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Bioactive compounds and antioxidant potential of mango peel extract

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Abstract

Bioactive compounds such as polyphenols, carotenoids and anthocyanins present in fruits and vegetables are receiving increased attention because of their potential antioxidant activity. Consumption of such antioxidants offers health benefits including protection against cardiovascular diseases and cancer. Mango peel is a major byproduct obtained during the processing of mango products such as mango pulp and amchur. In the present study, the antioxidant activity of mango peel extracts was examined. Polyphenol, anthocyanin and carotenoid contents in acetone extract of peels were determined. Ripe peels contained higher amount of anthocyanins and carotenoids compared to raw peels while raw mango peel had high polyphenol content. Antioxidant activity of ripe and raw mango peels extracted in acetone was determined using different antioxidant systems such as reducing power activity, DPPH free radical scavenging activity, iron induced lipid peroxidation of liver microsomes and soybean lipoxygenase inhibition. The IC_{50} values were found to be in the range of 1.39–5.24 μg of gallic acid equivalents. Thus, the mango peel extract exhibited good antioxidant activity in different systems and thus may be used in nutraceutical and functional foods.

Keywords: Mango; Mango peel; Bioactive compounds; Phenolics; Carotenoids; Anthocyanins; Antioxidant activity

1. Introduction

Diets rich in fruits and vegetables are gaining increased importance because of their significant role in reducing the risk of certain types of cancer, cardiovascular diseases and other chronic diseases (Criqui & Ringel, 1994; Hertog, Feskens, Hallman, Katan, & Kromhout, 1993; Joshipura et al., 2001). Fruits and vegetables contain many antioxidant compounds including phenolic compounds, carotenoids, anthocyanins and tocopherols (Bartosz, 1997; Naczk & Shahidi, 2006). Many studies have shown that free radicals in the living organisms cause oxidative damage to different molecules such as lipids, proteins, nucleic acids and these are involved in the interaction phases of many degenerative diseases. Antioxidants are substances that delay or prevent the oxidation of cellular oxidisable substrates. They exert their effect by scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS) or preventing the generation of ROS/RNS (Halliwell, 1996).

Synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in processed foods. It has been reported that these compounds have some side effects and are carcinogenic (Branen, 1975; Ito, Fukushima, Hasegawa, Shibata, & Ogiso, 1983). Natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic value. The increased interest in natural antioxidants has led to the antioxidant evaluation of many species of fruits, vegetables, herbs, spices and cereals (Kahkonen et al., 1999; Liyana-Pathirana & Shahidi, 2005; Velioglu, Mazza, Gao, & Oomah, 1998; Wolfe, Xianzhong, & Liu, 2003). Special attention has been paid to fruits, as they are rich source of phenolic

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compounds (Kalt, Forney, Martin, & Prior, 1999; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Wang & Lin, 2000).

Mango is one of the most important tropical fruits and India ranks first in the world production. As mango is a seasonal fruit, about 20% of fruits are processed for products such as puree, nectar, leather, pickles and canned slices, among others, which have worldwide popularity (Loelillet, 1994). During processing of mango, peel is a major byproduct. As peel is not currently utilized for any commercial purpose, it is discarded as a waste and becoming a source of pollution. It has been reported that mango peel contains a number of valuable compounds such as polyphenols, carotenoids, enzymes and dietary fibre (Ajila, Bhat, & Prasada Rao, 2007). Therefore, the objective of the present study was to evaluate the antioxidant activity of acetone extract of mango peel and estimate the major antioxidants such as polyphenols, carotenoids, and anthocyanin contents.

2. Materials and methods

2.1. Materials

Raspuri and Badami mango varieties used in this study were obtained from CFTRI campus, Mysore, India. Mango fruits were harvested at maturity and some fruits were kept to ripen at room temperature.

2.2. Chemicals

Gallic acid, butylated hydroxyanisole (BHA), Tris, adenosine diphosphate (ADP), ascorbic acid, thiobarbituric acid (TBA), soybean lipoxygenase, linoleic acid were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Himedia Laboratories Limited (Mumbai, India). All other chemicals used were of analytical grade. Glass double distilled water was used in all experiments.

2.3. Preparation of acetone extract of mango peel and estimation of total phenolics, carotenoids and anthocyanins

Both raw and ripe mango peels were removed from the fruits, homogenized with 80% acetone and centrifuged for 15 min at 8000*g* (Ajila et al., 2007). The clear supernatant so obtained was used for the estimation of total phenolic compounds, anthocyanin content, carotenoid content and evaluated for antioxidant activity.

