Research Article

Antioxidant activity of mangosteen peel (*Garcinia mangostana* L.) extracted using different solvents at the different times

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ABSTRACT

Background: Extraction is conducted to extract the important compounds in mangosteen peel using solvents at the different time. Extraction time needs to be calculated so that the bioactive compound can be extracted optimally using the most efficient solvent. The extraction of mangosteen peel was conducted by soaking in solvent at particular time (1-2 days in general) without heating. **Aim:** This study aims to evaluate the effect of different solvents and maceration time on antioxidant activity of mangosteen peel extract. **Materials and Methods:** The present research was conducted by extracting mangosteen peel using ethanol (EtOH), acetone (Ace), ethyl acetate (EtOAc), methanol (MetOH), hexane (HX), acetic acid (AcetAc) and aquadest (Aqua) at 24, 36 and 48h. Antioxidant activity was examined using visible UV spectrophotometer at the particular wavelength. **Result and Discussion:** The result showed that the type of solvent and extraction time significantly ($P<0.01$) affected antioxidant activity. Acetone extract of mangosteen peel is the most optimal solvent for antioxidant activity in extraction time for 24 hours (IC_{50} = 9,468 + 0,324 ppm). A further test was required to obtain a better result using HPLC method. **Conclusion:** It was evidenced that the properties of solvents, particularly polarity index and time, significantly affected antioxidant activity.

KEY WORDS: Antioxidant activity, Mangosteen peel extract, Solvents, Maceration time

INTRODUCTION

Mangosteen is a seasonal plant which can be found in almost all parts of Indonesia.[1] Nowadays, mangosteen is not only used as food but it is also used as functional food, especially its peel which has the highest level of xanthone compared to the other parts of the fruit.[2] Xanton is a bioactive compound in mangosteen which has many pharmacological properties because it contains very high antioxidant compounds.[3] Antioxidants are needed by the body to prevent free radicals and improve health and immunity.[4]

Antioxidant compounds can be isolated using extraction method with certain solvents[5] in a certain time.[6] Antioxidant compounds in mangosteen peel extract which have been isolated are reflected in antioxidant activity.[7] The testing of antioxidant activity in capturing free radicals can use several methods, but the most common one is diphenylpicrylhydrazyl (DPPH) method.[8] Antioxidant activity in mangosteen peel extract is largely determined by several factors, especially solvents and extraction time, which determine the extraction results.[9] The effectiveness and efficiency of the extraction process are largely determined by the type of solvent used because it contains different polarity indexes.[10] The other results report that DPPH antioxidant capacity in the extraction process is strongly influenced by the extraction time.[11]

The extraction method which will be carried out in this study is maceration method by immersing the sample either in single or mixed solvent with a certain duration (generally between 1 and 2 days) without heating.[12] Until now, there is no study which has been conducted about the effect of various types of solvents and extraction time on the antioxidant activity of the mangosteen peel extract. This study will extract mangosteen peel using seven types of solvents with different polarity indexes in different times. Therefore, this study aims to examine the effect of

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solvent and extraction time on the antioxidant activity of mangosteen peel extract.

MATERIALS AND METHODS

Extracting Mangosteen Peel

Mangosteen was obtained from Puspahiang area in Tasikmalaya Regency. The peels were removed and finely chopped and then oven-dried at 40°C for 24 h. The dried peels were put into a grinder to produce powder and then extracted in several solvents (96% ethanol, acetone, ethyl acetate, methanol, hexane, acetic acid, and aquadest) at 24, 36, and 48 h while being stirred. Extract (product of extraction) was sieved, and the yield filtrate was solidified using rotary evaporator to obtain the thick extract of mangosteen peel. The sample was freeze-dried to obtain mangosteen peel extract.

The Observed Variables

The observed variables in experiment stage 1 were as follows:

Antioxidant activity test

Antioxidant activity test on mangosteen peel extract was applying DPPH method using UV-visible spectrophotometer. The principle of DPPH is observing the reaction of hydrogen scavenge by DPPH from antioxidant compound. Hydrogen atom or the ability to donor electron from the mangosteen peel extract was measured from the vanishing purple color into transparent from DPPH solution in methanol. 6 mg of DPPH was dissolved in 200 mL ethanol to obtain DPPH solution with 30 bpj. Free radical inhibition from DPPH in percentage (I%) was calculated using the formula:

I% = (absorbance without sample – absorbance sample/absorbance without sample) × 100%.

