or weight. Orange peel essential oil is made up of a variety of ingredients, according to the

GC MS spectrum. The most prevalent constituents of lemon peel essential oil are limonene (38.48%), gamma-terpinene (7.69%), linalool (5.98%), beta-pinene (5.31%), and beta phellandrene (3.33%) (Fig.-1.a). Conversely, limonene (77.73%), beta myrcene (5.35%), beta-pinene (4.04%), and germacrene D 1.55% make up pamelo essential oil (Fig.-1b). The main components of lime essential oil are limonene (22.18%), citronella (14.37%), beta-pinene (11.60%), beta-phellandrene (9.80%), and 3-cyclohexane (9.05%) (Fig.-1c). Following the GC MS analysis of lemon peel essential oil, 37 peaks were discovered in the chromatogram; the five major components were identified as dl-limonene, gamma-Terpinene, 1-beta-pinene, linalool, and beta-phellandrene. The chromatogram of pomelo peel essential oil reveals 20 peaks, with the largest being beta-myrcene, beta-phellandrene, beta-limonene, and alpha-pinene. In contrast, the lime essential oil chromatogram shows 31 peaks, with the most prominent being dl-limonene, citronellal, beta-pinene, beta-phellandrene, and 3-cyclohexene.[24] The primary constituent of these essential oils is dl-limonene, which first appears at a retention time (Rt) of 7.617 minutes[25] (Fig.-1). Pomelo peels essential oil contains the highest percentage of limonene at 77.73%. Although the concentrations vary, the beta-pinene and beta-phellandrene contents are similar across the three types of orange peel essential oils. Therefore, the differences among the essential oils from pomelo, lemon, and lime peels lie in the other components and their concentrations, rather than in the primary component, dl-limonene. Orange peel essential oil is widely utilized in the chemical perfume industry, as a flavoring agent in beverages and food, and in the health sector as an antioxidant and anticancer agent.[26] Characterizing essential oils is crucial to prevent adulteration, which is a growing issue due to the expanding market. Adulteration occurs when pure essential oils are intentionally altered with foreign substances or contaminated by environmental pollution. Factors such as low production yields, poor raw material procurement, lack of quality standards, and difficulties in detecting counterfeit products contribute to this problem. To ensure purity, essential oils are evaluated based on organoleptic criteria such as color, odor, form, and taste. For example, essential oils extracted from lemon, pomelo, and lime peels are colorless with a distinct aromatic scent (Table-1). Most essential oils exhibit a white or yellowish tint, though some may appear reddish.[11] They can also absorb oxygen, causing them to darken and intensify in scent over time when exposed to sunlight and room temperature. To ensure the quality of essential oils, it is important to examine their refractive index (RI), specific gravity (SG), alcohol solubility, acid number (AN), and ester number (EN) (Table-2). The SG of orange peel essential oils ranges from 0.696 to 1.188 g/mL, indicating purity, as higher SG often correlates with more components in the oil.[9] The RI, which measures the ratio of light speed in air to light speed in the oil, also indicates purity and ranges between 1.471 and 1.475, meeting Essential Oil Association (EOA) standards. The RI increases with the density of the oil, which is influenced by the carbon chain length and the number of double bonds.[11] Furthermore, the acid number (AN) in essential oil characterization indicates the amount of free fatty acids present in the oil. It is expressed as the number of milligrams of KOH required to neutralize the free fatty acids in the essential oil. A higher AN signifies lower oil quality, while a lower AN indicates higher quality. For orange peel essential oils, the acid value is less than 4.0 mg KOH/g (Table-2), meeting the standard. This measurement reflects the free fatty acids formed due to hydrolysis during processing, which can cause the oil to become rancid more quickly.[27] Conversely, the ester number (EN) the amount of alkali needed to saponify the esters in the oil. A high EN suggests a better aroma quality in the essential oil. In addition to these quality assessments, the antioxidant activity of lemon, pamelo, and lime essential oils was evaluated using DPPH method, revealing IC_{50} values of 68.865 µg/mL, 143.294 µg/mL, and 348.668 µg/mL, respectively (Table-3). These findings highlight the superior antioxidant effectiveness of lemon peel essential oil, which correlates with its higher limonene concentration as detected in the GC-MS analysis. The high antioxidant activity of lemon peel essential oil can be attributed to its high limonene content, which has been shown in previous studies to possess strong radical scavenging properties.[3] This finding suggests that lemon peel essential oil could be a valuable natural antioxidant source for pharmaceutical or food preservation applications. Additionally, the xanthine oxidase inhibition activity of lime peel essential oil (IC_{50} 112.283 mg/mL) was found to be lower than that reported by Fahrurroji and Riza, who observed IC_{50} values of 80.45 mg/mL for lime essential oil from a different region. This difference may be due to variations in limonene and beta-pinene concentrations, as well as environmental factors such as soil quality and climate (Fig.-2). Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid, using oxygen as an acceptor for electrons or hydrogen.[18,28,29] The inhibition

