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A STUDY OF ANTIOXIDANT PROPERTIES FROM *GARCINIA MANGOSTANA* L. PERICARP EXTRACT

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Abstract. The present work was undertaken to assess the radical scavenging and antioxidant properties of the mangosteen (*Garcinia mangostana* L.) pericarp extracts obtained by various solvents and its mixtures of different polarity. The extracts were evaluated by FRAP (Ferric reducing antioxidant power), ABTS (2,2-azinobis (3-ethylbenzothiazoline--6-sulfonicacid) diammonium salt], DPPH (1,1-diphenyl-2-picrylhydrazyl), reducing power and chelating ability on ferrous ion. Among the different extracts studied, ethyl acetate and acetone extract were observed to have good antioxidant activity. The FRAP values for ethyl acetate and acetone extract were 1.30 and 1.01 mM TEAC respectively at 1 mg/ml. The TEAC values for ABTS assay were 38.21 and 38.15 μM respectively at 100 μg/ml. The IC_{50} values to quench the DPPH free radicals of these two extracts were 30.01 μg/ml and 33.32 μg/ml, but the chelating assay was observed to be better in aqueous fractions of Acetone: water (80:20) at 12 mg/ml. From the above results it can be inferred that ethyl acetate and acetone were suitable solvent to extract the antioxidant compounds form mangosteen.

Key words: mangosteen pericarp, xanthones, total antioxidant capacity, free radical scavenging

INTRODUCTION

Free radicals produced as a result of normal biochemical reactions in the body are implicated in various human diseases; therefore interest in reactive oxygen species (ROS) has substantially increased in direct relation to cellular abnormalities. The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) play an important role in scavenging oxidants and preventing injury to cellular macromolecules. However, quantities of ROS which overwhelm the capacity of the body's defense system may result in irreversible oxidative damage to DNA, proteins and lipids causing cellular and metabolic injury and accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation.

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Thus, supplementation of antioxidants and phytochemicals has attracted considerable attention due to their functional activity. Incorporation of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propyl gallate (PG) to foods is under strict regulation due to the potential health hazards caused by such compounds [Maisuthisakul et al. 2007].

Epidemiological studies have shown that fruits and vegetables rich in antioxidants can have complementary mechanism of action, including scavenging of free radicals, antibacterial and antiviral effects, stimulation of the immune system, regulation of cell proliferation and apoptosis [Etherton et al. 2002]. Therefore in recent years, considerable attention has been paid to plant sources as antioxidants.

In the present study, we investigated the antioxidant properties of the various solvent extracts of *Garcinia mangostana* L. pericarp. Mangosteen belongs to the family Guttiferae a tropical evergreen tree distributed in Thailand, India, Srilanka, Myanmar, Indonesia, Malaysia, Philippines and China. In the recent years mangosteen pericarp has received much attention as it contains high amounts of xanthones. Xanthones and its derivatives have been reported to have neuroprotective activity [Weecharangsan et al. 2006], antiulcer [Suksamrarn et al. 2003], and antioxidant activities [Yu et al. 2007, Jung et al. 2006], antimalarial [Nakatani et al. 2002], antimicrobial [Moongkarndi et al. 2004], antiacne activities [Mahabusarakam et al. 2000], cytoprotective activity [Kosema et al. 2007], anticancer [Akao et al. 2008] and histamine and serotonin receptor blockers [Chairungsrilerd et al. 1996].

Though there are many literatures on antioxidant activity of mangosteen the objective of our study was to find the most appropriate solvent which can extract active constituents from mangosteen pericarp and to evaluate the antioxidant activity. Standard methods like FRAP, ABTS, DPPH, reducing and chelating assay are used to measure the antioxidant activity. The results were compared with different conventional antioxidant like ascorbic acid, α-tocopherol, Trolox and BHA.

MATERIALS AND METHODS

Chemicals and equipments

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Aldrich (Steinheim, Germany), butylated hydroxyanisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, α-tocopherol, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS), 2,4,6-tris[2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma (St. Louis Mo, USA). Folin-Ciocalteu reagent, ferrous chloride, potassium ferricyanide were from Loba Chemie (Mumbai, India), anhydrous sodium acetate, acetic acid, hydrochloric acid, anhydrous sodium carbonate, iron (II) sulfate heptahydrate, L-ascorbic acid, potassium persulfate, ethylenediamine tetra-acetic acid (EDTA), trichloroacetic acid (TCA), and ferric chloride were from Merck (Mumbai, India). All other reagents were of analytical grade.