The obtained data of percentage inhibition were plotted on the concentration of sample extract. Inhibition concentration 50% (IC_{50}) value was obtained from the graph of extract concentrate on the DPPH inhibition percentage. IC_{50} indicated the required extract concentrate to inhibit 50% of DPPH free radicals.

Measuring the wavelength of DPPH

Nearly 3 mL of DPPH was dissolved in 2 mL of ethanol, then homogenized, and measured at 400–750 nm wavelength.

Drawing DPPH curve

DPPH solution was diluted into 5, 10, 15, 20, 25, and 30 bpj. DPPH solution of each concentration was let sit for 30 min and measured for the absorbability at the maximum wavelength to draw the standard curve.

Various concentration of mangosteen peel extract

Mangosteen peel extract was diluted at the concentration of 4, 8, 12, 16, and 20 ppm. About 2 mL extract was taken from each concentration and added with 3 mL of DPPH (30 ppm). The solution was then vortexed and let stand for 30 min at room temperature. The absorbability was measured at 512 DPPH wavelength that was obtained from wavelength measurement.

Measuring sample absorbability

About 2 mL of sample with 4, 8, 12, 16, and 20 ppm was added with 3 mL of DPPH, homogenized, and let stand for 30 min at room temperature. Absorbability

Table 1: Mean value of antioxidant activity of mangosteen peel extract extracted using different solvents at different extraction times

Treatment (h)	% Inhibition 4 ppm	% Inhibition 8 ppm	% Inhibition 12 ppm	% Inhibition 16 ppm	% Inhibition 20 ppm	Score IC_{50} (ppm)
EtOH, 24	35.020±0.019^{hi}	42.044±0.021^{gh}	48.021±0.064^{gh}	56.682±0.005^{hi}	64.609±0.017^{g}	12.398±0.134^{b}
EtOH, 36	32.157±0.022^{fgh}	38.847±0.022^{efg}	44.543±0.064^{ef}	53.171±0.007^{fg}	60.646±0.018^{defg}	14.321±0.579^{cde}
EtOH, 48	31.682±0.019^{fgh}	38.130±0.015^{ef}	43.236±0.076^{de}	52.147±0.010^{efg}	59.673±0.017^{cdef}	15.069±0.232^{cdef}
Ace, 24	37.955±0.022^{i}	44.096±0.022^{h}	49.324±0.065^{h}	57.245±0.009^{i}	64.108±0.022^{g}	9.468±0.324^{a}
Ace, 36	35,093±0.023^{hi}	41.537±0.023^{gh}	47.022±0.065^{fgh}	55.333±0.010^{ghi}	62.534±0.022^{efg}	9.668±0.453^{a}
Ace, 48	35.062±0.021^{hi}	41.496±0.021^{gh}	46.973±0.064^{fgh}	54.771±0.008^{ghi}	62.460±0.017^{efg}	9.922±0.469^{a}
EtOAc, 24	30.092±0.033^{def}	37.095±0.033^{de}	43.057±0.069^{de}	52.088±0.026^{efg}	59.914±0.032^{cdef}	14.492±1.991^{cde}
EtOAc, 36	27.636±0.021^{cde}	34.622±0.021^{cd}	40.570±0.064^{cd}	49.580±0.001^{de}	57.386±0.018^{cd}	15.516±1.769^{def}
EtOAc, 48	23.895±0.039^{b}	30.821±0.039^{b}	36.717±0.072^{b}	45.650±0.033^{c}	53.390±0.043^{b}	16.022±1.986^{ef}
MeOH, 24	34.416±0.020^{ghi}	41.034±0.021^{fgh}	46.668±0.064^{fgh}	55.202±0.001^{ghi}	62.598±0.017^{efg}	13.543±0.935^{bc}
MeOH, 36	31,737±0.035^{fgh}	38.922±0.024^{efg}	45.399±0.040^{efg}	53.654±0.012^{def}	59.818±0.011^{cdef}	13.917±0.852^{bcd}
MeOH, 48	30.834±0.021^{efg}	37.618±0.022^{de}	43.393±0.064^{de}	52.141±0.005^{cd}	59.721±0.019^{cdef}	14.484±1.171^{cde}
HX, 24	31.811±0.020^{fgh}	39.101±0.021^{efg}	45.307±0.064^{efg}	54.708±0.001^{ghi}	62.855±0.017^{fg}	15.719±0.529^{def}
HX, 36	25.661±0.020^{bc}	33.385±0.021^{bc}	39.961±0.064^{c}	49.922±0.001^{efg}	58.553±0.017^{cde}	15.953±0.417^{ef}
HX, 48	24.114±0.020^{b}	31.947±0.021^{bc}	38.616±0.064^{bc}	48.718±0.001^{cd}	57.471±0.018^{cd}	16.416±0.554^{f}
AcetAc, 24	33.302±0.021^{fgh}	40.101±0.022^{efg}	45.890±0.064^{efg}	54.659±0.005^{ghi}	62.257±0.017^{efg}	14.356±0.824^{cde}
AcetAc, 36	31.522±0.022^{fgh}	38.187±0.022^{ef}	43.860±0.065^{ef}	52.456±0.010^{efg}	59.903±0.021^{cdef}	14.767±0.966^{cdef}
AcetAc, 48	27.224±0.02^{bcd}	34.038±0.023^{c}	39.839±0.063^{c}	48.627±0.005^{cd}	56.241+0.018^{bc}	15.178±0.886^{cdef}
Aqua, 24	9.958±0.020^{a}	19.073±0.022^{a}	26.833±0.065^{a}	38.589±0.004^{b}	48.776±0.018^{a}	23.218±1.073^{g}
Aqua, 36	9.101±0.020^{a}	18.194±0.021^{a}	25.934±0.064^{a}	37.661±0.001^{ab}	47.823±0.018^{a}	23.941±0.623^{g}
Aqua, 48	8,340±0.018^{a}	18.123±0.010^{a}	24.406±0.057^{a}	35.279±0.033^{a}	47.385±0.018^{a}	24.318±1.021^{g}