test for xanthine oxidase measures hydrogen peroxide production, a byproduct of xanthine oxidase activity that can increase uric acid levels. Antioxidants can inhibit this process by donating electrons, thus preventing the formation of free radicals during the oxidation of hypoxanthine to xanthine and xanthine to uric acid.[7,28] This mechanism highlights the role of antioxidants in mitigating the effects of xanthine oxidase activity and reducing uric acid production in the body. [30,31]

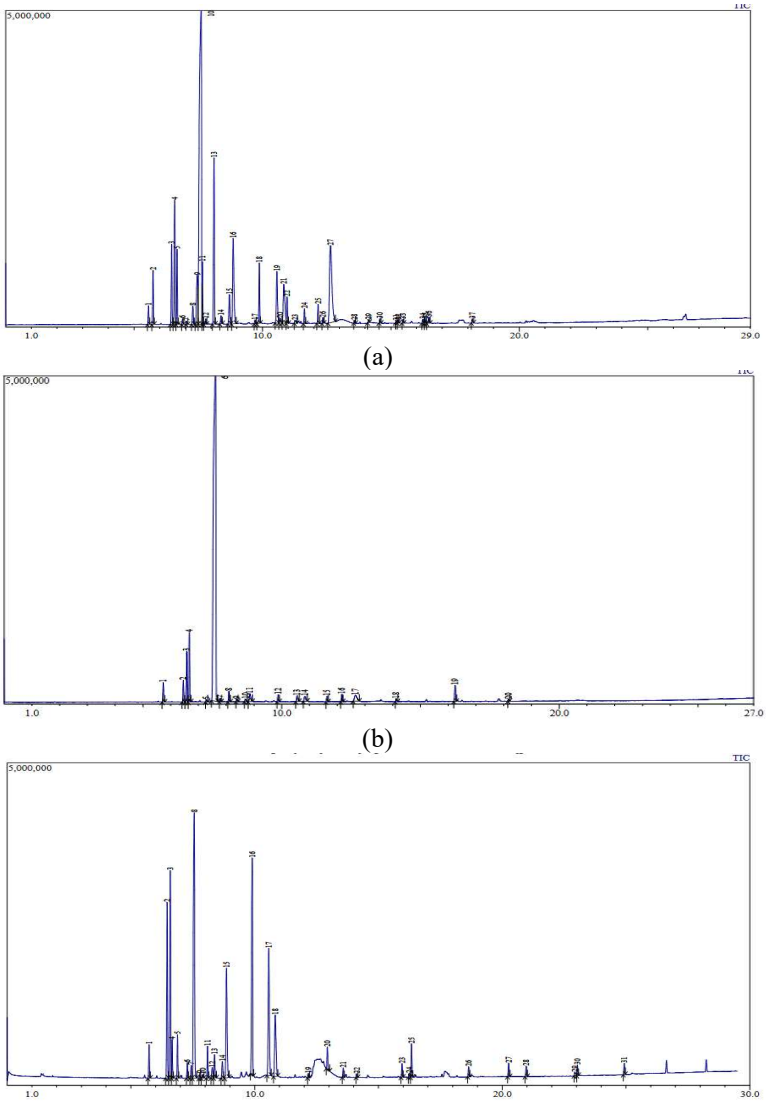
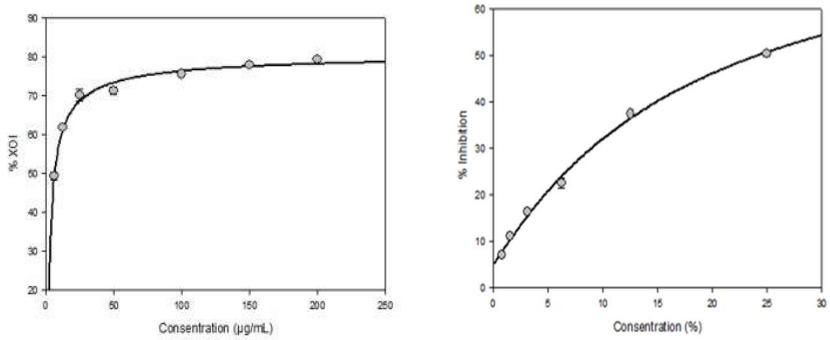
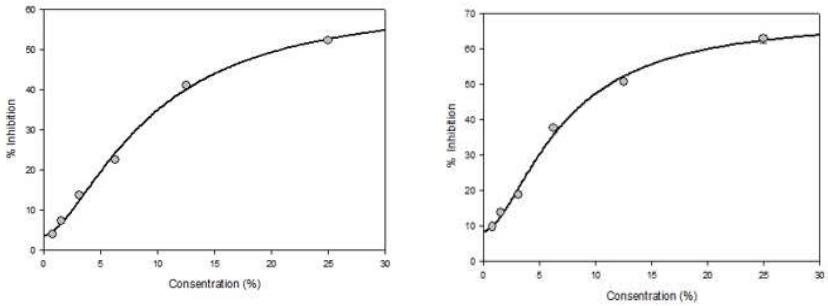


