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Toxicity Test of Essential Oil from Kintamani Slayer Orange Peel (Citrus Reticulata) With Zero Waste Application to Support Ecotourism

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A B S T R A C T

Kintamani slayer orange peel can be extracted into essential oil because it contains terpenoids, sesquiterpenoids, alkanes, alkyl alkanoates, and steroid alcohols. Orange peel contains active substances that can help heal wounds, such as essential oils, ascorbic acid, vitamin E, vitamin A, flavonoids, and polyphenols, and has antioxidant and antibacterial activity. Kintamani orange peel contains flavonoids, alkaloids, saponins, tannins, terpenoids, and essential oils. The main goal of this study is to identify the waste by-product characteristics of Kintamani slayer orange peel in order to achieve zero waste. Essential oils in Kintamani slayer orange peel (Citrus reticulata) are toxic to Artemia salina Leach shrimp larvae. The GC results on Kintamani slayer orange peel essential oil obtained twelve (12) compound peaks

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INTRODUCTION

Oranges are one type of fruit that is widely favored by the public. Oranges are needed by almost everyone because they are rich in vitamin C and other substances that are important for human health. Oranges are also fruits available throughout the year because orange cultivation does not recognize a specific fruiting season (Septiani, 2017).

Kintamani slayer orange is one type of orange widely cultivated in Indonesia, which ranks fourth as one of the largest producing commodities with a production contribution of 1,785,256 tons, or around 9.01 percent of national fruit production (Holtikultura, 2015). This Siamese orange is the most widely developed because it is relatively easy to care for, produces many results, and sells well on the market as fresh fruit.

Kintamani slayer orange fruit is recorded as a superior commodity in Bali. Kintamani slayer orange fruit is recorded as a superior commodity in Bali. In 2017, Bangli Regency was recorded as the largest producer of Siamese oranges and tangerines, with Kintamani District as its center. Bangli Regency is one of the potential Siamese orange plant development areas in Bali (B. P. S. P. Bali, 2018) because the environmental conditions (soil, climate, altitude, temperature) are very suitable for planting Kintamani slayer oranges (D. P. P. Bali, 2013). Catur Village, Kintamani District, Bangli Regency is one of the areas producing Kintamani slayer oranges. Almost the entire area is orange plantation land, amounting to 521.50 ha of the total area of 605,579 ha. This Kintamani Slayer orange is the most widely developed because it is relatively easy to care for, produces a lot of results, and sells well on the market as fresh fruit.

Kintamani Slayer oranges are generally only used for the flesh of the fruit for consumption purposes, but the skin is often ignored. Orange peel processing can be done by applying the principle of zero waste because unused fruit peels or leftover fruit from making food can be processed into useful by-products. Simply put, zero waste is a movement to not produce waste by reducing needs, reusing, recycling, and even making other products that have useful value (Destina, 2021). Orange peels are usually just thrown away and not used and become useless waste. So far, the use of orange peels has not been carried out intensively (Michiko, M., Manalu, C. V., 2020).

The peel of the Kintamani Slayer orange contains different compounds, but depending on the variety, it makes the type of aroma different. Siamese orange peel can be extracted into essential oils because it contains terpenoids, sesquiterpenoids, alkanes, alkyl alkanoates, and steroid alcohols. The benefits and functions of Siamese orange peel can be extracted into essential oils (Aji et al., 2018). It is known that orange peel contains active substances that can help heal wounds, such as essential oils, ascorbic acid, vitamin E, vitamin A, flavonoids, and polyphenols, and has antioxidant and antibacterial activity (Azizah & Widya Wati, 2018). Flavonoids also affect increasing wound healing by accelerating the rate of epithelialization (Azkiya et al., 2017). The ascorbic acid content in the orange peel can increase the strength and integrity of wounds (Cahyaningsih et al., 2021).

Essential oils are crucial to many different sectors. Essential oils can be used in a variety of ways. For example, they might be added to food as flavorings (spices) or taken orally as herbal medicine. used externally via massage, medication for wounds or bruises, and fragrances. either via the nose or by inhalation when using aromatherapy (Ni Luh Putu Putri et al., 2023).

