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Antioxidant activity of kintamani siamese orange peel extract (*Citrus nobilis*) different polar solvent: an in vitro experimental study

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Abstract Siamese oranges (*Citrus nobilis*) are one of Bali's most widely developed citrus fruits, especially in the Kintamani area. Siamese oranges (*Citrus nobilis*) contain many secondary metabolite compounds as a source of natural antioxidants. In this study, secondary metabolite compounds in plants were obtained through the extraction method. In addition to the extraction method, selecting the type of solvent is one of the main factors affecting the extraction results. Polar solvents tend to produce higher antioxidant activity, which aimed to determine the best polar solvent in producing Kintamani Siamese orange extract peel (*Citrus nobilis*) with the highest antioxidant activity. The research method used was experiment, where the orange peel was extracted through maceration using three polar solvents: methanol; ethanol; and water. Furthermore, antioxidant activity testing was carried out using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hidrate) method. Data from the analysis of antioxidants in IC_{50} was compared and classified according to BIOS Classification. The results showed that methanol solvent produced yield and antioxidant activity with the highest IC_{50}. Methanol solvent as a polar solvent could produce Kintamani Siamese orange peel extract (*Citrus nobilis*) with moderate antioxidant activity according to BIOS classification. Further research is needed to explore other potentials benefit of Kintamani Siamese orange peel waste (*Citrus nobilis*) as an application of zero waste system.

Keywords: antioxidant, orange peel, Kintamani, polar solvent

1. Introduction

Orange is one of the horticultural crops acting as a superior commodity. Its cultivation has been carried out for a long time in Bali Province. Siamese oranges *(Citrus nobilis)* are one of the most widely developed types of citrus fruit because they are relatively easy to take care of, produce abundant yields and have high sale figures in the market (Kristiandi 2020; Purba 2019). Among regencies in Bali, Bangli Regency is in the first position, producing the highest number of Siamese Orange *(Citrus nobilis)* in Bali at approximately 93,162.3 tons per year with a harvest area of 38,140.21 Ha and an average production of 24.42 Kw/Ha. According to the data issued in 2013, approximately 93,929 Siamese orange *(Citrus nobilis)* trees produced 4,200 tons of oranges per year in Sekaan Village, and Siamese orange production from year to year always fluctuates. The market demand for Kintamani Siamese oranges *(Citrus nobilis)* can be categorized as quite good when associated with an increase in population, income, and public awareness of the nutritional value contained in Siamese oranges *(Citrus nobilis)*. However, the increase in the amount of production greatly affects the potential increase in Siamese orange *(Citrus nobilis)* waste because the utilization of Kintamani Siamese orange peel *(Citrus nobilis)* waste is still not optimal (Bhola 2022). Due to this problem, Siamese orange (*Citrus nobilis*) must be managed properly, and the appropriate polar solvent used as well as a more important zero-waste system must be determined.

As a source of natural antioxidants, oranges contain many nutritional components. Currently, the potential utilization of fruits and vegetable waste as a source of micronutrients and antioxidants has increased (Malik 2021). The fruit juice in Siam Kintamani oranges *(Citrus nobilis)* contains approximately 20-60 mg of ascorbic acid per 100 ml and contains several other vitamins, such as vitamin A, thiamine, niacin, riboflavin, pantothenic acid, biotin, folic acid, inositol, and tocopherol (Sudiana 2008). Most of the nutritional components in oranges actually lie in the peel of the fruit, which is not utilized. The peel of the Kintamani Siamese orange *(Citrus nobilis)* is known to contain alkaloids, flavonoids, lycopene, and vitamin C, as well as pectin and tanning as the most dominant content in Siamese oranges *(Citrus nobilis)*.

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The content of essential oils in the leaves and peels of Siamese oranges *(Citrus nobilis)* can be used as an anti-acetylcholinesterase, while the content of flavonoids has anticancer properties in A549 cells (Zarrad 2015; Arinda 2022). Vitamin C in *Citurs nobilis* is believed to be a good source for vitamin C, which plays an important role in the proper functioning of the human body. It activates many enzymes and plays an important role in cell respiration. Vitamin C is also important for preventing and slowing the progression of many diseases, such as wounds, fractures, formation of bruises, and bleeding gums (Tada 2019).

