Antioxidant Properties of Sweet Orange Peels in Several Fractions of Methanolic Extract

Suhartomi[1], Kristin Natalia Gulo[1], Albert Daniel Saragih[2], Ahmad Raif Martinus[1], Refi Ikhtiari[1,2*]
[1]*Laboratory of Biomolecular Chemistry, Graduate School of Biomedical Science, Universitas Prima Indonesia*
[2]*Laboratory of Materials Engineering, Faculty of Industrial Engineering, Universitas Prima Indonesia*
Emails: (suhartomi, kristin, albert, ahmadraif, refiikhtiari)@unprimdn.ac.id

Keywords: *Citrus sinensis*, methanol, dichloromethane, n-hexane, Hydrogen peroxide, DPPH

Abstract: *Citrus sinensis* is a fruit that is widely consumed over the world and has potent natural antioxidant activity. This study was aimed to determine the potential of antioxidants from each partition of sweet orange peel methanol extract. The sweet orange peel methanol extract was made by the maceration method followed by fractionation through a separating funnel with n-hexane and dichloromethane solvents. Antioxidant activity of the methanolic extract was measured by H_2O_2 and DPPH methods. Results showed that the most potent H_2O_2 inhibition activity was the fraction of n-hexane (IC_{50}: 91.33 µg/ml), followed by dichloromethane (IC_{50}: 174 µg/ml), and methanol (IC_{50}: 189.63 µg/ml). While the most potent DPPH inhibitory activity was the n-hexane fraction (IC_{50}: 709.60 µg/ml), followed by the methanol fraction (IC_{50}: 790.64 µg/ml) and the dichloromethane fraction (IC_{50}: 895.58 µg ml). The fraction of n-hexane has higher antioxidant potential than the fraction of dichloromethane and methanol. These antioxidant properties might be related to bioactive compounds in the methanolic extracts which will be investigated further. However, the research results on several fractions might provide a preliminary study on citrus-based drug preparation in the pharmacy industry for antioxidants application.

1 INTRODUCTION

Free radicals are types of high reactivity molecules that have unpaired electrons and last only for a very short time (usually 10^{-9} to 10^{-12} seconds) before they react with other molecules and take or donate electrons to achieve stability. The most damaging main radicals in biological systems are oxygen radicals (sometimes called oxidative oxygen species), especially superoxide, O_2 *, hydroxyl, OH *, and per hydroxyl, O_2H *. Normally free radicals are formed in the body that are useful in cell signaling and especially the signaling process for cell apoptosis in damaged cells. However, these substances can also cause damage to nucleic acids, proteins, and lipids in cell membranes and plasma lipoproteins which then cause disorders such as cancer, atherosclerosis, and coronary artery disease, as well as autoimmune disease (Bander, 2015). More than half (54%) of deaths occurred during 2016 (56.9 million) due to the top 10 causes. Ischemic heart disease and stroke are the biggest killers, accounting for 15.2 million deaths in 2016 due to a combination of these diseases. Besides these two diseases are also the two most common causes of death in the world over the past 15 years (*Top 10 causes of death*, 2018).

Imbalance of oxidant and antioxidant lead to the impairment of signaling process, reduction-oxidation reaction control and/or molecular damage (Sies, 2015). The antioxidant is a chemical compound that prevents the oxidation of another chemical compound, Several phytochemicals that have potent antioxidant activity include tannin, flavonoid, and phenolic acid. The human body uses an antioxidant defense system to neutralize excessive Reactive Oxygen Species (ROS). This system consists of enzymatic and non-enzymatic antioxidants. Some antioxidant enzymes found to protect against ROS are superoxide dismutase, catalase, and glutathione peroxidase, in addition to that many small non-enzymatic molecules, are widely distributed in biological systems and are capable of cleaning free radicals. These non-enzymatic molecules include

Suhartomi, ., Gulo, K., Saragih, A., Martinus, A. and Ikhtiari, R.
Antioxidant Properties of Sweet Orange Peels in Several Fractions of Methanolic Extract.
DOI: 10.5220/0009515503710378
In *Proceedings of the International Conference on Health Informatics and Medical Application Technology (ICHIMAT 2019)*, pages 371-378
ISBN: 978-989-758-460-2

glutathione, tocopherol (vitamin E), vitamin C, β-carotene, and selenium (Shalaby and Shanab, 2013).

