Comparing the Effectiveness of Methods and Solvents on the Yield and Phytochemicals of Gerga Citrus Peel Essential Oil (*Citrus nobilis L.* Var RGL) from Kerinci Regency

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Abstract

Gerga orange was one of the leading commodities of Bengkulu province and also widely cultivated in Kerinci, Jambi. Nearly 75% of gerga orange peel was wasted without any processing. Orange peel was a plant that can be produced in to essential oil. However, the extraction effectiveness can be affected by the solvent and method. The purpose of this study was to determine the best solvent and method for extracting gerga orange peel essential oil. In addition, this research also aims to analyze the phytochemicals and yield. In this study, maceration and soxhletation methods will be compared to extract gerga orange peel essential oil with a variety of solvents with different polarities. The simplicia was macerated with ethanol, ethyl acetate and n-hexane solvents respectively for 3×24 hours. Then it was distilled to evaporate the ethanol. While the soxhletation method the simplicia are percolated using ethanol, ethyl acetate, and n-hexane solvents respectively for 4 hours. The results obtained yield of gerga orange peel essential oil by maceration method with ethanol, ethyl acetatee, n-hexane solvents, respectively, 23.04%; 16.05%; 11.80%, whereas with the soxhletation method 10.36%; 3.02%; and 2.04%. The phytochemical screening results of extractions using maceration and soxhlet methods indicate that all essential oils contain flavonoids and phenolics. The highest content of flavonoids and phenolics was obtained from extraction using ethanol solvent with the maceration method. The use of these three solvents in the maceration method, especially ethanol, was found to be more effective in extracting essential oils from bitter orange peel. Based on the phytochemical screening results using specific reagents with color changes indicating the presence of certain metabolites in the sample, it is revealed that the dominant secondary metabolites in the essential oil of bitter orange peel belong to the phenolic group. Furthermore, the essential oil of bitter orange peel also contains flavonoids and does not detect the presence of saponin compounds..

Keywords: maceration, soxhletation, Citrus nobilis L. Var RGL, phytochemicals.

Abstrak

Jeruk gerga merupakan salah satu komoditas unggulan provinsi Bengkulu dan juga banyak dibudidayakan di Kerinci, Jambi. Hampir 75% kulit jeruk gerga terbuang begitu saja tanpa ada pengolahan. Kulit buah jeruk salah satu tanaman yang dapat menghasilkan minyak atsiri. Akan tetapi, efektivitas ekstraksinya dapat dipengaruhi oleh pelarut dan metode. Tujuan penelitian ini untuk mengetahui pelarut dan metode terbaik untuk mengekstraksi minyak atsiri kulit jeruk gerga. Selain itu, penelitian ini juga bertujuan untuk analisis fitokimia dan rendeman hasil ekstraksi. Pada penelitian ini akan dibandingkan metode maserasi dan sokletasi untuk mengekstraksi minyak atsiri kuit jeruk gerga dengan variasi pelarut dengan kepolaran. Pada metode maserasi

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simplisia dimaserasi dengan masing-masing pelarut etanol, etil asetat dan n-heksana selama 3x24 jam. Kemudian maserat didistilasi untuk menguapkan etanol. Pada metode sokletasi, simplisia disokletasi menggunakan masing-masing pelarut etanol, etil asetat dan n-heksana selama 4 jam. Hasil penelitian menunjukan bahwa rendeman minyak atsiri kulit jeruk gerga dengan metode maserasi dengan pelarut etanol, etil asetat, n-heksana masing-masing yaitu 23,04%; 16,05%; 11,80%, sedangkan dengan metode sokletasi 10,36%; 3,02%; dan 2,04%. Hasil skrining fitokimia dari ekstraksi menggunakan metode maserasi dan sokletasi menunjukkan bahwa semua minyak atsiri mengandung flavonoid dan fenolik. Kandungan flavonoid dan fenolik tertingi diperoleh dari ekstraksi menggunakan pelarut etanol dengan metode maserasi. Penggunaan ketiga pelarut Metode maserasi dengan pelarut etanol lebih efektif untuk mengekstrak minyak atsiri kulit jeruk gerga. Berdasarkan hasil skrining fitokimia sesuai dengan indikator pada masing-masing reagen spesifik ditandai dengan perubahan warna pada sampel, menunjukkan bahwa kandungan metabolit sekunder pada minyak atsiri kulit jeruk gerga paling dominan adalah golongan fenolik. Selain itu, minyak atsiri jeruk gerga juga mengandung flavonoid dan tidak terdeteksi golongan senyawa saponin
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Kata Kunci: maserasi, sokletasi, Citrus nobilis L. Var RGL, fitokimia