Toxicity testing on Artemia salina is one way to do preliminary screening for cytotoxic compounds. Leach the larvae of shrimp. The objectives of a toxicity test are to identify the target organs and their sensitivity, ascertain the presence of toxicity in a chemical or material, and gather information on its dangers following acute compound delivery (Jabbar et al., 2019). In previous studies on toxicity tests on lemon peel extracts (Citrus limon L.), the highly toxic LC_{50} value was 1.77 ppm, making it the most dangerous substance ever studied (Aprilyanie et al., 2023). The LC_{50} value of the methanol extract of chili orange peel in this study was 234.42 g/ml, indicating that it is cytotoxic (Ayu et al., 2018).

Kintamani orange peel contains flavonoids, alkaloids, saponins, tannins, terpenoids, and essential oils. Testing the antibacterial activity of Kintamani orange peel essential oil on gram-positive bacteria, it was proven that the compounds contained in Kintamani orange peel essential oil can inhibit the growth of Staphylococcus aureus bacteria (Kindangen et al., 2018). These secondary metabolite compounds have antibacterial activity on Staphylococcus aureus by disrupting the process of cell membrane component composition or cell membrane permeability, causing shrinkage or components to exit the cell. In addition, the compound content in Kintamani orange peel can inhibit the formation of cell membranes and inhibit the growth process of bacteria (Amiliah, Nurhamidah, 2021).

The objectives to be achieved in this study in general are to obtain the characteristics (chemical and biological) of the by-products of Kintamani slayer orange peel waste to achieve "zero waste" as a basis for utilizing these by-products into various potential products in the future. Obtain screening results on all by-products of Kintamani slayer orange production to support ecotourism.

LITERATURE REVIEW

Kintamani slayer orange fruit is round, smooth, and thin with juicy flesh and is easy to peel from the flesh. Kintamani slayer orange is very popular because the fruit tastes sweet and delicious to eat. Usually used as a table fruit and sometimes also made into syrup or lemonade (Rai et al., 2016). This orange is also widely planted from seeds because it bears fruit quickly, which is an average of about four years (Purnamasari, 2010).

Oranges contain quite high levels of vitamin C, energy, protein, fat, carbohydrates, retinol, calcium, phosphorus, iron, and carbonic acid, where these nutrients are very much needed by the body. In addition to being a fresh fruit or processed food, oranges can be useful for preventing cancer, treating coughs, reducing the risk of heart disease, smoothing the digestive tract, maintaining healthy skin, preventing constipation, as an antioxidant, lowering cholesterol, and preventing anemia (Hafiz Maulana, 2023).

The ascorbic acid content in the Kintamani slayer orange peel can increase the strength and integrity of wounds (Cahyaningsih et al., 2021). The Kintamani slayer orange peel contains flavonoids, alkaloids, and tannins, which have activity in healing burns and can provide a healing effect on burns. Antioxidants have a working mechanism that can reduce the amount of damage to skin tissue caused by lipid peroxides and eliminate, clean, and withstand the effects of radicals. Antioxidant compounds are known to accelerate the healing of burns (Hariningsih & Hartono, 2022). Antioxidants can be used to regulate the continuous oxidation process in the body (Puspa Juwita et al., 2013).

Then a phytochemical screening test was conducted to determine the truth of the compounds contained therein (Septiana, 2016). Phytochemical screening tests on Kintamani slayer orange peel include alkaloid, flavonoid, tannin, terpenoid, and saponin tests. Based on the results of the phytochemical screening of Kintamani slayer orange peel, which showed the presence of flavonoid content, which was indicated by positive results during the phytochemical screening test, can be seen in Table 1.

Table 1. Results of Phytochemical Screening of Orange Peel

Testing	Orange Peel Results
Alkaloids	+
Flavonoids	+
Tannins	+
Terpenoids	-
Saponins	-

Orange peel contains flavonoid compounds that have antibacterial activity and stimulate the growth of new cells in wounds (Tuloli et al., 2020). In addition, flavonoids have a working mechanism to smooth blood circulation throughout the body and prevent blockages in blood vessels, as anti-inflammatory agents, also function as antioxidants, and help reduce pain in the event of bleeding or swelling (Wendersteyt et al., 2021). In addition to the flavonoid compound content, orange peel also contains alkaloid compounds and vitamin C, where alkaloids have anti-inflammatory activity, while vitamin C is a substance that can increase collagen production by hydroxylating lysine and proline to accelerate healing in burns (Rahma Zakiya, Lanny Mulqie, 2015).