Antioxidants are categorized in two synthetic and natural groups, which are mostly substituted phenolic compounds (Akbarirad *et al.*, 2016). The antioxidant content of plant ingredients acts as a free radical scavenger and helps convert radicals into less reactive species. Various free radical eradicating antioxidants found in food sources such as fruit, vegetables, and tea (Kumar, 2014; Zouaghi, Najar dan Abderrabba, 2018). While the synthetic compounds that have antioxidant properties must be non-toxic, have high activity at low concentrations (0.01-0.02%), and can be concentrated on the surface of fat or other oil phases, due to the non-protein nature of synthetic compounds. The synthetic antioxidant is relatively stable and usually can penetrate cells, so it can be given orally (Akbarirad *et al.*, 2016).

Citrus sinensis is a fruit that is widely consumed throughout the world and has strong natural antioxidant activity. Citrus plants are a group of plants originating from the Rutaceae family (Rafiq et al., 2016). The genus Citrus (Citrus L. of Rutaceae) is one of the world's most common fruit plants and is consumed mostly as a fresh product or juice because of its unique nutritional value and taste. The most popular in Europe and North America are grapefruits (Citrus paradisi), lemons (Citrus limon), limes (Citrus aurantifolia) and sweet oranges (Citrus sinensis). The level of consumption of citrus fruits or juices is found to be inversely related to the incidence of several diseases.

The health benefits of citrus fruits are mainly related to the presence of bioactive compounds, such as phenolics (for example, flavanone glycosides, hydroxylic acids), vitamin C, and carotenoids. Although these fruits are mainly used for dessert, they are also a source of essential oils due to their aromatic compounds. For example, the taste of lime is used in drinks, snacks, cakes, and desserts. Many authors have reported antioxidant and radical properties of essential oils and in some cases, applications that are directly related to food (Guimarães *et al.*, 2010).

Citrus plants promise various nutritional benefits as well as human life. The processing of Citrus by-products has the potential to represent a rich source of phenolic compounds and dietary fiber, as they are found in the skin. This orange fruit residue, which is generally disposed of as waste in the environment, can act as a potential nutraceutical resource. Because of their low cost and easy availability, the waste can offer significant low nutritional food supplements. Utilization of bioactive citrus-rich residues can provide an efficient, inexpensive, and environmentally friendly platform for the production of new nutraceuticals or for enhancements that already exist (Rafiq *et al.*, 2016).

Many studies have been conducted on the orange peel, especially sweet orange (Citrus sinensis) to examine its effects on health including antioxidants, antibacterial, and anti-inflammatory which are compatible with ascorbic acid, ciprofloxacin, and aspirin respectively. This could be related to the content of alkaloids, flavonoids, tannins, saponins, and steroids on extra sweet orange peels (Omodamiro dan Umekwe, 2013).

Flavonoid is a secondary metabolite that was found widely among plants and has some pharmacology properties. Mechanism of antioxidant that was had by donor the hydrogen ion or transferring single electron of flavonoid into reactive oxygen species and forms chelate complex (Banjarnahor and Artanti, 2014).

Previous studies conducted by Selvi et al. (2016) who conducted phytochemical screening and evaluation of antioxidant activity in Citrus sinensis skin extracts with several types of solvents reported the presence of alkaloids, flavonoids, saponins and other phenolic compounds that contribute to their antioxidant effects. Other studies conducted by Park et al. (2014) which aims to evaluate the antioxidant effect of orange peel extract and flesh with various variations show that the extract with acetone solvent has the best antioxidant effect on both types of extract with IC_{50} value of orange peel extract is 781.9 µg / mL (Park, Lee and Park, 2014; Selvi, Kumar and Bhaskar, 2016).

