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REVIEW

In vitro antioxidant properties of mangosteen peel extract

Weerayuth Suttirak · Supranee Manurakchinakorn

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Abstract The growing interest in the replacement of synthetic food antioxidants by natural ones has fostered research on the screening of plant-derived raw materials for identifying new antioxidants. The special attention of research today is focused on inexpensive or residual sources from agricultural industries. Fruit peels as sources of powerful natural antioxidants are often the waste parts of various fruits from consumption and food industry. Among the fruit peels, mangosteen peel is an important source of natural phenolic antioxidants. The mangosteen peel contains various bioactive substances, i.e., phenolic acids and flavonoids, which possess biological and medicinal properties, especially antioxidant properties. The aim of this review, after presenting analytical techniques for determining in vitro antioxidant activity of mangosteen peel extract, is to summarize available data on the factors affecting antioxidant activity of mangosteen peel extract. In addition, the potential antioxidant activity of mangosteen peel extract, the bioactive compounds identified from mangosteen peel extract and their antioxidant activity are presented. Potential applications of the mangosteen peel extract in food, pharmaceutical and cosmetic products are also discussed.

Keywords *Garcinia mangostana* L. · Antioxidant activity · Bioactive compounds · Fruit peel

W. Suttirak
School of Pharmacy, Walailak University,
Nakhon Si Thammarat 80161, Thailand

S. Manurakchinakorn (✉)
School of Agricultural Technology, Walailak University,
Nakhon Si Thammarat 80161, Thailand
e-mail: msuprane@wu.ac.th

Introduction

Oxidation reaction is a process where electrons are transferred from one atom to another, with the molecule losing an electron being oxidized. In the food systems, lipid oxidation is a major cause of food quality deterioration because it leads to the development of rancid odors and flavors, with a consequent decrease in nutritional quality, safety and shelf life caused by the formation of potentially toxic oxidation products (Maisuthisakul et al. 2007). The addition of antioxidants is required to retard the deterioration of food quality. Among the synthetic types, the most frequently used food additives are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), though carcinogenic effects of these antioxidants in the experimental animals are reported (Hocman 1988; Ito et al. 1983; Williams 1986). In addition, some synthetic antioxidants require high manufacturing costs but show lower efficiency than natural antioxidants. Currently, consumers have increasing demand for natural antioxidants due to concern about safety of the synthetic antioxidants. Consequently, discovering natural antioxidants from inexpensive and residual sources is the most necessary research for food industry.

Plants are recognized as major sources of natural antioxidants. They contain a variety of substances called "phytochemicals" (Céspedes et al. 2010). The antioxidant phytochemicals include flavonoids and other polyphenols. Among various parts of plant, fruits are important as a naturally antioxidant source. Interestingly, the peel fractions of some fruits, such as longan, orange, banana, mango, pineapple (Guo et al. 2003) and mangosteen (Fu et al. 2007; Naczk et al. 2011), have higher antioxidant activity than the pulp fractions. Antioxidant compounds have been identified in the peel of pomegranate (Devatkal et al. 2012; Iqbal et al. 2008), mango (Ajila et al. 2007), citrus (Oboh and Ademosun 2011; Singh et al. 2011; Zia-ur-Rehman 2006;), banana (Devatkal et

al. 2011; González-Montelongo et al. 2009), longan (Prasad et al. 2009), dragon fruit (Wu et al. 2006), rambutan (Okonogi et al. 2007), sapodilla (Devatkal et al. 2011) and mangosteen (Zadernowski et al. 2009).

Mangosteen (*Garcinia mangostana* L.) is known as "the queen of fruits" because it is one of the best tasting tropical fruits. The edible aril of mangosteen is milky white, while the peel is dark red and composes about two times of the edible portion (Fu et al. 2007; Zarena and Udaya Sankar 2012). The traditional use of mangosteen is manifold. Mangosteen is mainly consumed fresh as dessert. However, the production of wine, preserves, jam and puree from the aril are further traditional food applications (Bin Osman and Milan 2006). Processing of mangosteen generally involves separating the desired aril from the peel considered as waste. Recently, the mangosteen fruit extract is popularly used as a food supplement, while the fruit peel extract has been used in herbal medicines. The mangosteen peel has been reported to contain a variety of bioactive compounds with potential applications as therapeutic agents or as functional food additives such as phenolic acids (Zadernowski et al. 2009), tannins (Pothitirat et al. 2009), xanthones (Zarena and Udaya Sankar 2009b), anthocyanins (Palapol et al. 2009) and other bioactive compounds. These isolated compounds have various biological effects such as antioxidant (Jung et al. 2006), antimicrobial (Suksamrarn et al. 2002) and anti-inflammatory (Chen et al. 2008) activities. The objective of the present work is to review the methods for determining antioxidant activity of mangosteen peel extract with respect to its bioactive components. Factors influencing antioxidant property and the potential uses of the mangosteen peel extract are also included.

Methods for determining antioxidant activity of mangosteen peel extract

Antioxidants are substances that can delay or prevent the oxidation process by inhibiting the initiation or propagation of oxidation chain reaction. Antioxidants act through several mechanisms: free radical scavenging, chelating of metal ion, reducing activity and quenching of singlet oxygen. Antioxidants can be classified according to their protective properties at different stages in the oxidation process and since they act by different mechanisms, they are divided into two main types of antioxidants, i.e., primary and secondary antioxidants. Primary antioxidants can inhibit or retard oxidation by scavenging free radicals (i.e. reactive oxygen species, e.g. hydroxyl, superoxide, nitric oxide, thiyl and peroxyl) by donating of hydrogen atom or electron, which convert their radicals to more stable products. Secondary antioxidants display a function by many mechanisms including scavenging oxygen and hydrogen peroxide, absorbing UV radiation, deactivating singlet oxygen or binding of metal ion (Maisuthisakul et al. 2007; Shahidi and Naczk 1995; Tachakittirungrod et al. 2007).

In general, antioxidant assays are classified as single electron transfer and hydrogen atom transfer based assays. Selection of methods for evaluating antioxidant property depends on the oxidation sources. Thus, many analytical methods are necessary to evaluate different antioxidant effects. As shown in Table 1, the antioxidant properties of mangosteen peel extract can be divided into four groups including free radical scavenging, chelating ability, reducing power and lipid oxidation inhibitory ability based on the antioxidant measurements. The antioxidant activity of mangosteen peel extract has been reported using the following methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2-azinobis(3-ethylbenzothia-zoline-6-sulfonic acid) diammonium salt (ABTS) cation radical, superoxide anion radical ($O_2^{\bullet-}$), nitric oxide radical ($^{\bullet}NO$) and peroxynitrite ($ONOO^-$) scavenging activity, the deoxyribose assay, hydroxyl radical ($^{\bullet}OH$) scavenging activity, oxygen radical absorbance capacity (ORAC), ferrous ion (Fe^{2+}) chelating activity, ferric reducing antioxidant power (FRAP), phosphomolybdenum method, cytochrome c reducing capacity, ferric thiocyanate method (FTC) and thiobarbituric acid (TBA) assay.