Values bearing different superscripts within row show highly significant difference (P<0.01)

was measured at the maximum DPPH wavelength (512 nm) and measured triplet.

Statistical Analysis

The antioxidant activity of mangosteen peel extraction from each treatment was tabulated and analyzed through *t*-test/ANOVA with SPSS 25.0 and continued with DMRT test in case of significant difference.

RESULTS AND DISCUSSION

Antioxidant Activity

Table 1 shows the mean value of the antioxidant activity of different concentrations of mangosteen peel extract using different solvents at different times. Figure 1 shows the trend of the IC_{50} value of mangosteen peel extract.

Table 1 shows the antioxidant activity of several solvents at different extraction times using DPPH method. Antioxidant activity was tested at different concentrations, i.e., 4, 8, 12, 16, and 20 ppm. The higher the concentration, the higher the antioxidant activity. Furthermore, antioxidant activity could be observed from the value of IC_{50}, where IC_{50} indicated the value of extract concentration (ppm) that inhibited 50% of the oxidation. A compound is said to have a very strong antioxidant activity if the IC_{50} is under 100–150 ppm and weak if IC_{50} is 151–200 ppm. Thus, the lower the IC_{50} value, the stronger the antioxidant activity.[10] IC_{50} value was opposite the antioxidant capacity because it expressed the amount of antioxidant to lower 50% of DPPH concentration obtained from interpolation in the analysis of linear regression.[13]

Result revealed that acetone extracted for 24 h resulted in the optimum antioxidant activity (9.468 ± 0.324 ppm) compared to the other solvents and extraction time. Extraction using acetone at 36 and 48 h showed lower yield (9.668 ± 0.453 and 9.922 ± 0.469 ppm) than that of acetone for 24 h. Therefore, the longer the extraction time, the lower the antioxidant activity. It was in line with the previous study that mangosteen peel contained a very strong

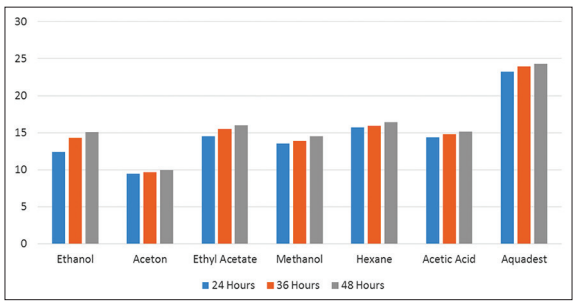

Figure 1: The chart of inhibition concentration 50% value of mangosteen peel extract extracted using different solvents at different extraction times.

antioxidant activity 5.94 µg/mL[14]and similar to BHT, synthetic antioxidant with IC_{50} = 7.5 ppm. Other studies reported that the highest antioxidant was using acetone solvent (33.32 µg/mL) compared to methanol (52.62 µg/mL), ethanol (69.43 µg/mL), and hexane (181.21 µg/mL).[15]