UV-visible spectrophotometer UV-160A, Shimadzu, Japan; Rota vapor RE111 Buchi, Switzerland; Soxtec system HT2 1045, Foss Tecator, Sweden.

Preparation of the extracts

The pericarp of mangosteen was tray dried at 40-52°C and ground into powder using hammer mill equipment. The fine powder (5 g) was extracted using Soxtec apparatus with various solvents. The solvent systems used were ethyl acetate (EtOAc), hexane (HX), acetone (Ace), and acetone: water (80:20; AW), methanol (MeOH) and ethanol (EtOH). Extractions were carried out for 2 h that include initial boiling for 30 min. After filtering the extract through Whatman No. 1 paper, each of the filtrates was concentrated using rota evaporator at 40°C, the weight of the each extract was noted and the final volume was made up to 25 ml in a volumetric flask. The extract were kept in air-tight amber bottles after flushing with nitrogen gas for 30 s and stored in freezer at –20°C until they were analysed.

Estimations

The moisture content of the mangosteen pericarp powder was determined by toluene distillation method [ASTA 1995]. The total sugar was estimated by phenol-sulphuric acid method [Dubois et al. 1956]. The yield of the evaporated dried extracts based on dry weight basis (%db) was calculated from the equation:

$$\text{Yield (\%)} = \frac{W_1}{W_2} \cdot 100,$$

where W_1 was the weight of extract after evaporation of solvent and W_2 was the dry weight of the pericarp powder.

Determination of total phenol content

Total phenol was determined by Folin-Ciocalteu reagent and was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight basis of the extract [Kujala et al. 2000].

Determination of the antioxidant activity by FRAP assay

The ferric reducing antioxidant power assay was carried out by using modified method of Benzie and Szeto [1999]. The FRAP solution, consist of 300 mM acetate buffer adjusted to pH 3.6, 20 mM ferric chloride hexahydrate dissolved in distilled water, 10 mM TPTZ dissolved in 40 mM HCl. The samples concentrations in the range of 0.02-1 mg/ml was made up to 3 ml by acetate buffer and incubated for 30 min. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Trolox was used to perform the calibration curve and the results were expressed as mM/mg Trolox.

ABTS cation radical-scavenging assay

The total antioxidant activity of the extracts was measured by the method of Re [1999]. Stock solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate and kept in dark at room temperature for 12-16 h before use to generate

the ABTS cation chromophore. The mixture was diluted with absolute ethanol to obtain an absorbance of ~0.700 ±0.01 at 734 nm. An aliquot of the extracts (10-100 μg/ml) in ethanol was added to 3 ml of ABTS reagent and was allowed to stand at 23°C the absorbance was taken after 6 min. The results were expressed μM/μg Trolox.

DPPH radical scavenging activity

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable radical DPPH accordingly to the method of Wang [2003]. The extracts in methanol at a concentration ranging from 20-400 μg/ml were mixed in the freshly prepared 0.5 mM DPPH in ethanol and 0.1 M acetate buffer (pH 5). Absorbance at 517 nm was determined after 30 min. The scavenging activity was calculated using the following equation.

$$\text{Scavenging activity}\,(\%) = \left[\frac{(\text{A}_{517}\text{of control} - \text{A}_{517}\text{of sample})}{\text{A}_{517}\ \text{of control}}\right]\cdot 100$$

The percentage of scavenging activity was plotted against the sample concentration to obtain IC_{50}, defined as the concentration of sample necessary to cause 50% inhibition.

Reducing power assay

The reducing power of the extracts was determined by the method described by Yen et al. [2000]. Different concentrations of the extracts (0.06-1 mg/ml) were mixed with phosphate buffer (0.2 mM, pH 6.5), ferric chloride solution (2 mM) and potassium ferricyanide (4 mM). To this, 100 mg/ml trichloroacetic acid was added to the reaction mixture and was made up to 1ml with water and incubated at 37°C for 10 min. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Test for ferrous ion chelating ability

Ferrous ion chelating activity was measured according to the method of Suter and Richter [2000] with minor modifications. The reaction mixture containing ferrous chloride (200 μM) and potassium ferricyanide (400 μM) without or with extracts (1-12 mg/ml) was made upto 1 ml with water and the reaction mixture was incubated at 20°C for 10 min. Formation of the potassium hexacyanoferrate complex was measured at 700 nm. Lower absorbance of the reaction mixture indicated higher iron chelating capacity. The control was without any chelating compound or test sample. The percent ferrous iron chelating effect was calculated from the following equation.