The level of orange consumption was reversely correlation with the incidence of some diseases. There are several health benefits of sweet orange due to the presence of some bioactive compounds, like phenolate, vitamin c, and carotenoid. However, this fruit was used as a dessert, this fruit is riched by essential oil due to its aromatic compound. Several studies have reported this antioxidant activity against free radicals as a food additive (Guimarães *et al.*, 2010). Based on the information above, this study was aimed to determine the potential of antioxidants from each partition of sweet orange peel methanol extract.

2 RESEARCH METHODS

2.1 Identification of Sample

The sample of sweet orange peel was obtained from the traditional market in Medan followed by an assessment of species identification at Herbarium Medan of North Sumatera University.

2.2 Extraction Process

Extract quality is influenced by several factors such as plant parts used as starting material, solvents used for extraction, extraction procedures, and ratio of plant to solvent. etc. From the laboratory scale to the pilot scale all parameters are optimized and controlled during extraction. Extraction techniques separate plant metabolites that can dissolve through the use of selective solvents (Gupta *et al.*, 2012).

Different solvent systems are available to extract bioactive compounds from natural products. Extraction of hydrophilic compounds using polar solvents such as methanol, ethanol or ethyl acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in a ratio of 1: 1 is used. In some instances, extraction with hexane is used to remove chlorophyll (Sasidharan et al., 2011).

Since the target compound may be non-polar to polar and thermally labile, the appropriateness of the extraction method must be considered. Various methods, such as sonification, heating by reflux, soxhlet extraction and others are usually used for extraction of plant samples. Besides, plant extracts are also prepared by maceration or percolation of fresh green plants or dry powder of plant material in water and/or organic solvent systems (Sasidharan et al., 2011).

Sweet Orange peels were washed, dried and mesh into simplicia powder. Amount of 218.46 gram of simplicia powder macerated using 1 liter of methanol for 24 hours and the residue was re-macerated for the next 2nd and 3rd day using 1 liter of methanol. While the filtrate from each maceration was evaporated using a rotary evaporator at 40oC. The concentrated form of the filtrate was named Methanol Extract of Sweet Orange Peel. The Methanol extract of sweet orange peel was then stratified by using separation funnel.

In the first step of the separation process, 10 gr of methanol extract of sweet orange peels was added and homogenized. The mixture was added 50 ml of n-hexane in the separation funnel then shook at the same time, the air from the funnel was then released slowly. After it formed 2 layers, the polar solvent (lower part) was withdrawn from funnel's stopcock and the hexane layer (upper part) was poured out from the funnel's stopper, at the same time remain polar solvent was separated again by same technique until they become colorless solvent.

In the second step of the separation process against the remaining polar solvent using dichloromethane was done by the same procedure. Finally, we obtained the n-hexane partition, dichloromethane partition and methanol partition (Andersen and Markham, 2006; Zouaghi, Najar and Abderrabba, 2018).

Each partition and ascorbic acid as positive control were dissolved into various concentrations by DMSO. A stock solution was 1,000 µg/mL serially diluted by using DMSO into some concentration including 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL.

The variations of these concentrations were done by making the mother solution first by mixing 50 mg of each fraction of sweet orange peel extract with 50 ml of DMSO so that the mother solution is found with a concentration of 1000 µg / mL. Then the mother solution was successively pipetted as much as 12.5ml, 6.25ml, 2.5 ml, and 1.25 ml into a 25 ml volumetric flask and added a DMSO solution to 25 ml so that a solution concentration of 50 µg / mL, 100 µg / mL, 250 µg was found. µg / mL, and 500 µg / mL. Making variations in the concentration of ascorbic acid as positive control is done in the same way (Park, Lee and Park, 2014).

2.3 H_2O_2 Assay

Hydrogen peroxide (H2O2) scavenging activity has been widely used to determine natural antioxidant activity by measuring H2O2 reduction and detected by spectrophotometer at a wavelength of 230 nm. The main drawback of this method is the possibility, disruption of secondary metabolites that are in the absorbance range. Optimum conditions for determining antioxidant activity by this method at 37oC and pH 7 (Fernando dan Soysa, 2015).