METHODOLOGY

Isolation of Essential Oils by Steam Distillation

Peeled orange peel is then chopped, then left (incubated) at room temperature for 1 day. water is added until the sample is submerged. Then heat to boiling until the water vapor rises. Distillation takes place at a temperature of 80-100 °C for 7-8 hours. The results of the essential oil distillation are separated using a separating funnel to separate the water from the oil that is still mixed. stored in a tightly closed brown bottle and protected from light, covered with aluminum foil, and stored at room temperature (Febrianti et al., 2019).

Toxicity Test on Artemia Salina Leach Shrimp Larvae

The toxicity test was carried out by preparing 10 test tubes; for each sample of young and old leaves, 9 test tubes were needed and 1 test tube as a control. Weighing each 10 mg of Orange peel essential oil, 2 mL of ethanol was then added to dissolve it. Moreover, 5 µL, 50 µL, and 500 µL of the resulting solution were pipetted into a test tube, and the solvent was allowed to evaporate for a whole day. Subsequently, 50 µL of dimethylsulfoxide (DMSO), 10 Artemia salina Each test tube received a yeast drop, one milliliter of saltwater, and leach shrimp larvae. To make a solution with concentrations of 100 ppm, 500 ppm, and 1000 ppm, 5 mL of saltwater was then added. Use 50 µL of dimethyl sulfoxide (DMSO) and 1 mL of seawater as a control or comparative solution, and 10 Artemia salina Leach shrimp larvae, then add seawater to a volume of 5 mL. Each test tube above is covered with aluminum foil with a small hole and repeated 3 times. After 24 hours, observations were made on the death of Artemia salina Leach shrimp larvae, and the quantity of deceased larvae was noted. Then the calculation of the dead Artemia salina Leach shrimp larvae was carried out, and the value (LC50) was calculated by taking a % probit graph.

Analysis Using Gas Chromatography and Mass Spectrometry (GC-MS)

The essential oil of Kintamani slayer orange peel obtained was analyzed by GC-MS to identify the chemical components that form the essential oil of Kintamani slayer orange peel. By comparing the mass spectra of recognized standard chemicals in the database that was programmed on the GC-MS equipment, this identification was accomplished.

RESULTS

Isolation of Essential Oils by Steam Distillation

The results of the isolation of essential oil from Slayer Orange peel are presented in Table 1. The results of the distillation of essential oil from Kintamani Slayer Orange as much as 10 ml with a sample weight of 20 kg of Kintamani Slayer Orange peel, which has physical properties, namely yellow, smells sour, and has a yield value of 0.05% (w/w). The distillation process of essential oil from Kintamani Slayer Orange peel with a steam distillation apparatus can be seen in Figure 1.

Figure 1. Steam Distillation Process of Kintamani Slayer Orange Peel Essential Oil

Test of Toxicity Using Artemia salina Take Off the Larvae of Shrimp

The outcomes of the test for toxicity of Kintamani slayer orange peel Artemia salina essential oil Table 1 displays leach shrimp larvae. It is possible to conclude that Kintamani Slayer Orange Peel Essential Oil is poisonous based on the test findings, which indicate that the oil has an LC50 value of 4.63 ppm is toxic.

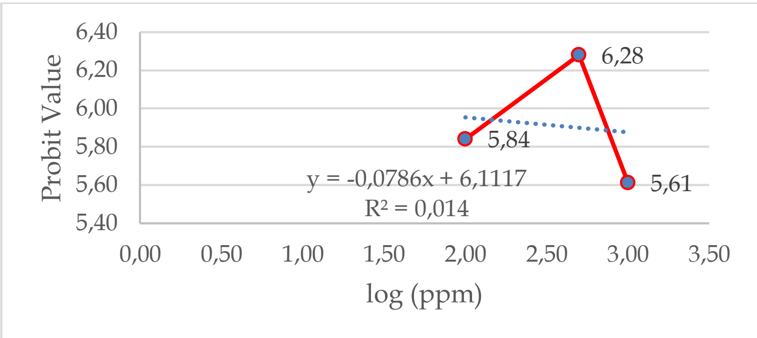


Figure 2. Graph of Probit Value Against LC_{50}

Analysis Using Gas Chromatography and Mass Spectrometry (GC-MS)

The essential oil of Kintamani slayer orange peel obtained from the distillation process was then analyzed for the compound components contained therein using a gas chromatography-mass spectrometer (GC-MS). The GC results obtained twelve (12) chromatogram peaks with compounds that match the Willey.Lib database in Figure 2.