1) The measurement techniques of free radical scavenging abilities

DPPH radical scavenging activity measures the antioxidant ability for donating hydrogen atom to capture the DPPH radical ($DPPH^{\bullet}$). As the $DPPH^{\bullet}$ are quenched by antioxidant, the color of DPPH solution changes from a deep purple to a light yellow and the absorbance at 515 nm decreases. (Bondet et al. 1997; Lim et al. 2007; Surveswaran et al. 2007). ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity measures the relative ability of antioxidant to scavenge the $ABTS^{\bullet+}$ similar to the DPPH method. In this analysis, the $ABTS^{\bullet+}$ are generated by reaction of a strong oxidizing agent (potassium persulfate) with the ABTS. The reduction of $ABTS^{\bullet+}$ concentration (decreased blue-green color of the solution) occurs by donating hydrogen atom to quench the $ABTS^{\bullet+}$, which can be measured by the absorption spectrum at characteristic wavelength (734 or 414 nm) (Surveswaran et al. 2007; Villaño et al. 2004). DPPH and ABTS assays are effective and frequently employed methods for evaluating the radical scavenging activity of antioxidant compounds because they are rapid and simple measurement techniques. Considering the occurrence of radical compound of both assays, the $ABTS^{\bullet+}$ are generated by reaction of a strong oxidizing agent with the ABTS whereas $DPPH^{\bullet}$ need not to be generated (Kedare and Singh 2011; Re et al. 1999; Villaño et al. 2004). It is revealed that $DPPH^{\bullet}$ is more stable than $ABTS^{\bullet+}$. However,

Table 1 Analytical methods used to assess antioxidant activity of mangosteen peel

Assay	References
Scavenging of radical	
1,1-diphenyl-2-picrylhydrazyl radical scavenging activity	Yoshikawa et al. (1994) Kosem et al. (2007) Maisuthisakul et al. (2007) Okonogi et al. (2007) Surveswaran et al. (2007) Yu et al. (2007) Pothitirat et al. (2009) Zarena and Udaya Sankar (2009a)
2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity	Okonogi et al. (2007) Surveswaran et al. (2007) Tachakittirungrod et al. (2007) Zarena and Udaya Sankar (2009a)
Superoxide anion radical scavenging activity	Kosem et al. (2007) Yu et al. (2007)
Nitric oxide radical scavenging activity	Kosem et al. (2007)
Peroxynitrite scavenging activity	Jung et al. (2006)
The deoxyribose assay	Zarena and Udaya Sankar (2009c)
Hydroxyl radical scavenging activity	Garcia et al. (2005) Kosem et al. (2007) Yu et al. (2007) Chin et al. (2008)
Oxygen radical absorbance capacity	Fu et al. (2007)
Chelating of metal ions	
Ferrous ion chelating activity	Zarena and Udaya Sankar (2009a) Kosem et al. (2007)
Reducing activity	
Ferric reducing antioxidant power	Kosem et al. (2007) Surveswaran et al. (2007) Zarena and Udaya Sankar (2009a)
Phosphomolybdenum method	Zarena and Udaya Sankar (2009b)
Cytochrome c reducing capacity	Zarena and Udaya Sankar (2009b)
Scavenging of lipid oxidation products	
Ferric thiocyanate method	Yoshikawa et al. (1994) Garcia et al. (2005) Kosem et al. (2007) Yu et al. (2007)
Thiobarbituric acid assay (TBA)	Zarena and Udaya Sankar (2009b)

DPPH$^{\bullet}$ can only be dissolved in organic media (especially in alcoholic media), not in aqueous media, which is an important limitation when interpreting the role of hydrophilic antioxidants (Arnao 2000). On the other hand, ABTS$^{\bullet+}$ can be solubilized in both aqueous and in organic media and is not affected by ionic strength, so the antioxidant activity can be measured due to the hydrophilic and lipophilic nature of the compounds (Arnao 2000). Floegel et al. (2011) reported that the high-pigmented and hydrophilic antioxidants were better reflected by ABTS assay than DPPH assay. $O_2^{\bullet-}$ scavenging activity is measured by electron spin resonance (ESR) spin-trapping technique. In the ESR spin-trapping, the $O_2^{\bullet-}$ generators are trapped by spin-trapping (5,5-dimethyl-1-pyrroline N-oxide, DMPO) to form the spin adducts (DMPO-OOH). The ESR signal intensities are dependent on the amount of DMPO-OOH, that measured by the ESR spectrometry under the specific condition (Kosem et al. 2007; Shimamura et al. 2007). $^{\bullet}NO$ scavenging activity is based on the amount of nitrite ion (NO_2^-). The $^{\bullet}NO$ generator (sodium nitroprusside) spontaneously generates $^{\bullet}NO$, which interacts with oxygen to produce NO_2^- and the subsequent NO_2^- reacts with Griess reagent. The reaction products are measured at 550 nm (Amano and Noda 1995; Kosem et al. 2007). $ONOO^-$ scavenging activity is determined by monitoring the oxidation of dihydrorhodamine 123 (DHR 123). In this assay, DHR 123 is oxidized by native $ONOO^-$ and $ONOO^-$-derived from the peroxynitrite donor 3-morpholinosydnonimine hydrochloride (SIN-1). The oxidized DHR 123 is evaluated by the luminescence spectrometer on the excitation and emission wavelengths at 480 and 530 nm, respectively. The $ONOO^-$ scavenging activity relates to the fluorescence intensities of oxidized DHR 123 (Shin et al. 2005; Zou et al. 2005). The deoxyribose assay measures the scavenging of $HO^{\bullet}$. This assay consists of deoxyribose, hydrogen peroxide (H_2O_2), ferric ion (Fe^{3+}), ethylene diamine tetraacetic acid (EDTA), reducing agent (ascorbate) and thiobarbituric acid (TBA). The deoxyribose is attacked by $HO^{\bullet}$, which is generated by the reaction of H_2O_2 and Fe^{2+}-EDTA complex. During heating with TBA at low pH, deoxyribose- $HO^{\bullet}$ complex is changed to a pink chromogen, which can be measured by the absorbance at 535 nm (Halliwell et al. 1987; Zarena and Udaya Sankar 2009c). ORAC contains a peroxyl radical ($RO_2^{\bullet}$) induced by 2, 2'-azobis(2-amidinopropane) dihydrochloride and the fluorescent marker protein, i.e., fluorescein. This method is based on the inhibition of $RO_2^{\bullet}$-induced oxidation of the fluorescein. The loss of fluorescence of fluorescein indicates the $RO_2^{\bullet}$ -induced free radical damage. The antioxidant efficiency is determined by assessing the area under the fluorescence decay curve of the sample as compared to that of the blank in which no antioxidant exists (Huang et al. 2002; Prior et al. 2003).

