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Mandarin citrus (*Citrus reticulata* Blanco cv. Terigas) peel essential oil as a potential nanoemulsion ingredient: Formulation, physicochemical characterization, and antibacterial activity

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Abstract. Citrus peel essential oil can be a complementary component in food and cosmetics. Volatile molecules, such as limonin, can be useful as antibacterial agents. The hydrophobic nature of essential oils causes problems when used in commodities under normal storage conditions. Nanoemulsion technology is the most effective method to increase hydrophilicity and facilitate the application of substances to various products. This study aims to utilize Mandarin cv. Terigas citrus peel waste into essential oils using hydrodistillation with aquades solvent. The essential oil is then processed into a nanoemulsion to characterise its physicochemical and antimicrobial properties against *Listeria monocytogenes* bacteria. Essential oils are formulated as nanoemulsions with several concentrations (1.5%, 2%, 2.5%). The nanoemulsions obtained were then tested for their physicochemical characteristics and antimicrobial activity. The results showed that the essential oil yield was 3.661%, and 40 volatile compounds were found from GC-MS analysis. Nanoemulsion at 2% essential oil concentration has a viscosity of 2,650 mPa.S, total dissolved solids of 4,877 brix, and activity to inhibit the growth of Listeria monocytogenes of 7.6 mm. Research on the utilisation of this waste can help increase the added value of waste into products with high economic value.

1 Introduction

The mandarin citrus processing industry produces waste in the form of fruit peels that have yet to be utilised. The pleasant, fresh scent of citrus makes them highly valued [1].

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Mandarin citrus peel essential oils, particularly those found in the flavedo (the orange peel's outer pigmented layer), are responsible for the scent of citrus. Although citrus peels are included in organic waste, if there is continuous accumulation, it will negatively impact the environment. According to the Indonesia National Waste Management Information System (SIPSN) data [2], Indonesia has the most significant accumulation of organic waste in food waste, at 40.65%. The Mandarin cv. Terigas citrus is one of the citruses widely used to make beverage products. Peel that isn't required for the industry must be processed to prevent trash from accumulating and polluting the environment. Waste mandarin peel can be converted into a beneficial essential oil [3].

Peel waste can be processed into an essential oil that has many benefits. Research on essential oils continues to grow, but a few specifically focus on producing nanoemulsions from citrus peel waste. The use of this waste offers opportunities to develop value-added products. Mandarin peel essential oil has received much attention in several fields due to its various therapeutic benefits and practical uses in aromatherapy, skincare, food preservation, and flavouring [4,5]. These days, many consumers are going towards the organic industry due to changes in their consumption habits. This makes essential oils a highly sought-after commodity since several studies have shown the health benefits. Essential oils have been confirmed to enhance health and wellness by lowering stress and elevating mood. They also have potent anti-inflammatory, antibacterial, and antioxidant properties [6–8]. The quality of strawberry fruit can be maintained during storage with edible coating equipped with essential oil [9]. Citrus essential oils are increasingly used in food to prevent oxidative damage and bacterial or fungal growth. Using pure citrus peel essential oil has disadvantages, including its overpowering aroma, high price, and low absorption rate [10,11].

Nanoemulsion essential oils are a type of emulsion that consists of small droplets of essential oils dispersed in water. The class of emulsions known as nanoemulsions, which has droplet sizes between 50 and 500 nm, has recently received much attention due to its distinctive properties [12]. These nanoemulsions have gained attention due to their potential antimicrobial properties and ability to enhance essential oils' solubility and stability [13]. One method for producing nanoemulsion essential oils is spontaneous emulsification, a simple and cost-effective technique. This method uses essential oil, carrier oil, and nonionic surfactant to titrate into an aqueous solution with continuous stirring. The composition of the oil phase, particularly the ratio of essential oil to carrier oil, affects the size of the droplets formed [13,14]. Particle characteristics such as composition, size, electric charge, aggregation state, physical state, and interfacial composition significantly determine a nanoemulsion's physicochemical and functional characteristics [15].