The content of total polyphenolics in acetone extract of mango peels was determined by the method of Swain and Hillis, 1959. The absorbance was recorded at 725 nm. Gallic acid was used as a standard. The content of total polyphenolics in the extract was expressed as gallic acid equivalents (GAE).

Peel extract (2.5 ml) was mixed with 40 ml of methanol containing 1 g of KOH and the carotenoids were extracted using the method described by Tee and Lim (1991). The total carotenoid content was estimated using the method described by Litchenthaler (1987) using following equations:

$$\text{Chlorophyll } a\ (C_a) = 12.25A_{663.2} - 2.79A_{646.8}$$

$$\text{Chlorophyll } b\ (C_b) = 21.50A_{646.8} - 5.10A_{663.2}$$

$$\text{Total carotenoid} = \frac{1000A_{470} - 1.82C_a - 85.02C_b}{198}$$

Monomeric anthocyanin contents of the mango peel extracts were measured using a spectrophotometric pH differential method of Wolfe et al. (2003). Anthocyanin content was expressed as mg cyanidin 3-glucosides equivalent/100 g mango peel for the triplicate extracts. The values were expressed as mean ± SD.

2.4. Measurement of antioxidant activity

2.4.1. Measurement of reducing power

The reducing power of the mango peel extract and the synthetic standard, BHA were determined according to the method of Yen and Chen (1995). The mango peel extract containing 5–20 μg of GAE was made up to 500 μl with 0.2 M phosphate buffer (pH 6.6), mixed with 1 ml of potassium ferricyanide (0.1%) and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (500 μl, 10%) was added to the reaction mixture and centrifuged at 3000*g* for 10 min. The supernatant obtained was mixed with equal volume of distilled water, 300 μl of 1% ferric chloride was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power. The antioxidant activity of the extract was compared with BHA.

2.4.2. Measurement of free radical scavenging activity

The effect of acetone extracts of mango peel on DPPH radical was determined according to the method described by Blois (1958) with modification described by Brand-Williams, Cuvelier, and Berset (1995). A 100 mM solution of DPPH in methanol was prepared and mango peel extract (200 μl) containing 1–5 μg of GAE was mixed with 1 ml of DPPH solution. The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance (A_s) of the resulting solution was measured at 517 nm. The control contained all the reagents except peel extract/BHA. The capacity to scavenge DPPH radical was calculated by following equation.

$$\text{Scavenging activity } (\%) = 1 - (A_s/A_0) \times 100$$

where A_0 is the absorbance at 517 nm of the control and A_s is the absorbance in presence of peel extract/BHA. The results were plotted as the % of scavenging activity against concentration of the sample. The inhibition concentration (IC_{50}) was defined as the amount of GAE required for 50% of free radical scavenging activity. The IC_{50} value was

calculated from the plots as the antioxidant concentration required for providing 50% free radical scavenging activity.

2.4.3. Measurement of inhibitory effect on microsomal lipid peroxidation

Liver microsomes were prepared according to the method of Kemp and Writz (1974). The protein content in the microsomes was determined according to the method described by Lowry, Rosenberg, Farr, and Randall (1951).

The liver microsomal lipid peroxidation was performed according to the method of Miller and Aust (1989) with some modifications. Peel extract (100 μl) containing 1–5 μg of GAE were added to 1 ml of microsomes (100 μg protein). Lipid peroxidation was induced by adding 25 μl ADP (2 mM), 25 μl of $FeSO_4$ (4 mM) and 25 μl of ascorbic acid (0.1 mM). After incubation for 1 h at 37 °C, the reaction was stopped by adding 2 ml of 0.25 N HCl containing 15% TCA and 0.375% thiobarbituric acid. The reaction mixture was boiled for 15 min, cooled, centrifuged and absorbance (A_s) of the supernatant was measured at 532 nm. Appropriate blanks and controls were run along with the test samples. The blank contained all the reagents and peel extract/BHA except microsomes while control contained all the reagents and microsomes except peel extract. Inhibition of lipid peroxidation (%) by the peel extract was calculated by the following equation:

$$\text{Lipid peroxidation inhibition}(\%) = 1 - (A_s/A_0) \times 100,$$

where A_0 is the absorbance value of the fully oxidized control and A_s is the absorbance in presence of extract.