Hydrogen peroxide (40 Mm) solution is prepared in a phosphate buffer (pH 7.4). Samples with different concentrations from each treatment group were added to hydrogen peroxide solution (0.6 ml, 40 Mm). The absorbance of hydrogen peroxide was measured using spectrophotometry at a wavelength of 230 nm with a blank solution in the form of a phosphate buffer without hydrogen peroxide. Percent inhibition by hydrogen peroxide with

Vitamin C (Ascorbic Acid) is calculated by this equation:

$$\% \text{ Inhibition} = [(A0-A1) / A0] \times 100$$

Where A0 is the absorbance of the control, and A1 is the absorbance of the sample and standard groups (Rekha and Bhaskar, 2013).

2.4 DPPH Assay

A fast, simple and inexpensive method for measuring the antioxidant capacity of foods involves the use of free radicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical cleaners or hydrogen donors and to evaluate the antioxidant activity of foods. It has also been used to measure antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to certain antioxidant components, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps to understand the functional properties of food (Shalaby dan Shanab, 2013).

The amount of 1 ml of each fraction and ascorbic acid as a group of the sample were added by 1 ml of 0.05 mM DPPH methanolic followed by incubation in the dark chamber for 30 minutes. The absorbance of the sample was measured at 518 nm and the percent of inhibition was calculated by using the following formulation (2).

$$\text{Percent of Inhibition} = ([a - b]/a) \times 100\%$$

a : absorbance of control
b : absorbance of sample

While the absorbance of control was the same as the group of sample however it was not added by either fraction or ascorbic acid. Those were repeated in triplicate (Selvi, Kumar and Bhaskar, 2016; Okoh *et al.*, 2017).

2.5 Data Analysis

All data analysis in this research is done by software IBM SPSS Statistics 25. Univariate analysis is intended to describe the central tendency and distribution of each variable. The variables in this study include the percent inhibition as an antioxidant potential through percent inhibition and IC_{50} values presented in tables and diagrams. Percent of inhibition was expressed analyzed based on the normality of data distribution. Percent inhibition that distributes normally was expressed as Mean ± SD and analyzed by one way ANOVA followed by Post Hoc test Tukey HSD. Meanwhile, percent inhibition that did not distribute normally was expressed as Median (Min-Max) and analyzed by the Kruskal-Wallis test and followed by the Mann-Whitney test.

3 RESULTS AND DISCUSSION

3.1 Determination of Orange Fruit

The sample of orange sweet fruit was collected from the traditional market in Medan, North Sumatera. The fresh fruit was then identified as a result of plant determination as well as the taxonomy below:

Kingdom: *Plantae*
Division: *Spermatophyta*
Class: *Dicotyledoneane*
Ordo: *Rustales*
Family: *Rutaceae*
Genus: *Citrus*
Species: *Citrus sinensis* (L.)
Local Name: Sweet Orange (*Jerukmanis*)

Citrus sinensis (L.) belongs to Rutaceae family fruit. It was widely consumed among humans over the world that had various health benefits include antioxidants, anti-bacterial, and anti-inflammation. Several types of citrus within the Rutaceae family are grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), and lime (*Citrus aurantifolia*). On the other hand, sweet orange peel is also riched by various content of phytochemicals include alkaloid, flavonoid, tannin, saponin, and steroid (Guimarães *et al.*, 2010; Omodamiro and Umekwe, 2013; Rafiq *et al.*, 2016).

3.2 DPPH Scavenging Activity of Various Fraction from Sweet Orange Peel Methanol Extract

Evaluation of the antioxidant activity by DPPH assay from various Fraction from Sweet Orange Peel Methanol extract was shown as percent inhibition. Due to the difference from data distribution in percent inhibition of hexane fraction and other fraction, then percent inhibition of hexane fraction was analyzed by non-parametric test while the others were the parametric test. The result of the analysis was shown in the Table 1.