The antimicrobial efficacy of nanoemulsion essential oils and different types of oils and microorganisms has been studied. Eucalyptus oil nanoemulsion killed *Staphylococcus aureus* and *Bacillus cereus* better than pure oil [16]. Nanoemulsion and pure essential oil of *Thymus daenensis* have better antibacterial properties than *Escherichia coli.* This is due to the increased solubility and penetration of essential oil into bacterial cells [17]. According to antibacterial and antibiofilm investigations, Nanoemulsion successfully eliminates both sessile and planktonic forms of *Salmonella sp.* and *Listeria sp.* in single- and multi-species culture settings [18]. In apple juice preservation research, citrus nanoemulsion inhibited mold growth [19]. The nanoemulsion delivery technology further enhances the solubility and bioavailability of essential oils, increasing their utility in various contexts [20]. This study will discuss the characteristics of essential oils in Mandarin citrus cv. Terigas and its nanoemulsion formulation to see the physical and chemical characteristics and antibacterial capabilities.

2 Material and methods

2.1 Material

Mandarin cv. Terigas peel waste (80 kg) was obtained from the production of KPRI Citrus in Batu City, East Java (-7.903398,112.534841). Chemicals such as distilled water, ethanol (Merck), tween 80 (Merck), NaOH (Merck), NaCl (Merck), and Listeria Selective Agar (Himedia) were purchased from the Kridatama Malang store. *Listeria monocytogenes* bacterial starter was obtained from Indilab.

2.2 Essential oil extraction

Distillation of Mandarin cv. Terigas peels essential oil using the hydro distillation method [21]. The distillation flask uses a size of 2 liters. The ratio of fresh peel to distilled water solvent is 1:2. Distillation was carried out for 2 hours, and oil separation was carried out with a separating funnel. The separated oil was then stored in a dark bottle for further analysis.

2.3 Preparation of nanoemulsion

The process of making a nanoemulsion requires a Mandarin cv. Terigas peel essential oil concentration (1.5%, 2%, 2.5%) and tween 80 surfactants in a ratio of 1:3 [22]. The mixture was stirred using a 250rpm magnetic stirrer for 5 minutes. Then, sterile distilled water was added to the mixture. The three solutions were homogenized at 10,000 rpm for 15 minutes. Furthermore, the solutions were sonicated using an ultrasonic bath for 30 minutes (40 Hz, room temperature).

2.4 Physical analysis

Particle measurements were carried out using a Particle Size Analyzer (PSA) Horiba SZ-100 (HORIBA, Ltd.). The viscosity test of the nanoemulsion solution was carried out using a vibro viscometer (A&D SV-10) and measured at room temperature in mPa.S units. A clarity test was conducted by dissolving 1 ml of the sample in a 100 mL measuring flask using distilled water. The distilled water was used as a blank. The sample solution was measured for its transmittance percentage using a UV-Vis spectrophotometer (Shimadzu, Japan) at 650 nm. The results of the clarity level measurement in the percentage range of 90%-100% showed that the sample had a clear and transparent appearance [23]. Total Soluble Solid (TSS) measurements were carried out using a hand-refractometer size N1 (Atago Tokyo, Japan) at 20°C. The results of TSS measurements are presented in degrees Brix (°Brix). The pH analysis was performed using a pH meter HI5221-02 (Hanna Instruments, Indonesia) at room temperature. The pH meter probe was dipped into the nanoemulsion solution, and the pH value measured on the screen was observed until a stable number was obtained.

2.5 Antimicrobial activity

Inoculation of *Listeria monocytogenes* bacterial cultures was performed using the pour plate method [24]. A total of 1 ml of physiological 0.9% NaCl solution containing bacterial culture was poured into a petri dish. The test was put into a Petri dish, and Listeria Selective Agar (LSA) was poured into it. The solid media was perforated using a cork borer with a diameter of 8 mm so that 4 wells were formed on the media. so that 4 wells are formed on the media.

The nanoemulsion solution of as much as 50 µl was injected into each well and allowed to diffuse for 1 hour at room temperature. The culture was then incubated at 37 ^{0}C for 24 hours. The negative control used was 50 µl of sterile distilled water, and the positive control used chloramphenicol. After incubation, the petri dishes' test cultures were observed for inhibition zones. The zone of inhibition was measured based on the diameter of the that formed around the wells.

3 Result and discussion

3.1 Result

3.1.1 Yield and chemical composition of essential oil

The quality of essential oils is determined by several parameters, including yield, specific gravity, and chemical composition. The value of these parameters can be influenced by the type of raw material and the extraction method used [6]. Table 1 shows the results of the physical component analysis of Mandarin cv. Terigas citrus essential oil.