Siamese oranges (*Citrus nobilis*) must be used as natural antioxidants since they are inexpensive and easy to obtain. However, before obtaining natural antioxidants, the fruits must be extracted, and the appropriate solvent must be determined. Secondary metabolite compounds in plants were obtained through the extraction method. In addition to the extraction method, selecting the type of solvent is one of the main factors affecting the extraction results (Permatananda 2020). According to the "like dissolves like" principle, a solvent can dissolve compounds with the same degree of polarity. Polar solvents will dissolve polar compounds and vice versa (Suryani 2016). Karimi stated that polar solvents tend to produce higher antioxidant activity (Karimi 2015). The antioxidant activity value of Balinese orange peel extract (*Citrus maxima*) extracted with ethanol solvent through the ultrasonic bath method is higher than the extract created using water solvent, while the lowest antioxidant activity lies in ethyl acetate solvent (Rafsanjani 2015). Apart from ethanol, other polar solvents that can be used are water and methanol (Sayuti 2017).

This study aimed to compare the antioxidant activity of Kintamani Siamese orange peel extract (*Citrus nobilis*) using different polar solvents. The polar solvents used were methanol, ethanol, and water. In this case, the best polar solvent for producing Kintamani Siamese orange peel extract (*Citrus nobilis*) with the highest antioxidant activity was determined

Practically, this research is the basis for determining the best solvent to extract Kintamani Siamese orange peel waste *(Citrus nobilis)* in terms of its potential as an antioxidant agent. This research is also a form of zero waste system application in utilizing organic waste and providing added value to domestic agricultural production.

2. Materials and Methods

The objective of this research was to determine the best polar solvent for producing Kintamani Siamese orange peel extract *(Citrus nobilis)* with the highest antioxidant activity. The orange peel was extracted by maceration using three polar solvents: methanol, ethanol, and water. Furthermore, antioxidant activity testing was carried out using the DPPH method. The research was conducted at the Faculty of Medicine and Health Sciences Research Laboratory and the Agricultural Laboratory of Warmadewa University. Current research employed several tools, including scales, dish, filter paper, blender, plastic bags, knive, jar, beaker, measuring cup, spatula, rotatory ovaporator, incubator, aluminum foil, spectrophotometer, UV–Vis, funnel vials, analytic balance, test tube, tube shelves, piper, micropipet, refrigerator, stirring rod, vortex, desiccator, furnace, and porselain dish.

Meanwhile, the materials used are Kintamani Siamese orange peel extract (citrus nobilis et reticulata) waste, Kintamani Siamese orange (citrus nobilis et reticulata) and Kintamani Siamese orange peel extract (citrus nobilis et reticulata) pulp waste.

As much as 10 kg of Kintamani Siamese orange (*Citrus nobilis*) that was used as a sample was taken from the Kintamani Siamese Orange *(Citrus nobilis)* Production Center, Bayung Gede Village, Kintamani District, Bangli Regency. Before being used in this research, the samples of Kintamani Siamese oranges *(Citrus nobilis)* were determined first at the Laboratory of the Faculty of Agriculture, Warmadewa University. Then, a total of 1.6 kg of Siam Kintamani Citrus *(Citrus nobilis)* peel waste was used to make the extract selected by purposive sampling. Selected orange peels are good, fresh, and not indicated to be affected by fungus or disease. Before being used, the peel of the Kintamani Siamese Orange *(Citrus nobilis)* was cleaned first using fresh water to free it from adhering dirt or sand. The clean orange peels were further dried using an oven at 60°C for 25 – 30 minutes (1) in the Faculty of Medicine and Health Sciences Laboratory, Warmadewa University. The sample was weighed again after the drying process because the sample will experience shrinkage due to decreased water content.

The dried orange peel was mashed using a blender to obtain a smoother texture. The refined samples were macerated in stages using different solvents, namely, methanol, ethanol, and water, at a ratio of 1:5 (w/v). This extraction was performed to produce a crude extract. The next step was to perform another extraction using the maceration or immersion method, carried out three times until the filtrate was close to a clear color. The results of this maceration extraction were then filtered using filter paper until the results were in the form of filtrate and residue (Putranti 2013). It is best to perform occasional stirring during the 3 x 24-hour maceration process (Senja 2014). The filtrate obtained was then concentrated with a vacuum rotary evaporator at a temperature of 40° or more until it reached the boiling point. A crude extract in the form of a paste was obtained (12). This crude extract was subjected to several tests, such as yield calculation and antioxidant activity testing with the *diphenylpicrylhydrazil* (DPPH) method. The extract yield formula is as follows:

$$\% \text{ Yield} = \frac{\text{Total weight of the extract in the form of a paste (g)}}{\text{Total dry weight (g)}} \times 100\%$$