2) The measurement technique of chelating ability

The metal chelating ability is an important antioxidant property. This technique evaluates the ability of substances to reduce the concentration of free transition metals ion that may act as a catalyst to generate the free radical to initiate the oxidation reaction. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Ferrous ion (Fe^{2+}) is the most powerful pro-oxidant among the various species of metal ions (Gülçin et al. 2004). The Fe^{2+}-chelating activity test is one of the most effective methods for measuring the chelating ability of antioxidant compounds because Fe^{2+} is an important catalyst for Fenton reaction and hydrogenperoxide decomposition that are cause of many diseases (Jayasri et al. 2009; Lim et al. 2007; Liu et al. 2010; Yeh et al. 2011). The Fe^{2+} chelating activity measures the ability of antioxidant to combine with Fe^{2+}, whereas residual Fe^{2+} can combine with particular compounds (ferrozine or potassium ferricyanide) to form the complex compounds (Ferrozine-Fe^{2+} or potassium hexacyanoferrate), which can be measured using absorbance at specific wavelength (562 or 700 nm) (Kosem et al. 2007; Zarena and Udaya Sankar 2009a). The Fe^{2+} chelating activity is calculated by Ferrozine-Fe^{2+} or potassium hexacyanoferrate absorbance which decreasing in absorbance indicates strong chelating ability.

3) The measurement techniques of reducing ability

FRAP measures antioxidant power with the assistance of an oxidant, i.e., Fe^{3+}. In the FRAP assay, the antioxidants reducing the Fe^{3+}-tripyridyltriazine complex at low pH causes the formation of a blue colored Fe^{2+}-tripyridyltriazine complex. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing standard antioxidant in known concentration (Surveswaran et al. 2007; Zarena and Udaya Sankar 2009a). Phosphomolybdenum method is based on the reduction of molybdate (Mo) ion from Mo^{6+} to Mo^{5+} by the action of electron donating antioxidants and the subsequent formation of a green phosphate-Mo^{5+} complex at acid pH, which can be measured at 695 nm (Prieto et al. 1999; Zarena and Udaya Sankar 2009b). The cytochrome c reducing capacity measures ferrocytochrome c quantity at 550 nm, due to its active heme group containing a Fe^{2+}, which has a characteristic spectrum at 550 nm. In this assay, cytochrome c is oxidized by oxygen saturated phosphate buffer to ferric cytochrome c, thereafter the antioxidants reduce ferric cytochrome c to ferrocytochrome c (Zarena and Udaya Sankar 2009b).

4) The measurement techniques of lipid oxidation inhibitory abilities

FTC measures the amount of peroxide produced during the initial stage of the lipid oxidation. The FTC assay consists of ammonium thiocyanate and Fe^{2+} in acidic solution. H_2O_2 induced by lipid oxidation oxidizes Fe^{2+} to Fe^{3+}, resulting in the formation of a red colored Fe^{3+}-thiocyanate complex. The levels of oxidation are determined by measuring the absorbance at 500 nm (Kosem et al. 2007; Yu et al. 2007). On the other hand, TBA measures the amount of malondialdehyde (MDA) produced during the second stage of the lipid oxidation. This method is based on the MDA reaction with thiobarbituric acid to obtain a red pigment, resulting from the condensation of two molecules of TBA with one molecule of MDA. The levels of oxidation are determined by measuring the absorbance at 535 nm (Fernández et al. 1997; Zarena and Udaya Sankar 2009b).

Factors affecting antioxidant activity of mangosteen peel extract

The quality of mangosteen peel extract and its antioxidative performances depends not only on the quality of mangosteen peel but also environmental and technological factors affecting the activities of antioxidants from mangosteen peel.

1) Maturation stage

Total bioactive compound content and antioxidant activity were found to be different in various maturation stages of mangosteen fruit (Pothitirat et al. 2009). The young fruit peel (green peel) contained higher total phenolic (42.57 g gallic acid equivalents/100 g) and total tannin contents (51.25 g tannic acid equivalents/100 g) than the mature fruit peel (dark reddish purple peel) (28.88 g gallic acid equivalents/100 g and 36.66 g tannic acid equivalents/100 g), whereas the mature fruit peel had higher total flavonoid content (4.08 g quercetin equivalents/100 g) than the young fruit peel (2.91 g quercetin equivalents/100 g). The results suggest that total phenolic and total tannin contents decreasing during the maturity stage of the mangosteen fruit may be indicated by the reduction of a bitter yellowish resin. In addition, total flavonoid content increasing during the maturity stage of the mangosteen fruit is indicated by dark purple color of anthocyanin accumulation. Congruently, it was found that total anthocyanin content (167, 278, 509, 1,111, 1,824 and 4,235 g/kg for stage 1–6, respectively) increased continuously during maturation and reached a maximum value (4,235 g/kg) at fully mature stage (Palapol et al. 2009). In consideration of antioxidant activity, the young fruit peel possessed about two-fold higher free radical scavenging activity (EC_{50}=5.56 μg/ml) than the mature fruit peel (EC_{50}=10.94 μg/ml) (Pothitirat et al. 2009). The higher antioxidant activity of the young fruit peel may be resulted from the higher total phenolic and total tannin contents. The results possibly indicate that the tannin present in the mangosteen peel mainly acts through the mechanism of free radical scavenging.