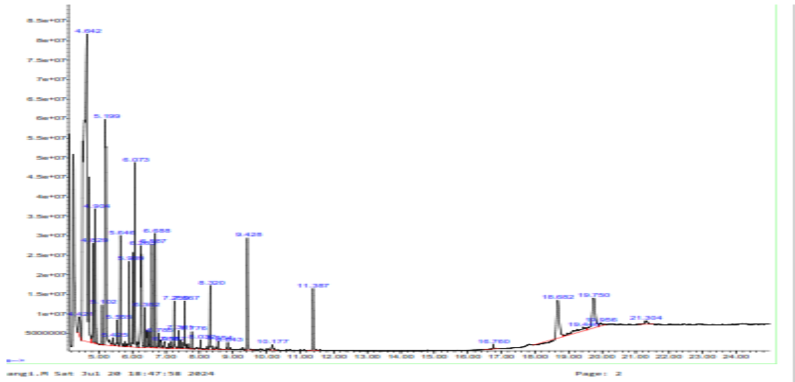
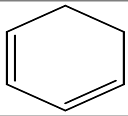
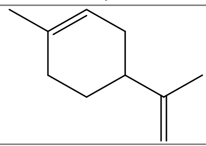
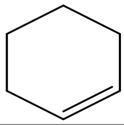
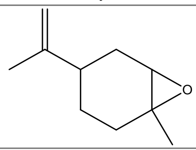
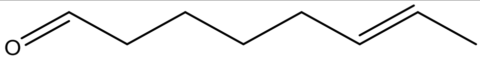
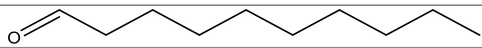
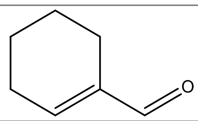
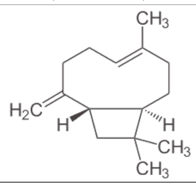
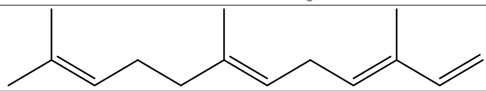
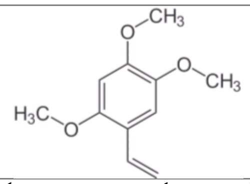
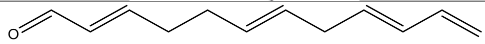
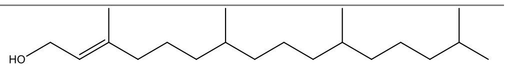


Figure 3. Chromatogram with Compounds Corresponding to the Willey.Lib Database

Table 2 displays the key compound estimate Artemia salina essential oil Table 2 displays leach shrimp larvae.

Table 2. Lists the Essential Chemicals in the Chromatogram of Kintamani Slayer Oranges Based on the WILEY229.LIB Database

No	Compound Name	Retention Time	% Area	Similarity (Quality) %	Compound Image
1	1,3-Cyclohexadiene	4.421	1.10	97	
2	D-Limonene	4.642	39.64	97	
3	Cyclohexene	5.199	0.71	98	
4	Limonene Oxide	5.555	0.58	97	
5	6-Octenal	5.646	2.30	97	
6	Decanal	6.073	5.98	97	
7	1-Cyclohexene-1-Carboxaldehyde	6.688	2.20	97	
8	Caryophyllene	7.776	0.35	99	
9	Alpha-Farnesene	8.320	1.60	98	

10	Asarone	9.428	3.50	99	
11	2,6,9,11-Dodecatetraenal	11.387	2.42	99	
12	Phytol	16.760	0.18	97	

DISCUSSION

The results of gas chromatography-mass spectroscopy (GC-MS) analysis show that the essential oil of Kintamani slayer orange peel contains twelve (12) peak compounds with relatively high abundance (quality), namely compounds 1,3-Cyclohexadiene, D-Limonene, Cyclohexene, Limonene Oxide, 6-Octenal, Decanal, 1-Cyclohexene-1-Carboxaldehyde, Caryophyllene, Alpha-Farnesene, Asarone, 2,6,9,11-Dodecatetraenal, Phytol.