Siamese orange peel waste sample extract (*Citrus nobilis*) was dissolved according to the solvent at concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Each of these concentrations was pipetted as much as 3 ml and dissolved with 1 ml of 100 µM DPPH solution (Putranti 2013). The mixture was then incubated at 30°C for 30 minutes in the dark. Then, the absorbance was measured using a UV – Vis spectrophotometer at a maximum wavelength of 517 mm (Sharma 2009). Antioxidant activity was expressed in units of % inhibition. This value was obtained using the following formula:

$$\%\text{Inhibition} = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100\%$$

This blank absorbance measurement aimed to calculate the inhibition percentage. A blank solution was prepared by reacting 3 ml of the solvent according to the solvent used in the sample with 1 ml of 100 µM DPPH solution in a test tube. After obtaining each treatment's inhibition value, the equation $y = A + Bx$ was determined by calculating the linear regression value, where x is the concentration (µg/ml) and y is the percentage of inhibition (%). The IC_{50} value was obtained from the x value after replacing y with 50. The smaller the IC_{50} value is, the higher the antioxidant activity. Data from the analysis of antioxidants in the form of IC_{50} are then displayed as the average and standard deviation. To make it easier to compare, IC_{50} data are also displayed as a bar chart. IC_{50} data are then also classified according to the Bios classification, which is a very strong antioxidant if the IC_{50} value is less than 50 ppm ($IC_{50} < 50$ ppm), strong (50 ppm < IC_{50} < 100 ppm), medium (100 ppm) < IC_{50} < 150 ppm), weak (150 ppm < IC_{50} < 200 ppm), and very weak ($IC_{50} > 200$ ppm) (Putranti 2013).

3. Results

A total of 10 kg of oranges were taken from the Kintamani Siamese Orange production center. The oranges were washed and sorted according to the inclusion and exclusion criteria and then peeled to obtain orange peel waste. The selected orange fruits should have clean, smooth skin and have no indications of disease or fungus. From 10 kg of oranges, 1600 grams of orange peel was obtained. The orange peel was then cleaned again and dried using an oven at 60°C for 20-30 minutes, blended, and macerated using polar solvents, namely, methanol, ethanol, and water, at a ratio of 1:5 (w/v). Maceration was carried out repeatedly until the filtrate approached a clear color. The filtrate was then concentrated using a rotatory evaporator at a temperature of 40°C until it became a crude extract or paste. Prior to testing, the extract yield calculation was carried out, which is contained in Table 1. It was further found that the highest yield was found in methanol solvent, namely, 30.46%, followed by ethanol solvent at 14.71% and water at 8.42% (Table 1).

Table 1 Yield Percentage of Kintamani Siamese Orange Peel Extract (*Citrus nobilis*).

Solvent	Paste weight (gr)	Powder weight (gr)	Yield (%)
Methanol	15.233	50	30.46
Ethanol	7.356	50	14.71
Water	4.214	50	8.42

Antioxidant activity testing using the DPPH method was carried out to determine the ability of Kintamani Siamese orange peel extract (Citrus nobilis) to capture radical compounds or their ability as antioxidant compounds. Figure 1 shows the DPPH solution color change after adding Kintamani Siamese orange peel extract (*Citrus nobilis*) with different solvents. From the picture, qualitatively, it can be seen that the highest decrease in absorbance is in methanol, followed by ethanol and distilled water, which are polar solvents. Meanwhile, in ethyl acetate and n-hexane, there was no significant decrease in absorbance, so they were considered to have weak antioxidant activity. Table 2 and Table 3 show antioxidant activity results using methanol and ethanol.

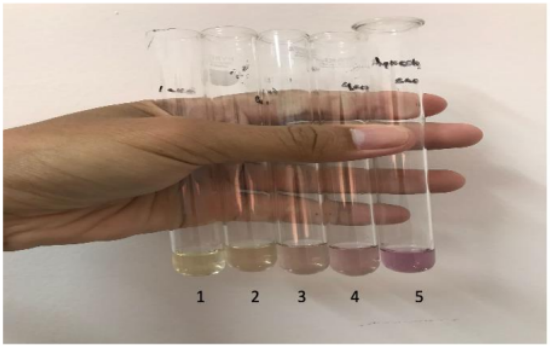

Figure 1 DPPH assay results (1: methanol, 2: ethanol, 3: water, 4: ethyl acetate, 5: n-hexane).

Table 2 Antioxidant activity result of Kintamani Siamese Orange (*Citrus nobilis)* using methanol solvent.