2) Extraction solvent

Solvent is more frequently used for isolation of antioxidant. Both extraction yield and antioxidant activity of the extract are strongly dependent on the solvent, due to the different antioxidant potential of compounds with different polarity. Various solvents have been used for extraction bioactive compounds from mangosteen peel as shown in Table 2. Furthermore, efficiency of the different extraction solvents has also been reported. Zarena and Udaya Sankar (2009a) demonstrated the antioxidant activities of mangosteen peel extracts obtained by various solvents, which are different polarities. The extraction of mangosteen peel in ethyl acetate and acetone showed higher free radical scavenging activity (38 and 38 μM Trolox equivalents for $ABTS^{\bullet+}$ scavenging activity, IC_{50}=30.01 and 33.32 μg/ml for $DPPH^{\bullet}$ scavenging activity, respectively) and reducing power (1.84 and 2.11, respectively), although lower chelating activities (17.75 % and 19.88 %, respectively) of the extracts were detected. In contrast, the 80 % acetone extract exhibited higher chelating activity (50.62 %) but showed lower free radical scavenging activity (36.20 μM Trolox equivalents for $ABTS^{\bullet+}$ scavenging activity, IC_{50}=50.45 μg/ml for $DPPH^{\bullet}$ scavenging activity) and reducing power (1.35) under the same condition. The results may be due to the different polarities of the extraction solvents. In general, 80 % acetone possesses higher polarity than acetone and ethyl acetate, respectively, resulting in the extraction of phenolic compounds with higher polarity in the 80 % acetone extract, compared with acetone and ethyl acetate extracts. The 80 % acetone extract exhibited the highest chelating activity because the chelating efficiency is ionized ability-dependent (Shahidi and Naczk 1995). In addition, the correlation between antioxidant activity and total phenolic content (TPC) were investigated (Suttirak and Manurakchinakorn 2009). The 50 % ethanol extract exhibited the highest TPC (152.52 g gallic acid equivalents/kg), $DPPH^{\bullet}$ (590.18 mmole ascorbic acid equivalents/kg) and $ABTS^{\bullet+}$ scavenging activity (727.48 mmole ascorbic acid equivalents/kg), and FRAP (491.23 mmole ascorbic acid equivalents/kg) followed by the 80 % (142.17 g gallic acid equivalents/kg, 525.79, 719.25 and 453.19 mmole ascorbic acid equivalents/kg for TPC, $DPPH^{\bullet}$ $ABTS^{\bullet+}$ scavenging activity and FRAP, respectively) and 100 % ethanol extracts (63.11 g gallic acid equivalents/kg, 181.62, 336.42 and 177.55 mmole ascorbic acid equivalents/kg for TPC, $DPPH^{\bullet}$ $ABTS^{\bullet+}$ scavenging activity and FRAP, respectively), and distilled water extract (16.05 g gallic acid equivalents/kg, 77.45, 103.85 and 117.25 mmole ascorbic acid equivalents/kg for TPC, $DPPH^{\bullet}$ $ABTS^{\bullet+}$ scavenging activity and FRAP, respectively), respectively (Fig. 1). The results suggest that 50 % ethanol extract is a preferred solvent for extracting $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging agents, and reducing agent from mangosteen peel. In addition, the major antioxidants present in mangosteen peel possess polarity between water and ethanol under the experimental conditions (Suttirak and Manurakchinakorn 2009). The above data indicate that the extraction solvent greatly affect the yield and antioxidant activity of mangosteen peel extract. However, the economic point of view and the treatment of generated effluents must be considered.

Table 2 The solvents for extraction bioactive compounds from mangosteen peel

Extraction solvents	References
Ethyl acetate	Bunsiri et al. (2003); Dangcham et al. (2008); Zarena and Udaya Sankar (2009a); Zarena and Udaya Sankar (2009b)
Dichloromethane	Chin et al. (2008)
Hexane	Asai et al. (1995); Zarena and Udaya Sankar (2009a); Zarena and Udaya Sankar (2009b)
Acetone	Asai et al. (1995); Ji et al. (2007); Zarena and Udaya Sankar (2009a); Zarena and Udaya Sankar (2009b)
70 % acetone	Fu et al. (2007)
80 % acetone	Zarena and Udaya Sankar (2009a)
Methanol	Yoshikawa et al. (1994); Suksamrarn et al. (2002); Jung et al. (2006); Kosem et al. (2007); Zarena and Udaya Sankar (2009a)
70 % methanol	Asai et al. (1995) ; Yu et al. (2007)
80 % methanol	Garcia et al. (2005); Surveswaran et al. (2007); Zadernowski et al. (2009)
80 % ethanol	Ketsa and Atantee (1998)
95 % ethanol	Maisuthisakul et al. (2007); Okonogi et al. (2007); Tachakittirungrod et al. (2007); Pothitirat et al. (2009)
Ethanol	Huang et al. (2001); Destandau et al. (2009); Zarena and Udaya Sankar (2009a)
Supercritical carbon dioxide	Zarena and Udaya Sankar (2009c); Zarena and Udaya Sankar (2011)
Benzene	Asai et al. (1995)
Methanol:HCl (99:1)	Palapol et al. (2009)

3) Material preparation

Mostly, mangosteen peel must be treated with drying prior to extraction of bioactive compounds or even storage to extend its shelf life. Suvarnakuta et al. (2011) reported that drying methods (hot-air drying, vacuum drying and low-pressure superheated steam drying) and conditions (60, 75 and 90 °C) immensely affected the xanthones content (i.e., α-mangostin and 8-deoxygartanin) and free radical scavenging activities of mangosteen peel extract. This research found that the amounts of xanthones and their free radical scavenging activities markedly decreased after all drying runs. Losses of xanthones after drying might be caused either by enzymatic or thermal degradations.