$$\text{Chelating effect}\ \ (\%) = \left(1 - \frac{\text{A}_{\text{sample (700 nm)}}}{\text{A}_{\text{control (700 nm)}}}\right)\cdot 100$$

Statistical analysis

Results are expressed as the means ±SD of three replicates. One-way analysis of variance (ANOVA) was used to determine the statistical difference. Statistical significance was $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

The moisture content of the dried pericarp powder was found to be 7 ±0.02%. Table 1, shows the yield of the extracts calculated on the dry weight basis in order to eliminate the influence of moisture content of the plant material. The higher yield in solvent extract with aqueous fraction may be due to the extraction of carbohydrates and flavanoids which occur mostly as glycosylated derivatives.

Table 1. Extraction yield, total phenols, total sugars in different extracts of mangosteen pericarp

Extract	Yield %db*	Polyphenol content mg GAE/g	Sugar content mg/g
EtOAc	15.4 ±0.024	269.9 ±0.02	0.035 ±0.015
HX	07.7 ±0.094	135.9 ±0.03	0.032 ±0.012
Ace	12.0 ±0.065	205.2 ±0.02	0.019 ±0.011
AW (80:20)	22.0 ±0.072	229.6 ±0.08	0.054 ±0.014
MeOH	18.8 ±0.056	315.7 ±0.01	0.129 ±0.023
EtOH	14.8 ±0.074	431.0 ±0.07	0.164 ±0.017

*Dry weight basis of original sample of pericarp.
The average values of three calculations are presented as mean ±S.D. (standard deviation).

Plant phenolics have multiple biological effects as they constitute one of the major groups of compounds acting as primary antioxidant or free radical terminator. From Table 1, we could infer EtOH (431 mg GAE/g), MeOH (315 mg GAE/g) and EtOAc (269 mg GAE/g) extract showed the highest phenolic content. The total phenolic content of these extracts were significantly higher than the non-polar solvent hexane (135 mg GAE/g). AW extract (80:20) had slightly higher phenolics (222 mg GAE/g) than the organic acetone (205 mg GAE/g), which may be due to the presence of water-soluble phenols. The results showed that the extract contained a mixture of phenolic compounds at different levels according to the polarity of solvent used in the extraction process.

The FRAP assay measures the Fe^{3+}/Fe^{2+} couple reducing ability of a complex matrix to a blue complex tripridyl triazine (Fe^{2+}/TPTZ); by the action of electron donating antioxidant. Results were calculated on the basis of Trolox, a water soluble analogue of vitamin E. Higher absorbance indicated higher activity, as the concentration of the extracts increased the intensity of the blue colour increased indicating the formation Fe^{2+}/TPTZ from colourless oxidized Fe^{3+} form, from Figure 1 we could infer all the extracts showed Fe^{3+} reduction, EtOAc extract showed strongest antioxidant property of 1.24 mM at 0.4 mg/ml (data not shown) and reached a maximum of 1.30 mM at 1 mg/ml, followed by Ace extract 1.01 mM. EtOH and MeOH at 1 mg/ml were within the range of 0.725-0.675 mM. The AW (80:20), HX extract at the same concentration was in the range of 0.636-0.623 mM respectively. The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process [Tachakittirungrod et al. 2007].

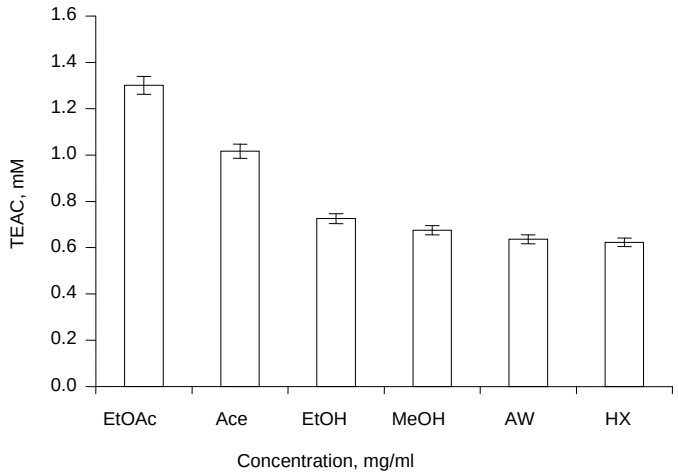

Fig. 1. Comparison of Ferric reducing antioxidant power of different extract of mangosteen pericarp: HX – hexane, EtOH – ethanol, MeOH – methanol, AW – acetone:water (80:20), Ace – acetone, EtOAc – ethyl acetate