The result of antioxidant activity indicated the amount of active antioxidant compound and the reduction of free radicals. It was evidenced that antioxidant compound was positively correlated with the quality of phenolic compound.[16] Phenolic compound in plant shows biological properties as primer antioxidant or free radicals terminator. Antioxidant has demonstrated to contribute hydrogen from phenolic hydroxyl group and break the chain of free radical oxidation to form the stable end product which does not initiate or spread oxidation.[15,17] DPPH radicals are stabilized by scavenging hydrogen from the hydroxyl group on phenolic compound. The obtained data showed that the extract scavenged free radicals and acted as the primer antioxidant to react with the free radicals by donating hydrogen.[15]

Acetone extract showed the highest antioxidant compared to the other solvents in mangosteen peel extract with IC_{50} <10 ug/mL. Acetone was effective to extract herbal materials, but it left residue.[10] On the contrary, ethanol known as the optimum solvent for extraction and contained lower toxicity[18] with IC_{50}value was 7,48 µg/mL.[19] Other studies observed that methanol extract of Buni fruit contained higher oxidant activity than the other solvents.[20] Based on the result, mangosteen peel extract with the highest antioxidant activity was obtained from the polar alcohol-based solvent.[21]

Ethyl acetate in this study (14.492 ± 1.991 ppm) was moderately optimum because it could restrain the sample by breaking the cell membrane of the herbs and improve the extraction of endothelium cells.[22] Antioxidant activity value from hexane extract was poor (15.719 ± 0.529 ppm) because hexane was non-polar solvent. It indicated that the active constituent in mangosteen had a moderate polarity and, therefore, easy to be extracted by acetone as the solvent.[15] The effectivity and efficiency of the extraction process were greatly influenced by the condition of extraction, polarity of the solvent, temperature, and extraction time.[23] The polarity of the solvent was directly correlated in extraction because it increased the solubility of antioxidant compound.[24,25] A solvent with higher polarity significantly increased phenolic compound and antioxidant activity.[26,27] It indicated that the extraction yield increase was linear with the polarity of the solvent in the extraction.

The polarity index of the solvents in the study was 5.2% in ethanol, 5.1% in acetone, 4.4% in ethyl acetate,

5.1% in methanol, 0.0% in hexane, 6.2% in acetic acid, and 9.0% in aquadest.[28] Polar solvents demonstrated a better performance to extract xanthone compared to the non-polar solvents due to the general principle of solubility "like dissolve like,"[20] in which the bioactive compound is more soluble in polar solvent compared to non-polar solvent. This study observed an anomaly where mangosteen peel extract using water solvent was not the highest antioxidant capacity. It may due to the different structure of the extracted phenolic which had a much lower hydroxy group (-OH); therefore, it was more difficult to donate hydrogen atom because of the higher activation energy compared to the phenolic extracted using different solvents. Hydroxy group in the antioxidant properties plays a role in the electron transfer to stabilize free radicals. The more hydroxy group in the antioxidant properties, the more electrons to be donated to stabilize free radicals. Furthermore, several components such as alkaloid and antioxidant vitamins play a role in the anomaly.

Figure 1 shows that most antioxidant properties of the mangosteen peel extract powder were highly degradable and damaged due to excessive extraction. Antioxidant and bioactive compound were abundant on day 10 extraction and declined on days 15 and 20.[29] It was in line with the previous study that antioxidant is a highly oxidized compound. Patras *et al*.[30] emphasized that degradation might occur due to oxidation, covalent bond breaking, or increasing oxidation rate. The oxidized antioxidant compounds would be damaged and incapable to donate electron to neutralize the free radicals. In general, the mangosteen peel powder extracted for 24 h contained the highest antioxidant activity. The longer the time of extraction, the lower antioxidant capacity which was generated by tannin reduction during extraction process. The depleting antioxidant activity was due to the loss of anthocyanin, ascorbic acid, and total phenolic.[31]

The other studies reported that the increase of antioxidant capacity of apple extract DPPH was linear with the time of extraction.[11,32] Extraction method conducted in a prolonged time generally coexisted with the increase of anthocyanin in grapes, while the other studies did not find an evident correlation between the time of extraction and Anthocyanin content. The cause might be the fixation of the solid properties and reduction in transparent form.[29]

CONCLUSION

The types of solvent and extraction time resulted in a highly significant effect ($P < 0.01$) on testing antioxidant activity. Acetone level of mangosteen peel extracted for 24 h showed the optimum antioxidant activity compared to the other treatments. It was evidenced that the properties of solvents, particularly polarity index and time, significantly affected antioxidant activity.

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