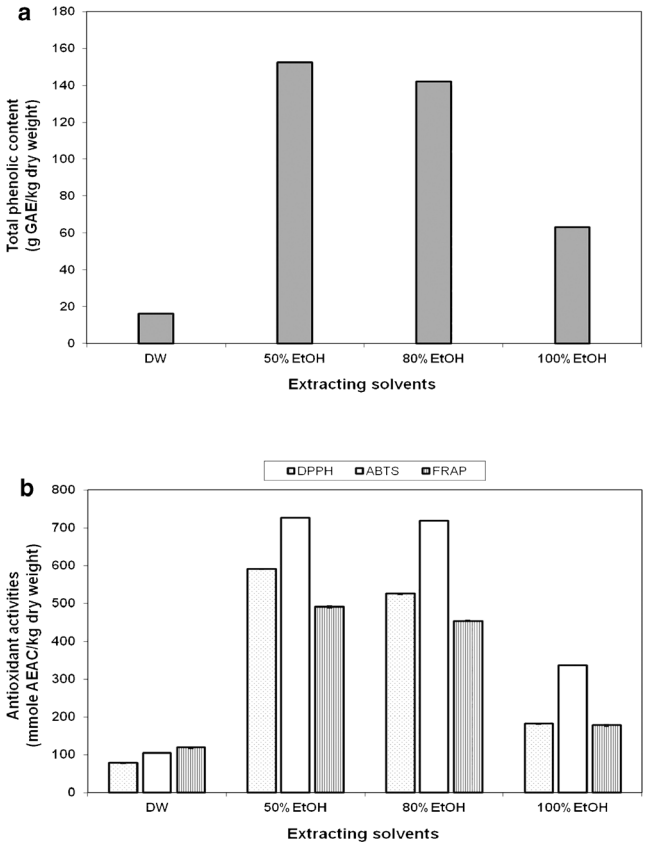

Fig. 1 Total phenolic content **a** and antioxidant activities **b** of mangosteen peel extract. DW stands for distilled water. EtOH stands for ethanol. GAE stands for gallic acid equivalents. AEAC stands for ascorbic acid equivalent antioxidant capacity. DPPH and ABTS stand for 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) scavenging activity. FRAP stands for ferric reducing antioxidant power. Vertical bars represent standard deviation of the mean (n=3). All means are significant difference at $P \leq 0.05$ (Suttirak and Manurakchinakorn 2009)

Degradative enzymes such as polyphenol oxidase (PPO) which is normally present in plant materials might contribute towards the degradation of xanthones in the early stage of drying, prior to their inactivation by the thermal treatment. On the other hand, degradation of xanthones could be continuously affected by the heat throughout the drying period. Losses of free radical scavenging activities after drying resulted from the degradation of xanthones. Hot-air drying (% retention of α-mangostin and 8-deoxygartanin= 78.1 and 72.7, respectively) or low-pressure superheated steam drying (% retention of α-mangostin and 8-deoxygartanin=78.3 and 72.3, respectively) at 75 °C is recommended to maximize the quantity and quality of xanthones in mangosteen peel extract because these drying methods involve the use of proper drying time and temperature to help inactivate PPO and also minimize thermal degradation of xanthones. Although other preparation processes of mangosteen peel may affect the antioxidant activity of the peel extract, no supportive research has been revealed.

Antioxidant efficiency of mangosteen peel extract

Efficiency of mangosteen peel extract for controlling oxidative reaction under in vitro condition has been reported, compared with other fruit peel extracts and commercial antioxidants. In the radical scavenging activity model system, mangosteen possessed better antioxidant activity than persimmon (Maisuthisakul et al. 2007), limonia (Surveswaran et al. 2007), banana, coconut, dragon fruit, long-gong (Okonogi et al. 2007; Tachakittirungrod et al. 2007), passion fruit (Okonogi et al. 2007) and kaffir lime (Tachakittirungrod et al. 2007). Correspondingly, the mangosteen peel extract exhibited higher reducing power on FRAP assay than limonia peel extract (Surveswaran et al. 2007). Surprisingly, the mangosteen peel extract in supercritical fluid carbon dioxide ($SC\text{-}CO_2$) showed inhibitory activity on lipid oxidation similar to α-tocopherol (Zarena and Udaya Sankar 2009c). Additionally, the higher effective treatments for retarding lipid oxidation than BHA were detected in the acetone and ethyl acetate extract (Zarena and Udaya Sankar 2009b). The results indicate that the higher antioxidant activity of mangosteen peel extract may be resulted from the variation in the quality and quantity of bioactive compounds present in the mangosteen peel extract.

Main bioactive substances of mangosteen peel

Many studies have been conducted to isolate and identify the main bioactive substances of mangosteen peel by several methods such as gas chromatography with mass spectrometry (GC-MS) (Zadernowski et al. 2009), high performance liquid chromatography with electrospray ionization mass spectrometry (HPLC-ESI-MS) (Zarena and Udaya Sankar 2011; Zarena and Udaya Sankar 2012; Zhou et al. 2011) and coupling centrifugal partition chromatography with ESI-MS (CPC-ESI-MS) (Destandau et al. 2009). The chemical compositions of mangosteen peel have been reported to contain complex phenolic compounds. Phenolic acid and flavonoid have been extensively studied by various research groups and they have shown the remarkable biological activity for many products. Consequently, the isolated compounds from mangosteen peel are discussed.

Ten phenolic acids were identified in mangosteen peel (Zadernowski et al. 2009). The inner and outer mangosteen peel contained six and eight phenolic acids, respectively. Of these, protocatechuic acid (3,4-dihydroxybenzoic acid) (Fig. 2a) was the major phenolic acid in the both inner and outer peels. *p*-coumaric acid and 3,4-dihydroxymandelic acid were only found in the inner mangosteen peel, whereas *m*-hydroxybenzoic acid, veratric acid, caffeic acid and ferulic acid were only detected in the outer mangosteen peel. Regarding flavonoids groups, i.e.,