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1. INTRODUCTION

Gerga Lebong Rimau Orange was one of the leading commodities of Bengkulu province which was the main ingredient for making fruit syrup. Utilization of this citrus fruit certainly produces waste in the form of fruit peels that cannot be handled properly. Wastes, if not handled well can be one of the causes of global warming even though fruit peels contain higher antioxidant compounds than other parts of the fruit (R.E.Abdelazem et al., 2021). Orange peel also contained phenolic compounds consisting of phenolic acids, falvonones, and polymethoxylated flavones, as well as carotenoids and ascorbic acid (N.A. Indrastuti et al., 2019).

Essential oils were important compounds that can be isolated from plants, from woods, seeds and leaves. Essential oil was colorless but has a distinctive aroma. Consumption of essential oils in the world increases every year because the use of essential oils were very wide in scope, namely in the manufacture of detergents, soaps, cosmetics, medicines, perfumes, soft drinks, and insecticides (E.A. Hassan et al., 2009; M.D. Asfaw et al., 2022). Indonesia produced 40 types of essential oils out of a total of 150 types worldwide (Salsabila et al., 2022). The essential oil components found in orange peel include limonene (95%), mircene (2%), noctanal (1%), pinene (0.4%), linanool (0.3%), deanal (0.3%), sabiena (0.2%), geranial (0.1%), dodecanal (0.1%), and other compounds (0.5%) (A. Kurniawan et al., 2008; A.S. Ananda et al., 2022).

The process of extracting essential oils from orange peels can be done by solid-liquid extraction methods, namely maceration, percolation, reflux, and soxhletation. Maceration and soxhletation methods were widely used because they were simple, economical and fast (R. Hasibuan et al., 2021). According to Febrina et al. (2017) by using distillation method, the yield of essential oil from Siamese orange peel was very small (about 0.5-1.7%). The same method was used by Wibaldus et al. (2016), the eyield of essential oil from lime peel was 0.23%. Compared with the study of Kawiji et al. (2015) essential oil from kaffir lime peel was 9.638%. Furthermore, Ananda et al. (2022) who used the maceration method for extraction, produced a higher yield reached 0.8496%. On the other hand

Salsabila et al. (2022) was also carried out the essential oil from lime by soxhletation method and the result was 3.25%.

The use of solvents in the orange peel extraction process will affect the effectiveness of the extraction. An ideal solvent can dissolve specifically, has a low boiling point, insoluble in water, inert, and cheap and easy to obtain. However, no solvent was ideal. Based on previous research, n-hexane was able to dissolve essential oils from lime because its polarity was close to that of essential oils (Salsabila et al., 2022). While, a study was reported by Adiyasa et al. (2015) found that the most effective solvent for producing mandarin orange peel extract was ethanol 96%. According to Rafsanjani and Putri (2015) ethanol, ethyl acetatee, and water can extract essential oils from mandarin orange peels. This study will compare the ability of n-hexane, ethyl acetatee, and ethanol to gain the maximum yield of essential oil from gerga orange peel. Gerga orange was widely consumed in Bengkulu and Kerinci. Lack information utilization of gerga orange peel. Therefore, further research needs to be carried out to determine the best method and solvent to utilize wasted peel of gerga to essential oil. This research will provide valuable information about the best method, solvent and also phytochemical of gerga orange essential oil.

2. MATERIALS AND METHODS

2.1. Materials

The tools used were erlenmeyer, analytical balance, a set of soxhleting tools, test tubes, sieve 60 mesh, beaker, stirring rod, spatula, a set of distillation apparatus, whatman filter paper no 1, dropper pipette and drip plate. The materials used were gerga orange peel obtained from Lolo Gedang, Kerinci Regency, ethyl acetate p.a (Supelco), ethanol p.a (Supelco), n-hexane p.a, methanol p.a (supelco), Mg powder (Merck), concentrated HCl (Merck), Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Liebermann Burchard's reagent (acetic acid anhydride-H_2SO_4), chloroform, and 1% $FeCl_3$.

2.2. Simplicia Preparation

Gerga oranges were obtained from Lolo Gedang, Kerinci Regency. As much as 8 kg of yellow gerga oranges separated by skin and fruit. Gerga orange peels were cleaned under running water and cut into ± 8 cm pieces. Gerga orange peels were dried for 3x24 hours, then crushed and sieved using sieve 60 mesh to obtain simplicia powder.