The percentage of inhibition was plotted against the concentration of the peel extract that was expressed in terms of GAE. The half-inhibition concentration (IC_{50}) value was defined as the amount of GAE required for inhibition of 50% of lipid peroxidation. The IC_{50} value was calculated from the plots as the antioxidant concentration required for providing 50% inhibition of lipid peroxidation.

2.4.4. Measurement of inhibitory effect on lipoxygenase activity

Lipoxygenase inhibiting activity was measured according to the method described earlier by Shobana and Naidu (2000) with some modification. The enzymatic lipid peroxidation was measured spectrophotometrically following an increase in absorbance of lipid hydroperoxide formation at 234 nm. The 1 ml reaction mixture contained 250 μM linoleic acid substrate solution, 5 nM soybean lipoxygenase and 50 mM Tris buffer (pH 9.0). Different concentrations of mango peel extracts (2.5–40 μg of GAE) were incubated with soybean lipoxygenase for 2 min prior to the initiation of the reaction with linoleic acid substrate. The decrease in hydroperoxide formation in presence of peel extract or BHA was calculated. Control contained all the reagents except peel extract/BHA. The IC_{50} value was determined by plotting a graph with concentration of peel extract versus percentage of inhibition of lipid peroxidation.

2.5. Statistical analysis

All analyses were performed in triplicate and data were reported as means ± SD. Duncan's new multiple range test was used to determine the difference of means, and $P < 0.05$ was considered to be statistically significant (Steel & Torrie, 1980).

3. Results and discussion

Phenolic compounds, and pigments are the major group of compounds that contribute to the antioxidant activity of vegetables, fruits, cereals and other plant based materials. Earlier, different solvents were used for the extraction of phenolic compounds from mango peel and we have reported that 80% acetone extracted maximum amount of phenolic compounds from the mango peel (Ajila et al., 2007). Therefore, in the present study the mango peel was extracted with 80% acetone and its antioxidant activity was determined. The bioactive compounds present in the acetone extract were also determined.

3.1. Antioxidant compounds in the acetone extract of mango peel

The antioxidant activity of phenolics is due to the reactivity of phenol moiety (hydroxyl group on aromatic ring). They have the ability to scavenge free radicals via hydrogen donation or electron donation (Shahidi & Wanasundara, 1992). Quantification of polyphenols was done using Folin-Ciocalteu (FC) reagent. In this method, phenols form the blue colored phosphomolybdic–phosphotungstic–phenol complex in alkaline solution (Singleton & Rossi, 1965). The total polyphenols content in 80% acetone extract of raw mango peels ranged from 90 to 110 mg/g peel, whereas it ranged from 55 to 100 mg/g in ripe peels depending on the variety.

Carotenoids are widely distributed in nature and they are liposoluble antioxidants. Carotenoids show good absorption at 470 nm; however, a small amount comes from chlorophyll b and negligible absorption from chlorophyll *a*. The concentration of total carotenoids content can, therefore, be determined by deducting the absorption of chlorophyll *a* and *b* from the absorbance read at 470 nm followed by division by the absorption coefficient of total carotenoids at 470 nm (Litchenthaler, 1987). The carotenoid content in the acetone extracts of both raw and ripe peels of Raspuri and Badami were determined. As shown in Table 1, the carotenoid content in the mango peel extracts ranged from 74 to 436 μg/g peel and it was found to be more in ripe mango peels compared to raw peels. Earlier, Ajila et al. (2007) reported the carotenoid content in the range of 547–3337 μg/g peel in the raw and ripe peels of the above varieties. However, the values obtained in the present study were much lower than that reported by Ajila et al. (2007). The low value for carotenoid content is due to the fact that in the present study peel was

Table 1
Total phenolic, carotenoid and anthocyanin contents in acetone extracts of mango peel[A]

Variety	Total phenolic content[B] (mg/g)	Carotenoid content (μg/g)	Anthocyanin content (mg/100 g)
Raspuri raw	109.70 ± 0.82^{d}	73.5 ± 0.53^{a}	203 ± 5.03^{a}
Raspuri ripe	100.00 ± 1.9^{c}	436.0 ± 0.22^{d}	360 ± 6.54^{b}
Badami raw	90.18 ± 0.57^{b}	81.0 ± 0.42^{b}	326 ± 3.05^{c}
Badami ripe	54.67 ± 1.5^{a}	194.0 ± 0.26^{c}	565 ± 3.51^{d}

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($P \leqslant 0.05$).
[A] Dry weight basis.
[B] Ajila et al. (2007).

extracted with 80% acetone and saponified while in the earlier report of Ajila et al. (2007), the mango peel was extracted directly after saponification with methanolic KOH.