Table 1: Percent Inhibition Against DPPH of Various Fraction from Sweet Orange Peel (Citrus sinensis) Methanol Extract and Ascorbic Acid as Positive Control Group

Conc. (µg/ml)	Percent Inhibition (%)			
	MF*	HF**	DF*	AA*
1000	47.22 (46.96-47.96)[a]	53.37 ± 0.90[a]	57.71 (54.39-57.77)[a]	77.38 (76.81-84.15)[a]
500	45.32 (44.91-59.03)[a]	45.15 ± 2.42[b]	47.46 (33.90-56.24)[a,b]	62.55 (61.91-64.87)[b]
200	16.52 (15.11-16.53)[b]	34.74 ± 2.90[c]	40.14 (23.25-46.55)[b]	52.10 (52.03-61.31)[c]
100	12.30 (11.08-13.88)[c]	25.20 ± 3.75[d]	35.53 (25.64-42.21)[b,c]	45.44 (32.03-49.50)[d]
50	8.83 (2.45-9.25)[d]	15.02 ± 0.97[e]	23.48 (22.53-27.69)[c]	15.00 (22.86-34.29)[d]

Conc. = Concentration; MF = Methanol Fraction; HF = Hexane Fraction; DF = Dichloromethane; AA= Ascorbic Acid. * Data was expressed as Median (Min-Max). The different of the small letter in the same column shows significant at $P < 0.05$ by Kruskal-Wallis and Mann-Whitney test. ** Data was expressed as Mean ± SD. The different of the small letter in the same column shows significant at $P < 0.05$ by Post Hoc Test Tukey HSD.

Based on data analysis in the table 1, there was no significant difference in DPPH scavenging among methanol fraction in the concentration higher than 500 µg/ml. Whereas hexane fraction at various concentrations showed a significant difference in DPPH scavenging activity. However, fraction dichloromethane showed a significant difference of percent inhibition in DPPH scavenging among 1000 µg/ml, 200 µg/ml, and 50 µg/ml. As a positive control, ascorbic acid showed a significant difference in DPPH scavenging activity at all concentrations, except at least 2 lower concentrations (100 µg/ml and 50 µg/ml).

Based on literatures, studies on Citrus aurantium (bitter orange) showed that the ethanol and aqueous media were comparatively more effective in extracting the antioxidant components. The total phenol content of the extracts ranged from 2.5 to 22.5 mg/g and 5.0 to 45.0 mg/g of pulp and peel fragments, respectively. The fruit components exhibited proton radical, oxyradical, and hydroxyl radical scaveng- ing abilities and were effective in preventing lipid peroxidation. Their analysis showed positive association between total phenolics and different antioxidant assays (Divya et.el. 2016).

Furthermore, the analysis was followed by linear regression to determine IC_{50} for each group of the sample. The IC_{50} value of each sample as shown in the Table 2.

Table 2: The IC_{50} Value against DPPH of Various Fraction from Sweet Orange (Citrus sinensis) Peels Methanol Extract and Ascorbic Acid as Positive Control.

Group of Sample	Equation	R^2	IC_{50} (µg/ml)
Methanol fraction	Y = 21.537 + 0.036	0.819	790.64
Hexana fraction	Y = 28.712 + 0.030x	0.652	709.60
Dichlorometane fraction	Y = 9.699 + 0.045x	0.738	895.58
Ascorbic acid	Y = 33.564 + 0.050x	0.715	328.72

Based on data in table 2, the lowest IC_{50} value was shown by ascorbic acid (328.72 µg/ml), followed by hexane fraction (709.60 µg/ml), methanol fraction (790.64 µg/ml), and dichloromethane fraction (895.58 µg/ml). The higher the IC_{50} value means the higher the concentration of the sample needed to decompose some of the free radicals tested.

The results of this study indicate that the results contradict with reported by Guimaraes et al. (2010) which states that the polar fraction of Citrus sinensis orange peel contains flavonoids at 0:21 ± 3.97 mg CE/g extract with IC_{50} values against DPPH amounted 0:31 ± 4.99 mg/ml. The difference in the results of this research could be due to the solvent and the way the extraction was carried out, in this study the fractionation process was carried out using separation and by using solvents while the research conducted by Guimaraes et al. (2010) carried out by stirring the orange peel powder samples for 12 hours at 25°C at a speed of 150 rpm using a methanol solvent (Guimarães *et al.*, 2010).