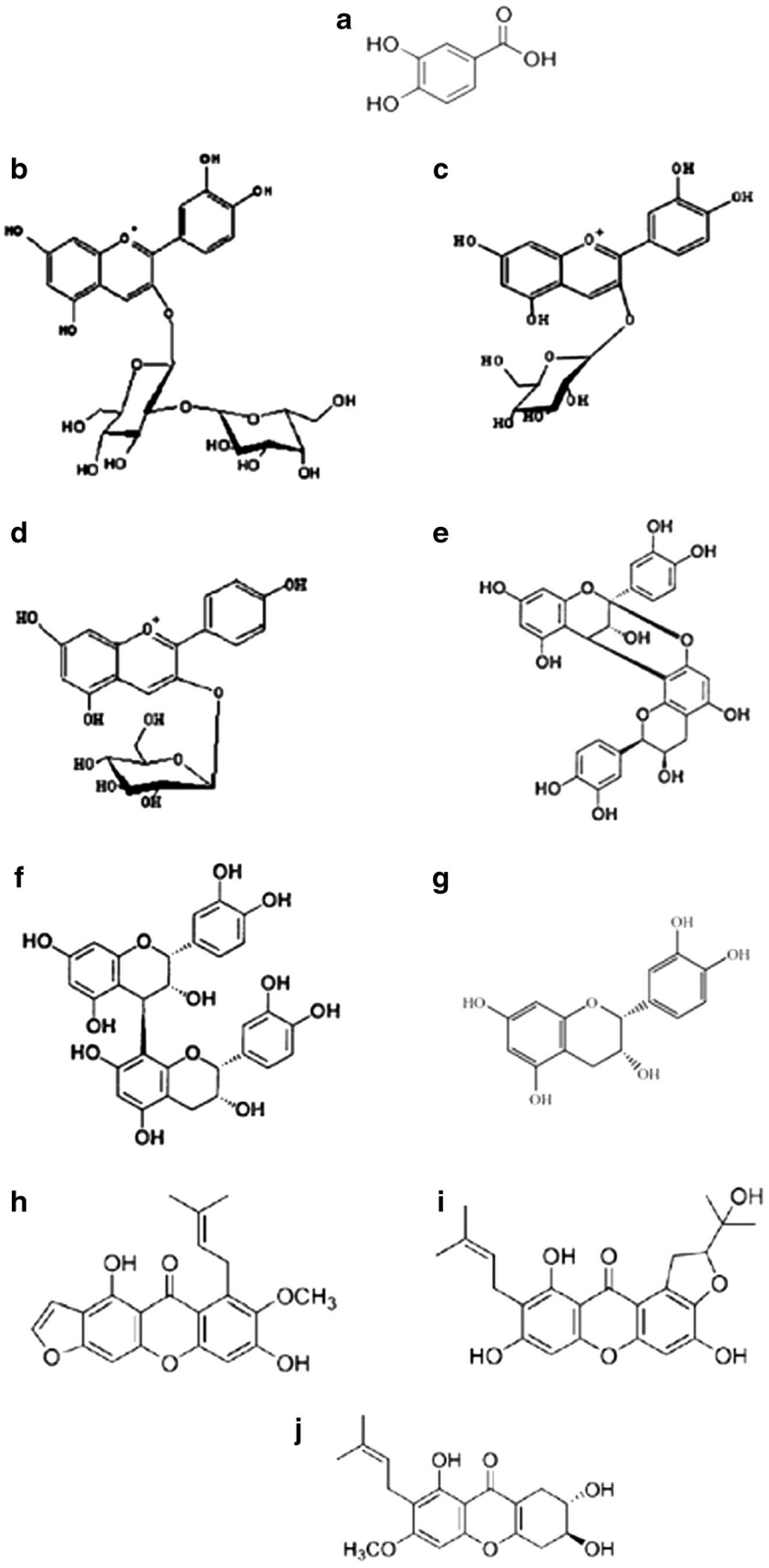

Fig. 2 Chemical structures of isolated compounds from mangosteen peel: protocatechuic acid **a**, cyanidin-3-sophoroside **b**, cyanidin-3-glucoside **c**, pelargonidin-3-glucoside **d**, procyanidin A-2 **e**, procyanidin B-2 **f**, (−)-epicatechin **g**, garcimangosxanthone A **h**, garcimangosxanthone B **i** and garcimangosxanthone C **j** (modified from Yoshikawa et al. 1994; Zhang et al. 2010; Zarena and Udaya Sankar 2012)

anthocyanins, proanthocyanidins, (−)-epicatechin and xanthones were noticed in the mangosteen peel. Anthocyanins are the main compounds for indicating the maturation stages of mangosteen. Cyanidin-3-sophoroside (Fig. 2b) and cyanidin-3-glucoside (Fig. 2c) were identified as the main anthocyanins in the mangosteen peel (Du and Francis 1977). Furthermore, the changes of these anthocyanins during mangosteen development are reported. The concentration of cyanidin-3-sophoroside and cyanidin-3-glucoside increased according to increasing of mangosteen maturation. In addition, other anthocyanins (cyanidin-glucoside-pentoside, cyanidin-glucoside-X, cyanidin-*X*2 and cyanidin-X, X denotes a residue of m/z 190 which is unified atomic mass units) were identified in the mangosteen peel of mangosteen (Palapol et al. 2009). Recently, pelargonidin-3-glucoside (Fig. 2d) was identified from the mangosteen peel. The molecular formula of this compound was $C_{21}H_{21}O_{10}$. In addition, the quantities of three anthocyanins were reported. Cyanidin-3-sophoroside was the largest amount (76.1 %) followed by cyanidin-3-glucoside (13.4 %) and pelargonidin 3-glucoside (6.2 %), respectively (Zarena and Udaya Sankar 2012). Fu et al. (2007) reported that the high quantity of anthocyanins in the peel related to the occurrence of proanthocyanidins or condensed tannins (Chen et al. 2009; Schwartz et al. 2007). Correspondingly, procyanidin A-2 (Fig. 2e) and procyanidin B-2 (Fig. 2f) were demonstrated as active principle compounds in the mangosteen peel (Yoshikawa et al. 1994). (−)Epicatechin (Fig. 2g) is one of the most flavan-3-ols (Shahidi and Naczk 1995), which are predominant monomeric unit of mangosteen proanthocyanidins; consequently, they are often found together in the mangosteen peel (Yoshikawa et al. 1994; Yu et al. 2007). On the other hand, the isolated xanthones from mangosteen peel are the most studied by several research groups. Fifty-two isolated xanthones from mangosteen peel were reviewed (Chin et al. 2008; Pedraza-Chaverrí et al. 2008). Recently, garcimangosxanthone A (Fig. 2h), garcimangosxanthone B (Fig. 2i) and garcimangosxanthone C (Fig. 2j) were identified from the mangosteen peel. The molecular formula of these compounds were $C_{21}H_{18}O_6$, $C_{23}H_{24}O_7$ and C_{19},$H_{22}O_6$, respectively (Zhang et al. 2010). Currently fifty-five isolated xanthones from mangosteen peel have been reported.