Saponification using KOH–methanol is carried out to avoid the effect of fat in the estimation of carotenoids. It has also been reported that organic acids present in tissues affect the estimation of carotenoids. In order to avoid such artifacts, during extraction, addition of alkaline compounds are suggested to neutralize the acids (Minguez-Mosquera, Hornero-Mendez, & Perez-Galvez, 2002). Mango fruit is rich in organic acids such as citric, malic and oxalic acids (Hulme, 1971). Mango peel is rich in polyphenols and it contains about 2.5% fat (Ajila et al., 2007), which may interfere with estimation of carotenoids in the absence of alkali. Thus, the results indicate that extraction using KOH–methanol may be a more suitable method to extract and estimate carotenoids in mango peel.

Anthocyanins are a group of phenolic compounds in the plant kingdom and they exhibit good antioxidant properties. Anthocyanins below pH 2 exist primarily in the form of flavyllium cation (Takeoka & Dao, 2002) and at this low pH, they absorb light around 510 nm, while degraded anthocyanins in the polymeric form absorb light below 2 pH and pH 4.5. Therefore, to avoid the absorbance of interfering substances, in pH differential method the difference in absorbance at two pH levels is measured at the same wavelength (Jackman, Yada, & Tung, 1987; Parry et al., 2006). As can be seen from Table 1, the anthocyanin content was more in ripe mango peel and ranged from 203 to 326 mg/100 g in raw peels and 360 to 565 mg/100 g in ripe peels. Anthocyanin content in apple peel was reported to be 2.1–26.8 mg of cyanidin 3-glucoside equivalent/100 g of apple peel depending on the variety (Wolfe et al., 2003).

3.2. Evaluation of antioxidant properties

3.2.1. Reducing power of mango peel acetone extract

The reducing power of a compound is related to its electron transfer ability and may, therefore, serve as a significant indicator of its antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995). Fig. 1 shows the reducing power of the Raspuri and Badami mango peel extracts. The reducing power increased with the concentration of peel extract. The reducing power of Badami peel extracts was more compared to Raspuri peel extracts and BHA. For example, the absorbance at 700 nm was found to be 0.358 for Badami raw peel extract at a dose level of 5 μg of GAE, while it was 0.125 for Raspuri raw extract and 0.136 for BHA. Phenolics, carotenoids and anthocyanins present in the peel are good electron donors and could reduce Fe^{3+}/ferricyanide complex to ferrous form, which indicates the antioxidant activity (Chung, Chang, Chao, Lin, & Chou, 2002; Yen & Chen, 1995).

3.2.2. Scavenging effect on DPPH radical

Scavenging the stable DPPH radical model is another widely used method to evaluate antioxidant activity. DPPH is a stable free radical with characteristic absorption at 517 nm and antioxidants react with DPPH and convert it to 2,2-diphenyl-1-picrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating ability (Van Gadow, Joubert, & Hannsman, 1997).

Fig. 2 shows the dose dependence curve for the radical scavenging activity of the mango peel extracts. The IC_{50} values ranged from 1.83 to 4.54 μg of GAE (Table 2). The mango peel extracts showed a concentration dependent scavenging of DPPH radical, which may be attributed to its hydrogen donating ability. Raspuri extracts showed low IC_{50} values (1.83–1.98 μg of GAE) compared to that of Badami peel extracts (3.67–4.54 μg of GAE). The free radical scavenging activity of mango peel extracts was compared with BHA. It was found that the acetone extracts of Raspuri raw and ripe mango peels showed higher scavenging activity than that of BHA (Table 2).

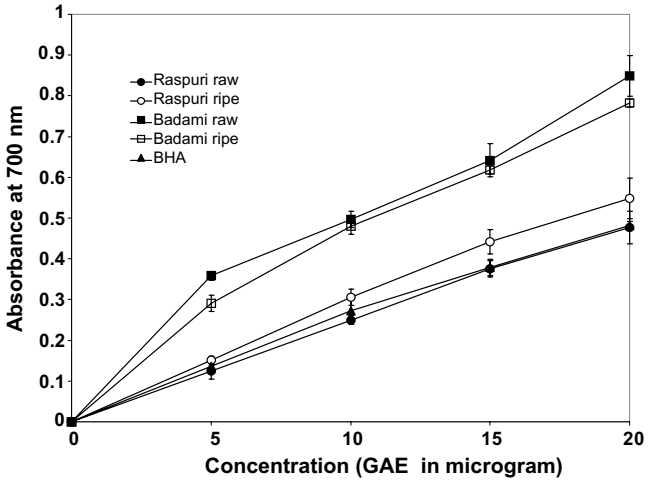

Fig. 1. Reducing power of the raw and ripe Raspuri and Badami mango peel extracts.