Replication	Concentration	Control Absorbance	Sample absorbance	Antioxidant activity	IC_{50}
	20		0.286	42.914	
	40		0.128	74.451	
1	60	0.501	0.076	84.830	146.4028
	80		0.048	90.419	
	100		0.021	95.808	
	20		0.281	43.912	
	40		0.133	73.453	
2	60	0.501	0.088	82.435	149.1948
	80		0.045	91.018	
	100		0.023	95.409	
	20		0.271	45.908	
	40		0.123	75.449	
3	60	0.501	0.098	80.439	112.9349
	80		0.035	93.014	
	100		0.029	94.212	

Table 3 Antioxidant activity results of Kintamani Siamese Orange (*Citrus nobilis)* using ethanol.

Replication	Concentration	Control Absorbance	Sample absorbance	Antioxidant activity	IC_{50}
	20		0.376	24.950	
	40		0.277	44.711	
1	60	0.501	0.167	66.667	464.7692
	80		0.098	80.439	
	100		0.061	87.824	
	20		0.346	43.912	
	40		0.237	73.453	
2	60	0.501	0.172	82.435	416.3095
	80		0.119	91.018	
	100		0.080	95.409	
	20		0.387	22.754	
	40		0.247	50.699	
3	60	0501	0.167	66.667	450.1836
	80		0.086	82.834	
	100		0.061	87.824	

Methanol had the highest antioxidant activity, with an average IC_{50} value of 136.17. In this case, according to the BLOIS classification, it belongs to the medium category, which ranges from 100-150 ppm. However, other polar solvents, such as ethanol and water, produce different antioxidant activities than methanol. Both ethanol and water solvents produce very weak antioxidant activity, as shown in Table 4 and Figure 2. Table 5 shows the result and interpretation according to BLOIS Classification.

Table 4 Antioxidant activity result of Kintamani Siamese Orange (*Citrus nobilis)* using water solvent.

Replication	Concentration	Control Absorbance	Sample absorbance	Antioxidant activity	IC_{50}
	20		0.421	21.455	
	40		0.279	47.948	
1	60	0.501	0.174	67.537	482.1769
	80		0.120	77.612	
	100		0.053	90.112	
	20		0.352	34.328	
	40		0.264	50.746	
2	60	0.501	0.176	67.164	407.351
	80		0.115	78.545	
	100		0.079	85.261	
	20		0.369	31.157	
	40		0.264	50.746	
3	60	0.501	0.185	65.485	417.415
	80		0.108	79.851	
	100		0.068	87.313	

Table 5 IC_{50} Results and Interpretation According to BLOIS Classification.

Solvent	IC_{50} (Mean ± Standard Deviation)	Interpretation
Methanol	136.17 ± 20.17	Medium
Ethanol	443.75 ± 24.86	Very weak
Water	435.64 ± 40.60	Very weak

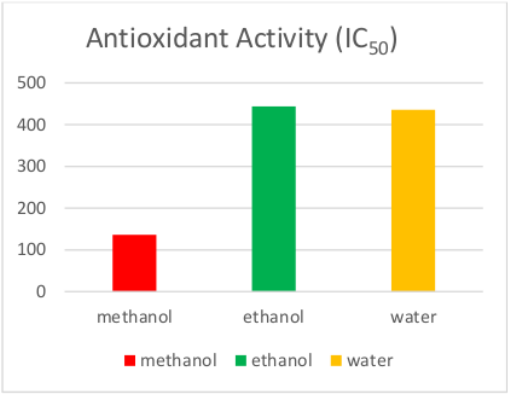

Figure 2 Comparison of IC_{50} Kintamani Siamese Orange Peel Extract *(Citrus nobilis)* using methanol, ethanol, and water solvents.

4. Discussion

Yield calculations are a simple method to predict the levels of secondary metabolites carried by the solvent but cannot determine the type of compound (Ukieyenna 2012). The yield was calculated by looking at the ratio between the weight of the paste and the weight of the powder (Putranti 2013). The higher the yield value, the higher the bioactive components contained (Nurjanah 2011). In this study, the highest yield was obtained with methanol as the solvent. This shows that the polarity of the compounds contained in Kintamani Siamese orange peel (*Citrus nobilis)* had a polarity that approaches the polarity of the methanol solvent so that more compounds can be extracted. The extraction process occurred by flowing the solvent into the cell, which caused the protoplasm to swell, and the material contained in the cell dissolved according to its solubility. This high dissolving power is related to the solvent's polarity and the compound being extracted (Sari 2015).