Other research conducted by Ghasemi et al. (2009) on several samples of Citrus sinensis variant extracted by methanol solvent percolation method showed similar results with this study where the total flavonoid content in sweet orange peel with Washinton Navel Variant contained 23.2 mg QE/gr extract powder with IC_{50} against DPPH 1.1 mg/ml whereas, in other variants namely, the variant shows a lower total flavonoid content of 2.1 mg QE/gr

extract powder with IC_{50} to DPPH only 1.7 mg/ml (Ghasemi, Ghasemi dan Ebrahimzadeh, 2009).

As a comparison result, Omodamiro and Umekwe (2013) states that the flavonoid which is owned by the ethanol extract of sweet orange peel with maceration using ethanol in the ratio of 1: 4 (g / ml) for 18 hours showed that the content of total flavonoids of the extract is only 2.5 ± 0:04 % and the extract has antioxidant activity through the inhibition of nitric oxide IC_{50} value of 1100 pg/ml and anti-lipid peroxidation IC_{50} of 1000 ug/ml. Other studies that can be a comparison of IC50 content of several fruit peel samples from the genus Citrus are shown in the Table 3 (Omodamiro dan Umekwe, 2013).

Table 3: Comparison of IC_{50} content of several fruit peel samples from the genus Citrus

Sampel	DPPH (IC_{50}) (µg/ml)
Sour Orange (SO)	742.7
Citrus macrophylla (CM)	946.4
Citrus carrizzo (CC)	985.4
Citrus volkameriana (CV)	585.4
Mandarin Cleopatra (MCL)	934.03
Citumelo swingle (Citru)	1095.1
Lemon rangpur (LR)	782.05
Poncirus trifoliate (PT)	827.49

However, another studies by Park et.al. 2014 reported antioxidant activity of orange (Citrus auranthium) flesh (OF) and peel (OP) extracted with acetone, ethanol, and methanol. Antioxidant potential was examined by measuring -diphenyl-1-picrylhydrazyl (DPPH). The results suggested that acetone is the best solvent for the extraction of antioxidant compounds from OF and OP. Furthermore, the high antioxidant activity of OP, which is a by-product of orange processing, suggests that it can be used in nutraceutical and functional foods.

3.3 H_2O_2 Scavenging Activity of Various Fraction from Sweet Orange Peel Methanol Extract

By assessing the H_2O_2 inhibitory activity of each fractionation result of methanol extract of sweet orange peel (Citrus sinensis (L.)), the data is presented in table 4. The data of H_2O_2 inhibitory activity of each fraction and positive control in the form of ascorbic acid were analyzed for normality in advance by the Shapiro-Wilk test and percent inhibition data owned by methanol extract fraction of sweet orange peel (Citrus sinensis (L.)) and acidic acid. abnormally distributed ascorbate. Then the data analysis continued to assess the differences in percent inhibition held by each concentration in each fraction and positive control. The results of the analysis of differences in each group of positive fractions and controls are shown in Table 4.

Table 4: Percent Inhibition Against H_2O_2 of Various Fraction from Sweet Orange Peel (Citrus sinensis) Methanol Extract and Ascorbic Acid as Positive Control Group.

Conc. (µg/ml)	Percent Inhibition (%)			
	MF*	HF**	DF*	AA*
1000	90.81 (56.12-92.03)[a]	97.42 (89.95-99.45)[a]	68.54 (67.36-69.14)[a]	90.81 (56.12 - 92.03)[a]
500	56.68 (50.72-61.43)[a, b]	95.05 (94.82-95.30)[a]	60.20 (59.66-60.20)[b]	56.68 (50.62 - 61.43)[a]
200	55.09 (55.03-55.26)[b]	53.20 (51.61-53.40)[b]	55.09 (55.03-55.26)[c]	37.95 (37.95 - 42.71)[b]
100	49.63 (49.58-50.16)[c]	49.28 (32.86-49.28)[c]	49.63 (49.58-50.16)[d]	34.26 (31.89 - 34.26)[c]
50	39.29 (38.76-44.21)[d]	45.99 (45.48-47.12)[c]	39.29 (38.76-44.21)[e]	24.47 (24.46 - 26.43)[d]