From the results of this study, it was found that the essential oil of Kintamani slayer orange peel is toxic to Artemia salina Leach shrimp larvae with an LC_{50} value of 4.63 ppm. According to Syaiful Anwar (2014), an extract bears the potential to function as an antibacterial if its LC50 value is between 30 and 200 ppm, and as an antipesticide if it is between 200 and 1000 ppm. This suggests that although the hot aquades extract (70^{O}C) has the potential to be an antibacterial, the room temperature aquades extract has the potential to be an antipesticide.

The mechanism behind these compounds' response to the death of Artemia salina Leach shrimp larvae, according to Cahyadi (2009), is that alkaloid, flavonoid, and triterpenoid compounds can be acutely toxic and can kill Artemia salina Leach shrimp larvae at specific concentrations. These substances operate by causing stomach poisoning as their mode of action. The larvae's digestive system will thus be disturbed if these substances get inside of them. Furthermore, these substances block the taste receptors in the larvae's mouth region. As a result, the larvae are deprived of taste cues and are unable to identify their food, which leads to their starved death (Carballo, 2002; Cahyadi, 2009). According to Kartikasari (2010), triterpenoid chemicals, which are poisonous substances, are thought to be the cause of Artemia salina Leach's mortality. These chemicals' entrance as triterpenoid compounds has the potential to harm membrane permeability and interfere with Artemia salina Leach's metabolic functions, leading to Artemia salina mortality. Diffusion occurs in the Artemia salina cell membrane. These substances have the potential to impair membrane permeability and interfere with Artemia salina Leach's metabolic functions, leading to Artemia salina death.

After using the steam distillation process, the essential oil of the Kintamani slayer orange peel revealed the presence of twelve (12) compound components in pretty great quantity. Most essential chemicals have several C atoms. Monoterpenes (C10) and sesquiterpenes (C15), and various aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones that have a distinctive aroma and smell. The presence of volatile monoterpene and sesquiterpene compounds or essential oils in plants provides an important defense strategy for plants, especially against herbivorous pests, insects, and pathogenic fungi. The potential of Kintamani slayer orange peel as a natural herb with antibacterial, anticancer, and antipesticide properties.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Essential oils in Kintamani Slayer Orange Peel (Citrus reticulata) are toxic to Artemia salina Leach shrimp larvae with an LC50 value of 4.63 ppm.
2. GC results on Kintamani Slayer Orange Peel essential oils obtained twelve (12) compound peaks. The MS results of the identified essential compounds were 1,3-cyclohexadiene, D-Limonene, Cyclohexene, Limonene Oxide, 6-octenal, Decanal, 1-cyclohexene-1-carboxaldehyde, Caryophyllene, Alpha-Farnesene, Asarone, 2,6,9,11-Dodecatetraenal, and Phytol. The potential Kintamani slayer orange peel can be an antipesticide, antibacterial, and anticancer.

Recommendations

To get more comprehensive data about the effectiveness and activity of Kintamani Slayer Orange fruit, it is recommended that this study examine the antioxidant activity of essential oils on Kintamani Slayer Orange peel, and pre-formulation research needs to be done to develop products based on Kintamani Slayer Orange essential oils, such as gels, creams, or ear drops, which can be applied topically or orally. Thus, the potential of this essential oil as an alternative treatment for bacterial infections can be further developed and utilized optimally.

FURTHER STUDY

The next stage will be directed at developing products based on Kintamani slayer orange peel essential oil. Product formulas such as hand sanitizers, surface disinfectants, or food preservatives will be developed and tested for effectiveness. Product stability tests will also be conducted to ensure the quality of the products produced. In addition, further toxicity studies will be conducted to ensure the safety of the products for humans and the environment.

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