Antioxidant activity of bioactive compounds from mangosteen peel

Effectiveness of oligomeric proanthocyanidins (OPCs) isolated from mangosteen peel for scavenging $RO_2^{\bullet}$ were reported, compared with Trolox (6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid) and commercially available sources. The mangosteen OPCs are excellent $RO_2^{\bullet}$ scavengers, showing higher ORAC value (1.7×10^4 μmol Trolox equivalents/g) than grape seed proanthocyanidins (1.0×10^4 μmol Trolox equivalents/g), pine bark proanthocyanidins (7.5×10^3 μmol Trolox equivalents/g) and Trolox (4.0×10^3 μmol Trolox equivalents/g) (Fu et al. 2007). The results may be due to the compositional difference and the difference of the degree of polymerization of the mangosteen proanthocyanidins, compared with other proanthocyanidins from different sources. In addition, the mangosteen OPCs contain (−)epicatechin as the predominant monomeric unit, reported as a better free radical scavenger (Fu et al. 2010; Schwartz et al. 2007; Yu et al. 2007). (−)Epicatechin is a polyphenol and the presence of two OH substitutions at the ortho position of the phenolic ring results in higher antioxidant activity. α-Mangostin was the first xanthone, which was isolated from mangosteen peel (Pedraza-Chaverrí et al. 2008) and has been reported as an antioxidant. Wittenauer et al. (2012) showed that α-mangostin was the major xanthone in mangosteen peel which was about 69 % of the total amount of xanthones. α-Mangostin was able to scavenge in a concentration-dependent way singlet oxygen (IC_{50}=4.6 μM), $O_2^{\bullet-}$ (IC_{50}=24.8 μM) and $ONOO^-$ (IC_{50}=23.3 μM). However, α-mangostin was unable to scavenge $^{\bullet}OH$ and H_2O_2 (Guzmán-Beltrán et al. 2008; Pedraza-Chaverrí et al. 2009). Chin et al. (2008) revealed that γ-mangostin was only found to be active for $HO^{\bullet}$ scavenging assay (IC_{50}=0.20 μg/ml) when compared with fifteen xanthones (IC_{50}>10 μg/ml). Similarly, γ-mangostin possessed $DPPH^{\bullet}$ scavenging activity similar to α-tocopherol. Additionally, in vitro effect of γ-mangostin on inhibitory ability of lipid oxidation exhibited higher antioxidant activity than BHA and α-tocopherol (Yoshikawa et al. 1994). Furthermore, the antioxidant activities of three mangosteen xanthones, i.e., 1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) (P_1), 1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl)xanthone (P_2) and (−)epicatechin (P_3), were evaluated by different tests. P_1 exhibited the highest $DPPH^{\bullet}$ and $HO^{\bullet}$ scavenging activity, and inhibitory ability on lipid oxidation, whereas P_2 and P_3 exhibited higher $O_2^{\bullet-}$ scavenging activity than P_1. The three phenolic compounds showed higher $DPPH^{\bullet}$ and $O_2^{\bullet-}$ scavenging activity than Trolox and BHA, respectively. P_1 showed higher $HO^{\bullet}$ scavenging activity than α-tocopherol, Trolox and BHA, and higher inhibitory ability on lipid oxidation than α-tocopherol and Trolox. Correspondingly, P_2 and P_3 showed higher inhibitory ability on lipid oxidation than Trolox (Yu et al. 2007). Additionally, the potential antioxidant activities of fourteen mangosteen xanthones were demonstrated (Jung et al. 2006). Of these, 8-hydroxycudraxanthone G (Fig. 3b), smeathxanthone A (Fig. 3c) and gartanin (Fig. 3d) possessed better $ONOO^-$ scavenging activities. Suvarnakuta et al. (2011) reported that 8-deoxygartanin (Fig. 3e) revealed higher antioxidant activity than α-mangostin (Fig. 3f). The results are possibly due to the specific form of chemical structure.

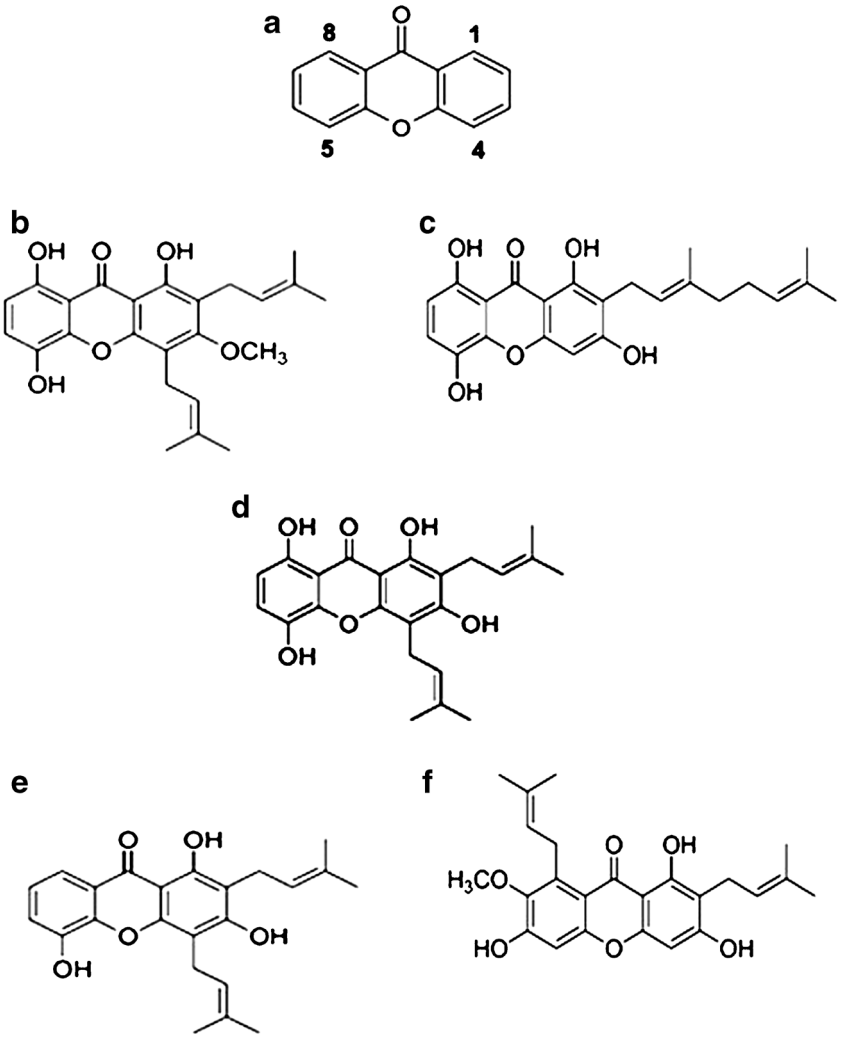

Fig. 3 Chemical structures of isolated xanthone and xanthone derivatives from mangosteen peel: xanthone **a**, 8-hydroxycudraxanthone G **b**, smeathxanthone A **c**, gartanin **d**, 8-deoxygartanin **e** and α-mangostin **f** (modified from Jung et al. 2006; Suvarnakuta et al. 2011)