Fig.-1: GCMS Chromatogram of Essential Oils of Lemon (a), Pamelo (b), and Lime (c)



(a) (b)



(c) (d)

Fig.-2: Inhibition of Essential Oils of Allopurinol (a), Lemon (b), Pamelo Peel(c), and Lime Peel(d) Against Xanthine Oxidase Enzyme

Table-1: Organoleptic Parameters of Orange Peel Essential Oil

Parameters	lemon	Pamelo	Lime
Color	clear	clear	Clear, slightly yellowish Lime smell
Smell	Smell of lemons	Grapefruit smell	Liquid
Form	Slightly viscous liquid	Liquid	Bitter, there is a warm sensation
Taste	Bitter	Bland	

Table-2: Characterization Test of Orange Peel Essential Oil

No	Parameter	Result			Standard
		Lemon	Pamelo	Lime	
1	Refractive index (24,2^{0}C)	1.476	1.4773	1.4670	1.471-1.475 (EOA)
2	Specific gravity (g/mL, 25^{0}C)	6.9397	6.8112	6.9023	0.696-1.188
3	Solubility in alcohol (1: 6)	Soluble	Soluble	Soluble	Soluble
4	Acid number (mgKOH/g)	2.1	1.05	1.46	Maximal 4.0 (SNI, 2015)
5	Ester number (mgKOH/g)	1.19	0.58	1.25	

Table-3: Antioxidant Activity

Essential oil	IC_{50} (µg/mL)[a] (DPPH)
Lemon peel	68.865 ±0.441
Pamelo peel	143.294±0.478
Lime peel	348.668±1.003
Vitamin E	7.238±0.373
Allopurinol	

IC_{50} values were analyzed with SigmaPlot ver. 12 and a four-parameter logistic model.
[a]Data are presented as mean±SD of triplicate

CONCLUSION

The essential oils from lemon, pomelo, and lime peels were identified in this study. Limonene was found to be the most prevalent chemical in all of the samples by GC-MS analysis. The essential oil extracted from lemon peels had the highest antioxidant activity, whereas the oil extracted from lime peels showed the highest inhibition of xanthine oxidase. These results shed important light on the bioactive characteristics of citrus peel essential oils and point to their possible uses as natural antioxidants and xanthin oxidase enzyme inhibitors in medicine and food preservation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC- 9016/2024]