2.3. Maceration Method

Maceration method according to study conducted by Handayani (2014) and Damayanti et.al (2021). 20 grams of each simplicia was macerated with ethanol, ethyl acetate and n-hexane (1:10) in a closed container to be stored for 3 days (in a closed room at 28ºC and protected from sunlight). During the maceration process, stirring is also carried out every day. After 3 days of maceration, filtering was carried out using filter paper. The resulting filtrate was then distilled to separate the oil and solvent (distillation temperature; ethanol 78°C, n-hexane 70°C and ethyl acetate 77°C) for 3 hours. The distilled extract oil was evaporated again with the oven based on solvent temperature (ethanol 78°C, n-hexane 70°C and ethyl acetate 77°C) to remove the remaining solvent and weighed (Ramli et al., 2019).

2.4. Soxhletation Method

Each of 20 grams of gerga orange peel simplicia was put into filter paper that has been formed into a cylinder and tied. Then it was inserted into the socket with 300 mL of solvent (respectively ethanol, ethyl acetate and n-hexane). Then heated with heating mantle depend on solvent temperature (ethanol 78°C, n-hexane 70°C and ethyl acetate 77°C) extraction for 4 hours. The socket that has been assembled with cooling back is closed on top with grease-free cotton (Adam et al., 2019; Dewi et al.,

2022). Essential oil yields determination according to Chen et al. (2015).

2.5. Phytochemical Screening Test

Phytochemical tests such as alkaloids, flavonoids, phenolics, saponins, terpenoids and steroids refer to Harborne (2013). The standard used refers to Noviarni et al. (2020).

Alkaloid Test

Test for alkaloid compounds using dragendorf, wagner, and mayer reagents which were dripped into the extract. The positive result for the dragendorf reagent produced a red precipitate, the Wagner reagent produced a brown precipitate, and the Mayer reagent produced a white precipitate.

Flavonoid Test

A small quantity of sample was put on the drip plate then added Mg powder and 2 drops of concentrated hydrochloric acid into the same plate, observed the color change. Formation of orange to red color indicated the presence of flavonoids.

Phenolic Test

A small quantity of the sample put on the drip plate, then added 1-2 drops of 1% FeCl3 reagent into the plate, observed the color change. The presence of phenolic was indicated by a color change to green or blue-black.

Saponin Test

Took a few mL of the water layer then put it in a test tube and shaked it, and observed the changes that occur. The presence of saponins was indicated by the formation of foam which remained for 5 minutes.

Terpenoids and Steroids Test

The sample was dissolved in chloroform then added Lieberman Buchard's reagent (acetic acid anhydrous-H_2SO_4), observed the changes that occur. A positive result for the presence of steroids is indicated by a change in color to green-blue and red-purple for terpenoids.

3. RESULTS AND DISCUSSION

3.1. Determination of Yield Essential Oil

Extraction of gerga orange peel essential oil was carried out using various solvents (ethanol, ethyl acetate, n-hexane) and methods (maceration and soxhletation). The essential oils had different colors. The essential oil extracted using ethanol and ethyl acetate was yellow-orange in color. The color of essential oils on extraction with ethanol was more concentrated than ethyl acetate. While the extraction using n-hexane essential oil was yellow. Based on the study that conducted, the highest yield was in the maceration method with ethanol solvent, as shown in (Table 1.) and the lowest was in n-hexane. This is in line with the research reported by Hossain et al. (2011), which suggests that essential oils produced from more polar solvents (such as ethanol) are superior to those extracted using ethyl acetate and n-hexane (methanol extract > ethyl acetate extract >chloroform> butanol > hexane extract)

Table 1. Yield (%) of Essential Oil

Solvents	Ethanol	Ethyl acetate	*n*-hexane
Maceration yield (%)	23,04	16,05	11,80
Soxhletation yield (%)	10,36	3,02	2,04

The differences in extraction methods affect to efficiency and percentage of yield. In line with the research conducted by Ramli et al. (2017), maceration was more effective in producing a higher yield than the soxhletation method. It was because the soxhletation method involved the use of heat which resulted in the possibility of heat-resistant compounds to be degraded. While the maceration method had not involved heat therefor it was suitable for the extraction of easily degraded compounds. Adam et al. (2019) also reported that essential oils from extracts of Sudan's medicinal plants using the maceration

method produced a higher yield than the soxhletation method. The solvents used were methanol, chloroform, and n-hexane. The highest yield was obtained from extraction using methanol solvent with the percentage of yield of the maceration method 7.123% while the soxhletation method was 6.137%.