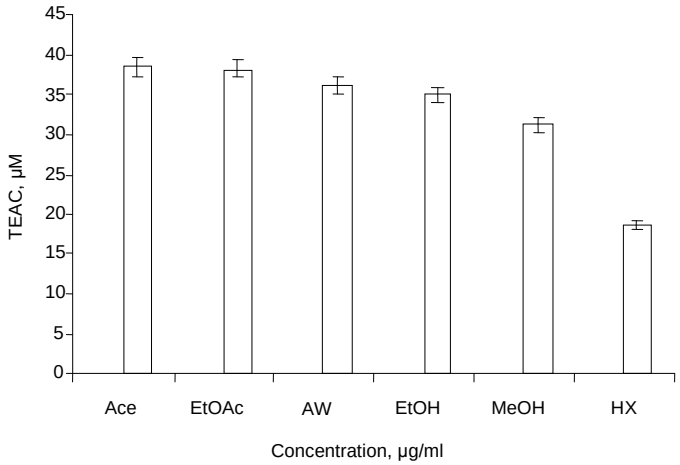

Fig. 2. Comparison of Trolox equivalent antioxidant capacity of mangosteen pericarp extract by ABTS method: HX – hexane, EtOH – ethanol, MeOH – methanol, AW – acetone:water (80:20), Ace – acetone, EtOAc – ethyl acetate

The ABTS method gives the measure of the antioxidant activity determined by the decolorization of the $ABTS^{\bullet+}$ through measuring the reduction of the radical cation at 734 nm. The extent of inhibition of $ABTS^{\bullet+}$ was plotted as a function of concentration in order to determine the TEAC (Trolox equivalent antioxidant capacity). As shown in Figure 2, Ace and EtOAc extract at a TEAC of 38 µM completely decol-

ourised the blue green $ABTS^{\bullet+}$ solution followed by AW extract which had a TEAC of 36.20 μM at 100 μg/ml. EtOH and MeOH extract showed maximum TEAC activity of 34.95 μM and 31.25 μM at 100 μg/ml. HX a non-polar solvent showed the least activity of 18.64 μM at 100 μg/ml. It was observed that higher the TEAC value of the sample, the stronger was the antioxidant activity. This is an excellent method for determining the antioxidant activity of a broad diversity of substances, such as hydrogen-donating antioxidants or scavengers of aqueous phase radicals and of chain-breaking antioxidants or scavengers of lipid peroxylradicals [Re et al. 1999].

DPPH radical scavenging activity represents the free radical reducing activity of the extract based on one electron reduction. The scavenging potential was compared with known antioxidants, such as BHA and α-tocopherol. Each of these antioxidants showed an IC_{50} of 14.23 and 18.01 μg/ml respectively. The IC_{50} (Fig. 3) of EtOAc (30.01 μg/ml), Ace (33.32 μg/ml), AW (50.45 μg/ml) MeOH (52.62 μg/ml), EtOH (69.43 μg/ml) extract showed the maximum radical scavenging activity. Although HX (181.21 μg/ml) extract showed good radical scavenging activity it showed lower IC_{50} compared to pure solvent extracts. The antioxidants are believed to donate hydrogen from the phenolic hydroxyl groups and break the free radical chain of oxidation forming a stable end product, which does not initiate or propagate further oxidation [Sherwin 1978]. The DPPH radicals get stabilized by accepting the hydrogen donated by the hydroxyl groups present on the phenolic compounds. The data obtained show that the extracts are free radical scavengers and may act as primary antioxidants, which can react with free radicals by donating hydrogen.