Considering the correlation between antioxidant activity and chemical structure of bioactive compounds, some conclusions can be summarized. Firstly, polyphenol structure shows higher antioxidant activity than monophenol structure. For example, hydroquinone, catechol and resorcinol expressed higher $ONOO^-$ scavenging activity than phenol (Fig. 4) (Heijnen et al. 2001). Correspondingly, it was reported that increasing the number of OH groups increased the antioxidant activity (Cao et al. 1997). Secondly, the position of the OH substitution at the conjugated ring contributes to the antioxidant activity. Considering the one position of OH substitution, the substitution at position C-5 (Fig. 3a, 3e) exhibits better free radical scavenging activities than the substitution at position C-6 (Fig. 3a, f). Beyond the two position of OH substitution, the substitution at position C-6 and C-3 (Fig. 3a, f) can decrease the antioxidant activities due to the steric hindrance of the molecule structure (Suvarnakuta et al. 2011). Furthermore, Heijnen et al. (2001) reported that the most negative Hammett Sigma constant as electron donating effect was observed when the OH is at the *o*- or *p*-positions of conjugated ring. In contrast, positive Hammett Sigma constant as electron withdrawing effect was observed when the OH is at the meta (*m*-) position (Fig. 5). The results are possibly due to the appearance of two OH groups at the *o*- or *p*-positions of conjugated ring resulting in the decrease of the O-H bond strength and the subsequent excellent hydrogen or electron

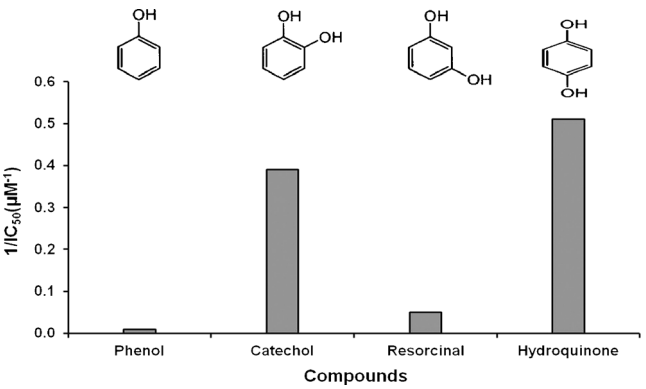

Fig. 4 Peroxynitrite scavenging activity of monophenol and polyphenol. IC_{50} stands for the concentration giving 50 % inhibition of the oxidation of dihydrorhodamine-123 (modified from Heijnen et al. 2001)

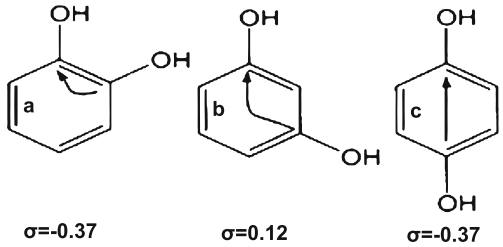

Fig. 5 The Hammett sigma constant (σ) of substitution of an OH group in ortho **a** meta **b** and para positions **c** of a conjugated ring (modified from Heijnen et al. 2001)

donors. Furthermore, the OH substitution at the *o*- or *p*--positions of conjugated ring might result in the stable radical intermediate due to the occurrence of resonance delocalization of unpaired electrons around the conjugated ring and the lack of suitable sites for attacking by molecular oxygen through intramolecular hydrogen bond (Shahidi and Naczk 1995).

Potential applications of mangosteen peel extract in food, pharmaceutical and cosmetic products

Nowadays, there is a growing interest in substances exhibiting antioxidant properties. Oxidation reactions are not an exclusive concern for the food industry, and antioxidants are widely needed to prevent deterioration of other oxidizable products such as cosmetics and pharmaceuticals. Consequently, antioxidants have become an essential part of food preservation technology and contemporary health care. Considering the potent antioxidant activity of mangosteen peel extract, it could be used as food additives for increasing the shelf life of food by preventing lipid peroxidation. The data in this review also suggest possible pharmaceutical and cosmetic applications that relate to the antioxidant activity of mangosteen peel extract for protecting oxidative damage in living systems. Recent trends in anti-aging research projected the use of bioactive compounds from natural resources against skin aging (Mukherjee et al. 2011). Most promising topical treatments of skin aging includes herbal extracts, vitamins and antioxidant food supplement, which have been accepted widely to scavenge free radicals from skin cells and to restore skin elasticity (Pinnell 2003). The crude ethanol extract of mangosteen peel, α-mangostin and γ-mangostin has been revealed to possess potent peripheral and central antinociceptive effects in mice, suggesting that xanthones from the mangosteen peel may be developed as novel analgesics and anti-inflammatory drugs (Cui et al. 2010). Potential therapeutic applications of the bioactive substances from mangosteen peel have also been summarized (Pedraza-Chaverrí et al. 2008). In contrast, interactions of plant phenolics with proteins may lead to the formation of soluble or insoluble complexes (Shahidi and Naczk 2004). These interactions may have a detrimental effect on the in vivo bioavailability of both phenolics and proteins (Lowry et al. 1996; Wollgast and Anklam 2000). Naczk et al. (2011) reported that crude extract of phenolics isolated from mangosteen peel displayed a strong protein-precipitating potential. The results suggest that these phenolics are prone to form insoluble protein-phenolic complexes. Ampasavate et al. (2010) found that the ethanolic extract of mangosteen peel had strong cytotoxic effects on leukemic cell lines but it was toxic to the normal peripheral blood mononuclear cells. Likewise, mangosteen peel extracts showed potent antiproliferative activity on cancer cell lines. However, the extracts had cytotoxic action on normal peripheral blood mononuclear cells (Khonkarn et al. 2010). This limits the application of these extracts as a source of novel anticancer agents. Consequently, further researches need to be conducted in order to investigate the effects of mangosteen peel extract in humans. The primary safety issue is the popular misconception that being natural is equal to being safe. However, being natural does not mean an absence of toxicity or potential human health risks. There has been a significant and possibly increasing incidence of intoxications with plant-derived ingredients used as food supplements and herbal drugs (Fu et al. 2009; Jordan et al. 2010). In vivo studies are also required to evaluate that the potential antioxidant detected by in vitro assays really acts in the same way in biological systems.

Conclusion

Mangosteen peel is one of the most important sources of natural antioxidants, which possesses various forms of antioxidant activities according to the analytical techniques for determining antioxidant activity of the extract. The comparative studies of maturation stages, extraction solvents and material preparation reveal the significant contribution of these factors to the yield and performance of antioxidant property of the mangosteen peel extract. Regarding antioxidant activity of the crude extract, mangosteen peel mostly shows higher antioxidant power than the extracts from many fruit peels. Similarly, the bioactive compounds isolated from mangosteen peel exhibit higher antioxidant power, compared with some commercial antioxidants. Therefore, several potential applications in food, pharmaceutical and cosmetic products can be considered for the mangosteen extract. However, the safety of mangosteen peel extract must be thoroughly assessed.

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