Conc. = Concentration; MF = Methanol Fraction; HF = Hexane Fraction; DF = Dichloromethane; AA= Ascorbic Acid. * Data was expressed as Median (Min-Max). The different of the small letter in the same column shows significant at $P < 0.05$ by Kruskal-Wallis and Mann-Whitney test. ** Data was expressed as Mean ± SD. The different of small letters in the same column shows significant at $P < 0.05$ by Post Hoc Test Tukey HSD.

Based on the analysis of the sample groups of the methanol, n-hexane, and dichloromethane fractions,

with a difference in percent inhibition of hydrogen peroxide at concentrations ≥ 500 µg/ml, 200 µg/ml, 100 µg/ml, and 50 µg/ml. Whereas in the positive control group in the form of ascorbic acid it was found that the difference in inhibition of hydrogen peroxide was at concentrations ≥ 500 µg/ml, 200 µg/ml, and ≤ 100 µg/ml.

To compare antioxidant activity through percent inhibition of hydrogen peroxide in each fraction and positive control, an analysis was performed using linear regression to determine the IC_{50} of each fraction of sweet orange peel (Citrus sinensis (L.)) and positive control. The statistical analysis of the antioxidants of fractions are presented in Table 5.

Table 5. IC50 Inhibition of Hydrogen Peroxide from Each Sweet Orange (Citrus sinensis (L.)) Skin Fraction and Ascorbic Acid.

Group of Sample	Equation	R^2	IC_{50} (µg/ml)
Methanol fraction	Y = 43.363 + 0.035x	0.669	189.63
Hexane fraction	Y = 44.520 + 0.060x	0.776	91.33
Dichloromethane fraction	Y = 45.824 + 0.024x	0.828	174
Ascorbic acid	Y = 26.634 + 0.55x	0.846	42.48

From the statistical data, it was showed that the IC_{50} value of each fraction of sweet orange peel (Citrus sinensis (L.)) and ascorbic acid as a positive control that the smallest IC_{50} value was 42.48 µg/ml namely ascorbic acid while the most IC_{50} value was in the methanol fraction that is 189.63 µg/ml. So that the antioxidant activity through IC_{50} value inhibition of hydrogen peroxide was found that the most potent antioxidant effect is owned by ascorbic acid as a positive control and the weakest is the methanol fraction. While the antioxidant activity of each orange peel fraction from the strongest to the weakest was the fraction of n-hexane (91.33 µg/ml), dichloromethane (174 µg/ml), and methanol (189.63 µ/ml).

4 CONCLUSION

The most potential hydrogen peroxide inhibition activity was the fraction of n-hexane, followed by dichloromethane, and methanol. While the most potent DPPH inhibitory activity was the n-hexane fraction, followed by the methanol fraction, and the dichloromethane fraction. Hence, the fraction of n-hexane is the best antioxidant potential instead of dichloromethane and methanol. This research result might give valuable preliminary information on the efficacy of Citrus sinensis extracts for pharmacology industry applications.

ACKNOWLEDGEMENTS

This research was fully supported by the Ministry of Research, Technology and Higher Education by Funding Contract No. 7/E/KPT/2019 and No. T/63/L1.3.1/PT.01.03/2019.

REFERENCES

Andersen, O. M. and Markham, K. R. (2006) *Flavonoids. Chemistry, Biochemistry and Applications.*, *Angewandte Chemie International Edition*. DOI: 0-8493-2021-6.

Bander, D. A. (2015) "Free Radicals & Antioxidant Nutrient," in *Harper's Illustrated Biochemistry Thirtieth Edition,*. US: McGraw Hill Education Lange.

Banjarnahor, S. D. S. and Artanti, N. (2014) "Antioxidant properties of flavonoids," *Medical Journal of Indonesia*, 23(4), pp. 239–244. DOI: 10.13181/mji.v23i4.1015.