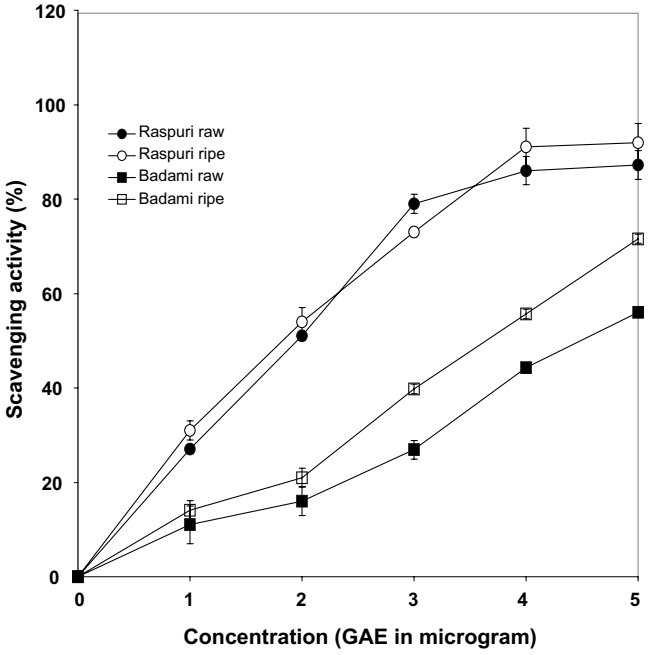

Fig. 2. DPPH radical Scavenging effects of raw and ripe mango peel extracts of Raspuri and Badami.

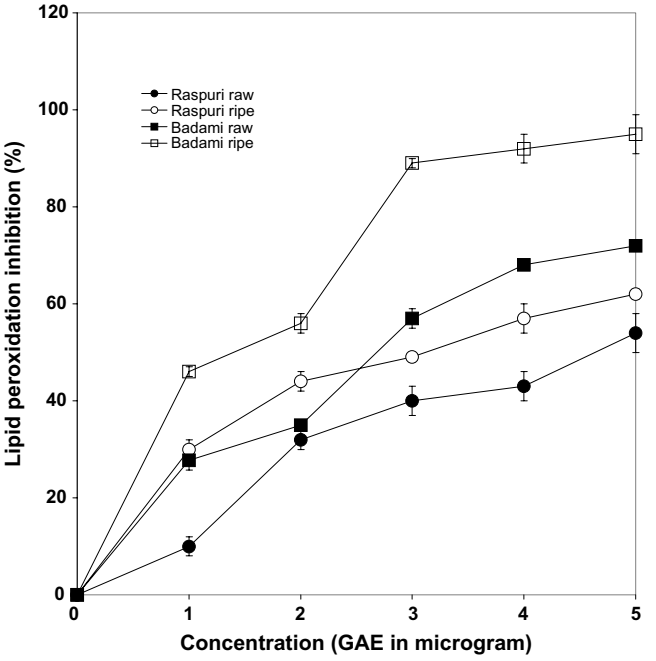

Fig. 3. Inhibition of lipid peroxidation effects of raw and ripe mango peel extracts of Raspuri and Badami.

Table 2
IC_{50} values of acetone extract of Raspuri and Badami raw and ripe mango peel on different antioxidant model systems

Mango variety	DPPH· (μg of GAE)	LPO (μg of GAE)	Soybean Lipoxygenase (μg of GAE)
Raspuri Raw	1.98 ± 0.05^{b}	4.59 ± 0.04^{e}	5.14 ± 0.08^{d}
Raspuri Ripe	1.83 ± 0.02^{a}	3.13 ± 0.02^{d}	5.24 ± 0.09^{d}
Badami Raw	4.54 ± 0.02^{e}	2.68 ± 0.01^{c}	2.02 ± 0.10^{a}
Badami ripe	3.67 ± 0.06^{d}	1.39 ± 0.02^{b}	4.73 ± 0.12^{c}
BHA	3.40 ± 0.08^{c}	0.80 ± 0.04^{a}	2.82 ± 0.14^{b}

IC_{50}values were calculated from the dose responses curves. All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($P \leqslant 0.05$).