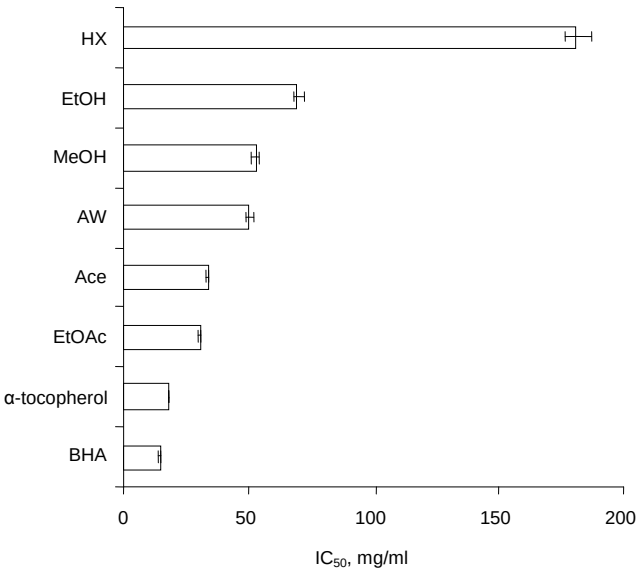

Fig. 3. Comparison of IC_{50} value of different extracts of mangosteen pericarp by DPPH assay: BHA – butyl hydroxyanisole, α-tocopherol, HX – hexane, EtOH – ethanol, MeOH – methanol, AW – acetone:water (80:20), Ace – acetone, EtOAc – ethyl acetate

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [Rice-Evans et al. 1995]. The reducing power increased with the increasing dosage of the extracts. All the extracts showed significantly higher activities than the control sample but lower than standard L-ascorbic acid. L-ascorbic acid (20-100 μg/ml) which is a well known reducing agent showed maximum reducing value of 2.2 (data not shown) at 60 μg/ml and remained constant on further increase in the concentration. As shown in Figure 4, the Ace extract had a value of 2.11 at 1mg/ml indicating good activity, EtOAc, EtOH and MeOH reached the maximum heavy metal reducing capacity of 1.84, 1.69 and 1.47 at 1 mg/ml. The absorbance of the other extracts were of the order AW > HX (1.35 > 1.18) at 1 mg/ml. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

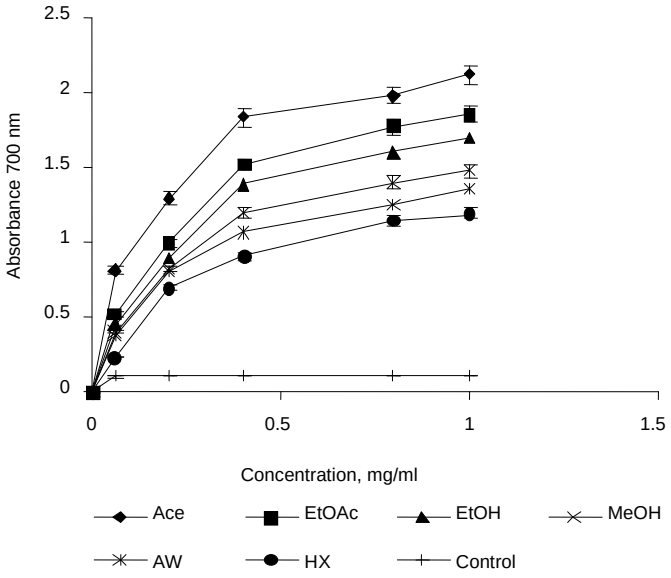

Fig. 4. Comparison of reducing power of mangosteen pericarp on iron ion

Iron is an extremely reactive metal and will catalyze oxidative changes in lipid, proteins, and other cellular components. In addition, lipid peroxidation induced by Fenton reaction iron is a reactant hence, the ability of the mangosteen pericarp extracts to chelate iron was studied. Figure 5, shows the chelating effect increased with increasing concentration of the extracts; of all the extracts tested the strongest chelating effect was of the order; AW (50.62%) > MeOH (38.14%) > HX (31.86%) > EtOH (22.43%) > Ace (19.88%) > EtOAc (17.75%) at 12 mg/ml EDTA (20-100 μg/ml) which is well-known chelator showed maximum chelation of 95% at 50 μg/ml and remained constant (data not shown). The results indicate the activity increased in aqueous fractions with the progression of hydrophilicity. The solvent extract showed poor chelation on ferrous ion suggesting that they minimized the concentration of ferrous ions to lesser extent.