The yield of an extract can be influenced by various factors, especially the type of solvent and its concentration. This type of polar solvent is considered capable of dissolving almost all organic compounds and has volatile properties, so it is easily released from the extract (Andayani 2015). Suryanto et al (2008) stated that polar solvents produce the highest yield compared to nonpolar solvents (Suryanto 2019). In this study, the solvents used were methanol, ethanol, and water, which are all polar solvents. Ethanol is polar because ethanol is a primary alcohol with one hydroxyl group and an alkyl group, namely, ethyl ethanol, so it can form hydrogen bonds with water. Meanwhile, methanol is slightly more polar than ethanol because it has fewer C atoms (Loekitowati 2003). This causes the methanol solvent to produce more yield than ethanol and water solvents. Similar results were also obtained by Suryani (2016), who found that methanol produced the highest extract yield compared to other polar solvents (Suryani 2016).

Antioxidant activity testing using the DPPH method was carried out to determine the ability of Kintamani Siamese orange peel extract (*Citrus nobilis)* to capture radical compounds or their ability as antioxidant compounds. DPPH is a stable free radical compound used to evaluate free radical scavenging in natural products. The principle of the reaction of this method is that DPPH will be reduced by a hydrogen or electron donation process so that the color will change from violet to yellow with a change in color intensity that is proportional to the number of electron donations followed by a decrease in the absorbance of DPPH (Rumiantin 2011). Figure 2 shows the DPPH solution color change after adding Kintamani Siamese orange peel extract (*Citrus nobilis*) with different solvents. From the figure, qualitatively, it can be seen that the highest decrease in absorbance is in methanol, followed by ethanol and distilled water, which are polar solvents. Meanwhile, in ethyl acetate and n-hexane, there was no significant decrease in absorbance, so they were thought to have weak antioxidant activity.

The parameter used to determine the ability of antioxidant compounds is IC_{50}. IC_{50} is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%. The smaller the IC_{50} value, the more active the extract or test fraction as a DPPH radical scavenging compound or antioxidant compound (Putranti 2013).

Methanol solvent had the highest antioxidant activity, with an average IC_{50} value of 136.17. In this case, according to the BLOIS classification, it belongs to the medium category, which ranges from 100-150 ppm. This shows that the bioactive compounds in Kintamani Siameme orange peel extract (*Citrus nobilis)* have relatively the same polarity as methanol. According

to the “like dissolve like” principle, the acquisition of chemical compounds is based on the similarity of the polarity of the solvent used (Suryani 2016).

Methanol is considered a universal polar solvent capable of binding all chemical components found in natural plant materials, including semipolar and nonpolar components. Methanol easily enters the cell through the cell wall material so that the secondary metabolites present in the cytoplasm will dissolve in the solvent, and the compounds will be extracted perfectly (Sayuti 2017). These results are slightly different from the research conducted by Febrianti et al (2018), which found that the antioxidant activity of Banjar Siamese orange peel extract with methanol solvent has an IC_{50} value of 264 ppm, which is relatively weak (Febrianti 2018). This difference can be caused by environmental factors, differences in age, geographical location, and climate that can influence the content of secondary metabolites. These differences can affect the content of bioactive compounds in plants. In addition, the age and maturity of plants also impact the content of active secondary metabolites in plants (Supriatna 2019; Amir 2023).

Compared with ethanol and water solvents that are included in food-grade or GRAS (generally recognized as safe) solvents, methanol is a chemical nonfood grade solvent. Therefore, developing antioxidants from Kintamani Siamese orange peel extract (*Citrus nobilis)* with methanol solvent could be difficult even though this study produced quite good antioxidant activity. The use of chemical nonfood grade solvents is rarely done because of doubts about their safety and their possible capability to increase production costs due to the longer processing stages, the need for more equipment, and the higher price of solvents compared with food-grade solvents (Widayanti 2009).

This research sums up that polar solvents produce the highest residue compared to nonpolar solvents. The polar solvents used in this research are ethanol, methanol, and water. However, based on the research results, the highest residue was obtained from methanol. Therefore, the use of methanol will produce more compounds to be extracted. Further research is needed to explore other potential benefits of Kintamani Siamese orange peel waste (*Citrus nobilis*) as an application of a zero-waste system.

4. Conclusions

This research sums up that polar solvents produce the highest residue compared to nonpolar solvents. The polar solvents used in this research are ethanol, methanol, and water. However, based on the research results, the highest residue was obtained from methanol. Therefore, the use of methanol will produce more compounds to be extracted. Further research is needed to explore other potential benefits of Kintamani Siamese orange peel waste (*Citrus nobilis*) as an application of a zero-waste system.

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Ethical considerations

Ethic for Wamadewa University Number 2022.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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