Bintang, M. (2010) *Biokimia Teknik Penelitian*. Jakarta: Erlangga.

Dewi, S. R., Ulya, N. and Argo, B. D. (2018) "Kandungan Flavonoid dan Aktivitas Antioksidan Ekstrak Pleurotus ostreatus," *Jurnal Rona Teknik Pertanian*, 11(1), pp. 1–11.

Divya, P. J. (2016) "Antioxidant properties of flesh and processed Citrus aurantium fruit," *Cogent Food and Agriculture,* 2:1184119.

Ghasemi, K., Ghasemi, Y. and Ebrahimzadeh, M. A. (2009) "Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues," *Pakistan Journal of Pharmaceutical Sciences*, 22(3), pp. 277–281.

Guimarães, R. *et al.* (2010) "Targeting excessive free radicals with peels and juices of citrus fruits: Grapefruit, lemon, lime and orange," *Food and Chemical Toxicology*. DOI: 10.1016/j.fct.2009.09.022.

Okoh, S. O. *et al.* (2017) "Bioactive constituents, radical scavenging, and antibacterial properties of the leaves and stem essential oils from Peperomia pellucida (L.) kunth," *Pharmacognosy Magazine*, 13(51), pp. 392–400. DOI: 10.4103/pm.pm_106_17.

Omodamiro, O. D. and Umekwe, C. J. (2013) "Evaluation of anti-inflammatory, antibacterial and antioxidant properties of ethanolic extracts of Citrus sinensis peel and leaves," *Journal of Chemical and Pharmaceutical Research*, 5(5), pp. 56–66.

Park, J. H., Lee, M., Park, E. (2014) "Antioxidant activity of orange flesh and peel extracted with various solvents," *Prev. Nutr. Food Sci.* 2014;19(4):291-298.

Prihantini, A. I. and Tachibana, S. (2017) "Uji Aktifitas Antioksidan dari Ekstrak Daun Tilia kiusiana," *Jurnal Faloak*, 1(2), pp. 59–66.

Rafiq, S. *et al.* (2016) "Citrus peel as a source of functional ingredient: A review," *Journal of the Saudi Society of Agricultural Sciences*. The Authors. DOI: 10.1016/j.jssas.2016.07.006.

Rekha, S. S. and Bhaskar, M. (2013) "In Vitro screening and identification of antioxidant activities of orange (citrus sinensis) peel extract in different solvents," *International Journal of Pharma and Bio Sciences*, 4(4), pp. 405–412.

Selvi, S., Kumar, R. Z. A. and Bhaskar, A. (2016) "Phytochemical investigation and in vitro antioxidant activity of citrus sinensis peel extract," *Der Pharmacia Lettre*, 8(3), pp. 159–165.

Shalaby, E. a and Shanab, S. M. M. (2013) "Antioxidant compounds , assays of determination and mode of action," *African Journal of Pharmacy and Pharmacology*. DOI: 10.5897/AJPP2013.

Sies, H. (2015) "Oxidative stress: A concept in redox biology and medicine," *Redox Biology*. Elsevier, 4, pp. 180–183. DOI: 10.1016/j.redox.2015.01.002.

Simaremartye, E. S. (2014) "Skrining Fitokimia Ekstrak Etanol Daun Gatal (Laportea decumana (Roxb.) Wedd)," *Pharmacy*, 11(1), pp. 98–107.

Top 10 causes of death (2018) *WHO*. DOI: 10.1787/9789264306202-graph31-en.

Wardana, A. P. and Tukiran (2016) "Skrining Fitokimia dan Aktvitas Antioksidan Ekstrak Kloroform Tumbuhan Gowok (Syzygium polycephalum)," in *Prosiding Seminar Nasional Kimia dan Pembelajarannya, Jurusan Kimia FMIPA Universitas Negeri Surabaya*, pp. 1–6.

Zouaghi, G., Najar, A. and Abderrabba, M. (2018) "Rootstocks effect on flavonoid content and antioxidant properties of Maltese (Citrus sinensis L .) ethanolic extracts," 49(2), pp. 2956–2960.