Table 1. Physical properties of lemon essential oils

Parameter	Terigas Essential Oil
Yield (%)	3.661
Specific gravity (g/ml)	0.84
Optical Rotation (^{0})	6.90

The yield of this study was 3,661%. Mandarin cv. Terigas citrus essential oil has a specific gravity value of 0.84 g/ml. The optical rotation value of Mandarin cv. Terigas essential oil obtained in this study was 6.90°. Chemical compounds with the potential to be antimicrobial can be analyzed using GC-MS. Table 2 shows that 40 components have been identified in the Mandarin cv. The compound D-limonene was found in the highest amount in Mandarin cv. Terigas citrus essential oil at 56.58%, followed by 39 other compounds with various compositions.

Table 2. Components of volatile constituents of Mandarin cv. Terigas

No	Compound Name	Concentration (%)
1	α-Pinene	4.3171
2	Camphene	0.1376
3	Sabinene	1.6578
4	Pinene	12.6254
5	β-Myrcene	9.7061
6	Octanal	0.5397
7	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.2459
8	D-Limonene	56.5854
9	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	0.7541
10	γ-Terpinene	0.3815

Table 2. Components of volatile constituents of Mandarin cv. Terigas

No	Compound Name	Concentration (%)
11	Formic acid, octyl ester	0.0562
12	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.3232
13	Linalool	2.5452
14	1,3,8-p-Menthatriene	0.0315
15	Octanoic acid, methyl ester	0.1123
16	6-Octenal, 3,7-dimethyl-, (R)-	0.1564
17	Isoborneol	0.0453
18	Terpinen-4-ol	0.8112
19	Octanoic acid, ethyl ester	0.0952
20	α-Terpineol	0.9706
21	Decanal	0.8602
22	6-Octen-1-ol, 3,7-dimethyl-, (R)-	0.351
23	(-)-Carvone	0.1066
24	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	0.1529
25	Dodecanal	0.1105
26	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	0.4793
27	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	0.12
28	Geranyl isobutyrate	0.0531
29	Copaene	0.1112
30	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	1.1845
31	Dodecanal	0.2611
32	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	0.3118
33	Germacrene D	0.2751
34	Humulene	0.2662
35	Germacrene D	0.5506
36	α-Farnesene	1.1886
37	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	0.206
38	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	0.1134
39	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	0.1387
40	2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl-, (E,E,E)-	0.218

3.1.2 Characteristics of essential oil nanoemulsion

Nanoemulsion is an emulsion solution that has a nanometer particle size. The oil-in-water (O/W) emulsions category includes nanoemulsions produced from citrus peels. The dispersed phase is the oil phase (essential oil), and the dispersed phase is the water phase (aquades) with the help of tween 80 surfactants. Tween 80 is a surfactant to reduce surface tension, making the nanoemulsion more stable. The properties of nanoemulsions produced at varying concentrations of 1.5%, 2%, and 2.5% are shown in Table 3. The table includes total soluble solids (TSS), viscosity, pH, percentage transmittance, mean droplet size, and polydispersity index (PI) for each nanoemulsion concentration.

Table 3. Characteristic of Nanoemulsion

Nanoemulsions (%)	TSS (^{0}brix)	Viscosity (mPa.S)	pH	Percentage Transmittance (%)	Mean Droplet size (nm)	PI
1.5	3.867^b±0.306	2.480^a±0.200	6.121^a±0.114	99.157^a±0.839	14.1	0.573
2	4.877^b±0.751	2.650^a±0.240	5.860^a±0.097	98,197^a±0.210	13.5	0.583
2.5	6.487^a±0.338	2.720^a±0.310	5.707^a±0.319	98.157^a±0.726	19.9	0.514

*Values are means (±) standard deviations. In each column, means with different uppercase letters are significantly different (P < 0.05)

Total Soluble Solids (TSS) values increase with increasing nanoemulsion concentration, ranging from 3.867 to 6.487. The viscosity of the nanoemulsions shows a slight increase with increasing concentration, ranging from 2.480 to 2.720. The difference in the concentration of essential oils in nanoemulsions did not significantly ($p > 0.05$) increase the viscosity. The pH values of the nanoemulsions drop as the concentration increases, ranging from 6.121 to 5.707. The variation in the concentration of essential oils in nanoemulsions did not result in a significant ($p > 0.05$) reduction in pH.