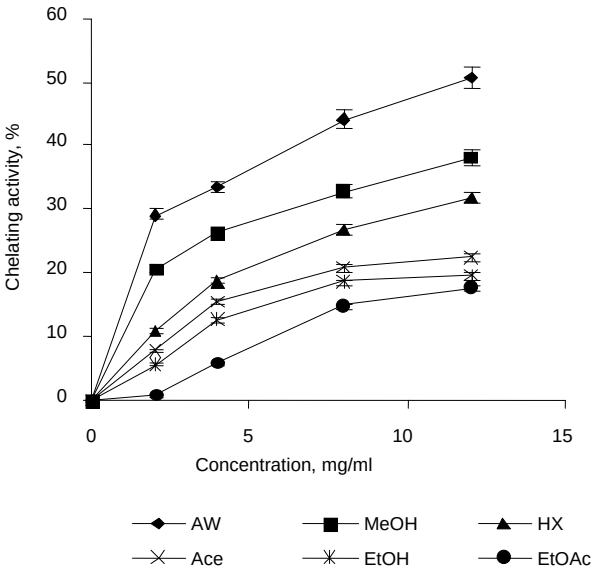

Fig. 5. Chelating effects of mangosteen pericarp extracts on Fe^{2+}

In the above studies ethyl acetate and acetone extract showed good antioxidant activity except in the chelating assay, though the hexane extract showed good radical scavenging property it could not match the antioxidant activity of the other extracts. This may be due to the variation of distribution of xanthone in different extracts. Since the extraction yield and antioxidant potential of the compounds in the extract is strongly dependent on the solvent polarity [Marinova and Yanishlieva 1997]. The poor antioxidant activity of hexane extract could be attributed due to its non-polar nature. The results indicate the active constituents in mangosteen have intermediate polarity hence can be easily extracted by ethyl acetate and acetone as extraction solvent.

Antioxidant properties of single extract within same group can vary in such a way that the same levels of phenolics do not necessarily correspond to the same antioxidant responses; thus, the radical scavenging activity of an extract cannot be predicted on the basis of its total phenolic content [Ka¨hko¨nen et al. 1999]. However, our results showed a weak correlation between antioxidant activities and total phenolic contents, similar results were also observed by Ka¨hko¨nen et al. [2001] and Lafka et al. [2007]. Extracts are very complex mixtures of many different compounds with distinct polarity as well as antioxidant and prooxidant properties, it also depends on their chemical structure of the compound present in these extract. On the basis of the data in this study there is likelihood that mangosteen pericarp extracts may provide the nutritional and health benefits in reducing oxidative stress.

CONCLUSIONS

To sum up, this work highlights the importance of mangosteen which has been traditionally used in an indigenous medicine as a rich source of antioxidant. Seven extracts were carried using various solvent of different polarity. The extraction of mangosteen peicarp in acetone or ethylacetate extract showed maximum antioxidant activity and free radical scavenging activities *in vitro* conditions. In addition, the antioxidant activity was found to be different for different kinds of solvent extracts. From the above antioxidant assay it could be concluded that the extracts are a good primary antioxidant and showed much less secondary antioxidant property as inferred by chelating assay. As the interest in antioxidant has increased, so have the number of methods for measuring their activity. Over all comparison of the results among different assay method is encouraging. These results support the possibility that the extracts can contribute to protective effects on human health and to the current growing pharmaceutical and food industries.

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OCENA WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCYCH EKSTRAKTÓW OWOCÓW MANGOSTANU WŁAŚCIWEGO – SMACZELINY (*GARCINIA MANGOSTANA* L.)

Streszczenie. W pracy podjęto próbę oznaczenia aktywności przeciwrodnikowej i przeciwutleniającej ekstraktów owoców mangostanu właściwego – smaczeliny (*Garcinia mangostana* L.) uzyskanych na drodze ekstrakcji rozpuszczalnikami o różnej polarności. Ocena obejmowała oznaczenie FRAP, $ABTS^{+\cdot}$, $DPPH^{\cdot}$, siły redukującej oraz zdolności chelatowania jonów żelaza. Spośród badanych ekstraktów największą aktywność przeciwutleniającą stwierdzono w próbach ekstrahowanych octanem etylu i acetonem. Wartości oznaczeń FRAP dla stężenia 1 mg/ml ekstraktów octanu etylu i acetonu wynosiły odpo-

wiednio: 1,30 i 1,01 mM TEAC, natomiast dla oznaczeń ABTS wynosiły 38,21 i 38,15 mM. Stwierdzono, że wartości IC_{50} zmiatania rodników DPPH obu wymienionych ekstraktów wynosiły odpowiednio 30,01 i 33,32 μ/ml. Analiza wyników dotyczących zdolności chelatowania jonów żelaza wskazała na większą aktywność frakcji wodnej ekstraktów aceton – woda (80:20). Na podstawie uzyskanych wyników badań można stwierdzić, że zarówno octan etylu, jak i aceton są odpowiednimi rozpuszczalnikami wykorzystanymi do ekstrakcji związków przeciwutleniających z owocni mangostanu.

Słowa kluczowe: owocnia mangostanu, ksantony, całkowita pojemność przeciwutleniająca, zmiatanie wolnych rodników

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