3.2.3. Inhibition on microsomal lipid peroxidation

In biological systems, lipid peroxidation (oxidative degradation of polyunsaturated fatty acids in the cell membrane) generates a large number of degradation products such as malonaldehyde (MDA), which are found to be an important cause for cell membrane destruction and cell damage (Kubow, 1992). MDA, one of the major products of lipid peroxidation, has been extensively used as an index for lipid peroxidation and as a marker for oxidative stress. The reaction of MDA with TBA has been widely adopted as a sensitive assay method for lipid peroxidation (Ohkawa, Ohishi, & Yagi, 1978).

Fig. 3 shows the dose dependence curve for the inhibition of lipid peroxidation. Badami extracts showed IC_{50} values from 1.39 μg to 2.68 μg of GAE compared to that of Raspuri peel extracts (3.13–4.59 μg GAE). The mango peel extract showed a concentration dependent inhibitory effect on lipid peroxidation. The lipid peroxidation inhibitory activity of mango peel extracts was compared with BHA (Table 2). In this method, ripe mango peel extracts showed higher inhibition on lipid peroxidation than raw peel extracts and these peel extracts showed higher IC_{50} values compared to BHA.

3.2.4. Inhibition on lipoxygenase activity

Lipoxygenase is a biological target for many diseases such as asthma, atherosclerosis, cancer (Mogul & Holman, 2001) and tumor angiogenesis (Nie & Honn, 2002). Lipoxygenase (E.C. 1.13.11.12) constitutes a family of non-heme containing dioxygenase group of enzymes that are widely distributed in plants and animals. In mamma-

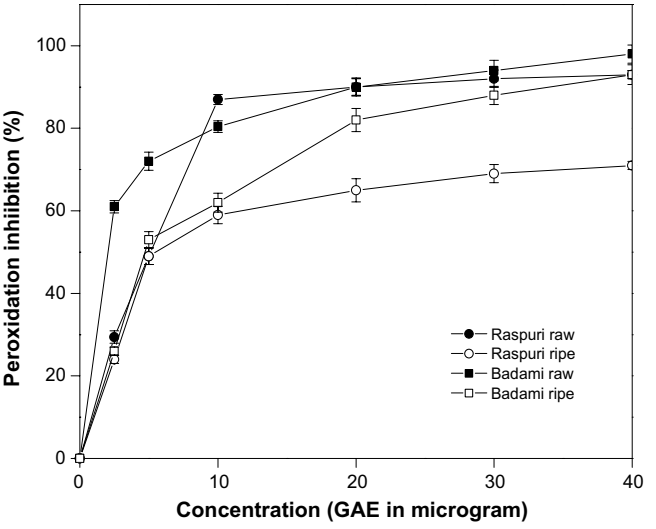

Fig. 4. Inhibition of soybean lipoxygeanse activity by raw and ripe mango peel extract of Raspuri and Badami.

lian cells, these enzymes have key role in the biosynthesis of a variety of bio-regulatory compounds such as hydroxyeicosatetraenoic acid, leukotrienes, lipoxins and hepoxylines (Lands, 1985). Lipoxygenases are, therefore, potential targets for the rational drug design and discovery of mechanism based inhibitors for the treatment of a variety of disorders and autoimmune diseases. Antioxidants interact non-specifically with lipoxygenase by scavenging radical intermediates and/or reducing the active heme site (Cao, Sofie, & Prior, 1996).

Acetone extracts of mango peel showed concentration dependent inhibition of lipoxygenase activity (Fig. 4). Raw mango peel extracts showed higher inhibition of lipoxygenase activity compared to that of ripe peel extracts (Table 2). Of the different extracts, Badami raw peel extract showed maximum inhibition activity with an IC_{50} value of 2.02 μg of GAE, which was better than BHA (2.82 μg of GAE, Table 2).

4. Conclusion

Acetone extracts of mango peel contained polyphenols, anthocyanins and carotenoids and these extracts exhibited good antioxidant activity by effectively scavenging various free radicals such as DPPH radicals, hydroxyl radicals, peroxyl radicals and reducing the ferric to ferrous ion in different antioxidant systems. The difference in antioxidant activity of the peel of different varieties at different stages of maturity may be due to variation in composition and content of antioxidants such as polyphenols, carotenoids and anthocyanins. The antioxidant potential of mango peels could be due to synergistic actions of bioactive compounds present in them.

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