The percentage transmittance values are high for all nanoemulsion concentrations, ranging from 98.157% to 99.157%. The difference in the concentration of essential oils in nanoemulsions did not significantly ($p > 0.05$) decrease the transmitance. The mean droplet size of the nanoemulsions varies with concentration, with values of 14.1 nm, 13.5 nm, and 19.9 nm.

3.1.3 Antimicrobial activity

Listeria monocytogenes is a pathogenic bacteria that can cause contamination of food and foodborne disease, causing Listeriosis in humans and animals. Therefore, the presence of these bacteria must be prevented. The antimicrobial activity of nanoemulsion Mandarin cv. Terigas are shown in Table 4.

Table 4. Antimicrobial activity of nanoemulsion

Essential oil (%)	Inhibition (mm)
1.5	6.8
2	7.6
2,5	6.6

In this study, the inhibition zone produced by the nanoemulsion was 6.6-7.6 mm. Essential oil at a concentration of 2% produced the highest inhibition zone of 7.6 mm. The inhibition decreased to 6.6 mm at a 2.5% concentration.

3.2 Discussion

Another study on tangerine peels using the hydro distillation method produced a yield of 2.53% with a distillation time of 150 minutes [25]. Some factors that usually affect the yield value of essential oils are the origin of the material, the preliminary treatment of the material, and the distillation time. The distillation time of 2 hours produced a yield of Citrus reticulate Blanco (Ponkan) essential oil of 3.1% [6]. Distillation of essential oil from unmatured *Citrus deliciosa* citrus using hydro distillation produced a yield of 0.09% [26]. The time of harvest and maturity of the citrus fruits can affect the essential oil content. In this study, the Mandarin cv. Terigas used were in optimum ripe condition. This is thought to cause the yield to be higher than previous studies on other mandarin cultivars.

The field of component compounds can affect the specific gravity of essential oils [27]. Terpene compounds are the main components of essential oils, and processing conditions and extraction methods can also affect them. Previous studies on patchouli essential oil showed that the type of drying or the size of the cuttings did not affect the specific gravity of the oil; this suggests that these components do not change the density or composition of the oil [28].

The optical rotation of essential oils may be measured, which is useful for characterizing the variety of optically occurring components in these natural products. The optical rotation of essential oils is a fundamental physicochemical characteristic that provides valuable data on the oils' quality, purity, and genuineness. Optical rotation is the capacity of a substance to rotate plane-polarised light, and it is quantified using a polarimeter [29]. Dextrorotatory, or right-handed rotation, is denoted by a positive (+) symbol, whereas levorotatory, or left-handed rotation, is denoted by a negative (-) symbol [28]. The right-handed optical rotation exhibited by Mandarin cv. Terigas essential oil is primarily attributed to the presence of specific chiral compounds, particularly D-limonene.

Determining essential oils' volatile component content is crucial to ascertaining their chemical composition. The research shows that mandarin essential oil mainly comprises monoterpenes, with D-limonene being the primary component, making up 56.5854% of the entire complex. Limonene is a distinctive chemical in citrus essential oils and is renowned for its delightful, lemony fragrance. The mandarin essential oil possesses a notable fragrance and flavor profile due to its elevated limonene concentration. The hydrodistillation process yields sour orange blossom essential oils with a concentration of 9.16% [30].

The composition of the substance also includes noteworthy molecules such as beta-Myrcene (9.7061%), alpha-Pinene (4.3171%), and linalool (2.5452%) in large quantities. Additional monoterpenes, including sabinene, γ-terpinene, terpinene-4-ol, and α-terpineol, contribute to the essential oil composition. Although found in smaller amounts, these chemicals influence the aroma of the essential oil. The chemical constituents of these compounds indicate that they have essential oils that can exhibit antifungal and antibacterial properties [9]. Limonene is a major contributor to the antibacterial activity of mandarin essential oil. Other compounds in the oil, such as linalool, α-pinene, and β-pinene, also possess antibacterial properties. The synergistic and complementary effects of these compounds contribute to the overall antimicrobial efficacy of the essential oil [31,32].

The difference in essential oil concentration in nanoemulsions significantly ($p < 0.05$) increased TSS. Higher nanoemulsion concentrations lead to a higher content of soluble solids in the system. TSS can impact the stability of nanoemulsions by affecting the osmotic pressure and interfacial tension between the droplets and the continuous phase. Higher TSS levels can increase the osmotic pressure, which may lead to droplet shrinkage or swelling, affecting the overall stability of the nanoemulsion system [33] . The higher the viscosity value, the more difficult it is for the fluid to move. An increased dispersed phase concentration leads to an associated rise in the system's viscosity [34]. Increasing viscosity causes the movement of globules contained in the nanoemulsion to decrease to maintain the stability of the nanoemulsion [35].