The highest yield produced from both methods was by using ethanol solvent. According to Adiyasa et al. reaserch in Dewi et.al (2022), ethanol was the most effective solvent among n-hexane, ethyl acetate, and ethanol. The yield of essential oil was 47.69% from mandarin orange peel. The difference in yield was due to the polarity of ethanol which was higher than other solvents since the secondary metabolites in the sample were more extracted to ethanol solvents (R.E Abdelazem et.al., 2021).

3.2. Phytochemical Screening

The results of the phytochemical tests showed that each essential oil extracted using ethanol, ethyl acetate and n-hexane contained phenolic. Tables 2 and 3 showed the results of the phytochemical tests using the maceration and soxhletation methods. The highest phenolic detected in essential oils extract with ethanol and the lowest in ethyl acetate. It was indicated by a change in color intensity to blackish green which was more concentrated in the essential oil extracted with ethanol.

Essential oil extracts had not contained saponins and there had no color change when Dragendorf reagent had added as shown in Tables 2 and Table 3. Steroid groups in Gerga orange essential oil was not detected in extraction using ethanol and ethyl acetat solvents. However, in ethanol solvents, triterpenoid groups were detected. It was indicated by a red-purple color change when Lieberman Buchard's reagent was added.

Steroids typically have higher solubility in polar solvents, such as ethanol, compared to nonpolar solvents like ethyl acetate and n-hexane. Therefore, in extractions using ethyl acetate and n-hexane, steroid compounds are less likely to be extracted or may not be extracted at all. More polar solvents have a better affinity for polar compounds like steroids.

Polar ethanol will dissolve steroids in the first stage of extraction, whereas with n-hexane solvent, steroids are not detected. Abdelazem et al. (2021) reported a similar finding, stating that based on phytochemical screening results on orange peel, saponins were not detected in samples extracted using ethyl acetate and other nonpolar solvents.

Table 2. Phytochemical Test Results of Essential Oil Produce by Maceration Method

Phyto-chemical	Reagent	Ethanol	Ethyl acetate	*n*-hexane
Alkaloid	Mayer	-	+	++
	Wagner	+++	++	+++
	Dragendroff	-	-	-
Flavonoid	Mg + HCl	+++	++	+
Phenolic	$FeCl_3$	+++	+	++
Saponin	Distillated water	-	-	-
Triterpenoids and Steroids	Lieberman Buchard			
	Triterpenoids	++ -		-
	Steroids	- -		++

Explanation: (-): not detected. (+) detected. Amount of (+) show the color intensity

Table 3. Phytochemical Test Results of essential oil produce by Soxhlet Method

Phyto-chemical	Reagent	Ethanol	Ethyl acetate	*n*-hexane
Alkaloid	Mayer	-	++	++
	Wagner	++	+	+++
	Dragendroff	-	-	-
Flavonoid	Mg + HCl	++	+	+
Phenolic	$FeCl_3$	+++	+	++
Saponin	Distillated water	-	-	-
Triterpeno-ids and Steroids	Lieberman Buchard			
	Triterpenoids	+	+	-
	Steroids	-	-	+

Explanation: (-): not detected. (+) detected. Amount of (+) show the color intensity

The differences of secondary metabolites detected depend on the type of solvent and method used. In addition, the more concentrated the color intensity change when added with the reagent, showed the higher concentration of the compound group. The more intense the color changed that appeared, illustrated the higher content of phtyochemical in essential oil (Table 3).

Besides the phenolic group, the essential oil from gerga orange peel which has been extracted by the maceration method contains flavonoids. The highest flavonoids in essential oils extracted with ethanol solvent were characterized by a more intense orange color intensity. Based on research by Oikeh et al. (2020) the generally in orange peels were flavonoids and phenolics. In addition, Hasibuan et.al reported that lemon peel contains a high content of limonene. Syamsuhidayat and Hutapea (1991) found that the essential oil content present in lime peel was siral, limonene, feladren, and hedperidin glycosides.

4. CONCLUSIONS

Based on research, the maceration method was more effective for extracting essential oil from gerga orange peel than the soxhletation method. Among ethanol, ethyl acetate, and n-hexane, the effective solvent was ethanol with yield value was 23.04%. The phytochemical screening of essential oil from gerga orange peel showed the most dominant group were phenolics and flavonoids, which were the higest phytochemical contained on extract that produced by maceration method and solvent was ethanol.

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