The decrease in pH could be attributed to the acidic constituents in the emulsified phase or to interactions between the components of the emulsion and the aqueous phase. The surfactant used in this research was Tween 80, a non-ionic surfactant with a negative charge ￼. Low pH values can be beneficial for certain applications. However, low pH conditions can interfere with various metabolic processes in bacterial cells. Acidic pH can affect bacterial enzyme activity, disrupting the proton gradient across the cell membrane [36].

An optimal nanoemulsion formulation exhibits a visually clear and transparent appearance, characterized by a high transmittance percentage within the 90% to 100% range. The clarity measurements indicated all nanoemulsion formulations in this investigation were transparent. A clarity level close to 100% indicates that the droplets generated in the nanoemulsion preparation have reached a size of a few nanometers [37]. Lime essential oil nanoemulsion showed the highest transmittance percentage, 99.0%, among the lime varieties studied [38]. Nanoemulsions with consistent transmittance values indicate steady quality, making them appropriate for various applications in the food and beverage sectors [39].

The low droplet size in nanoemulsion makes the preparation appear transparent and more stable when stored for a long period of time. Another advantage of the nanoemulsion system is the increased bioactivity of essential oil compounds due to their good diffusion ability [18]. The addition of surfactants can form stable and uniform nanoemulsion droplets by reducing the surface tension between the oil and water phases. The tween 80 surfactant binds the non-polar groups of the oil phase and the polar groups of the water phase in solution simultaneously. The uniformity of the nanoemulsion particle size is also due to the assistance of ultrasonic waves and homogenizers. Ultrasonic waves can reduce particle size, while homogenizers prevent particles from settling so that non-polar compounds are well-dispersed [20,40]. The droplet size is in the nanometer range, confirming the formation of nanoemulsions. The variation in droplet size may be influenced by factors such as the emulsification process, surfactant concentration, and the physicochemical properties of the emulsified components [41,42].

The antibacterial effects of essential oils do not increase with their maximum concentration. This is assumed to be the case because essential oils contain chemicals that, at a certain concentration, will suppress *Listeria monocytogenes* growth. Based on earlier research, the pure citral compound nanoemulsion with a formulation of span 85 and surfactants can inhibit bacteria growth. The inhibitory activity of *Listeria monocytogenes* is 14.4 mm, then in Escherichia coli, it is 9.4 mm [43]. The difference in a clear zone in each essential oil concentration treatment shows that the amount of concentration used affects the antibacterial activity produced.

The antibacterial activity of Mandarin cv. Terigas peel essential oil nanoemulsion was related to the contained chemical compounds. The higher the essential oil concentration, the higher its chemical compound content. The antibacterial compounds in Mandarin cv. Terigas peel essential oil are limonene, β-pinene, sabinene, and α-pinene. These compounds are the monoterpene hydrocarbon group that has an antibacterial mechanism by disintegrating the outer membrane of bacteria. Nanoemulsions and emulsions incorporated with lemon essential oil were studied to determine the effect of particle size on antimicrobial activity. The results showed that nanoemulsions exhibited stronger antimicrobial activity against *E. coli* and *Listeria innocua* [44]. Mandarin cv. Terigas essential oils' antimicrobial properties and nanoemulsion make them stand out as special food industry additives. The efficiency of nanoemulsions can be influenced by their particle size. The particle size can change at different nanoemulsion concentrations, increasing antibacterial activity. This is because smaller particles have a larger surface area, which allows them to interact better with the bacterial cell membrane.

4 Conclusion

GC MS research shows that Mandarin cv. Terigas essential oil has 40 volatile components. Limonene is the chemical with the highest content. The chemical combination has antibacterial properties. *Listeria monocytogenes* can grow less than 7.6 mm in the nanoemulsion solution when adding 2% essential oil. This study's findings may provide helpful information when applying essential oils as antibacterials. More research is required to assess the effectiveness against other infections, particularly those resistant to antibiotics. Further research is required to evaluate the formulation's long-term stability and shelf life and to conduct comprehensive toxicological tests on various cell lines.

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