

Full Length Research Paper

Comparison of antioxidant potential in pulp and peel extracts of *Manilkara zapota* (L.) P. Royen

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Accepted 17 July, 2013

Free radical scavenging ability of both pulp and peel of *Manilkara zapota* (sapota) were tested against 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS⁺), peroxy nitrites and lipid peroxidation generated *in vitro*. Both ethanolic and methanolic extracts of pulp and peel of sapota were subjected to *in vitro* free radical scavenging assays like DPPH, ABTS⁺, nitric oxide and lipid peroxidation inhibition assays. Total antioxidant capacity assay to confirm the antioxidant potential and preliminary phytochemical screening and phytochemical content like total phenols and flavonoids was also carried out by standard methods. In this study, the results indicates that peel extracts of sapota showed highest radical scavenging potential and high antioxidant activity compared to pulp extracts. In addition, ethanolic extract of peel exhibited highest flavonoid and total phenol contents. Among the ethanolic and methanolic extracts of sapota, ethanolic peel exhibited least IC₅₀ value which indicates high potential for multiple radical scavenging activity. The preliminary study carried out confirms the significant free radical scavenging potential of the sapota peel extracts which indicates its potential health benefits.

Key words: Free radicals, antioxidants, 2,2-diphenyl-1-picryl hydrazyl (DPPH), ABTS, lipid peroxidation, flavonoids, total phenols

INTRODUCTION

Free radicals are produced by normal aerobic metabolism and are necessary to life. Our immune system needs free radicals to fight against invading bacteria and virus. In biological system, the main free radicals or precursors of free radicals include reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxide or hydrogen peroxide, hydroxyl radical (HO[·]) and reactive nitrogen species. Increasing evidence suggests that these free radicals may bring about oxidative damage to DNA, proteins and lipids and hence play a major role in the development of many diseases, like cancer, cataracts, heart diseases and

aging in general (Shui and Leong, 2006). Although all cells have some capacity of repairing oxidative damage to proteins and DNA, they are not able to cope with excessive damage. Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction triggered by free radicals, seem to be very important in the prevention of these diseases. Some of the important synthetic antioxidants of this class are butylated hydroxyanisole (BHA), butylated hydroxyl toluene, tertbutyl hydroquinone (TBHQ), propyl gallate (PG) and tocopherols. In recent years, the use of some synthetic antioxidants has been restricted because of

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Abbreviations: ROS, Reactive oxygen species; BHA, butylated hydroxyanisole; TBHQ, tertbutyl hydroquinone; PG, propyl gallate; ABTS, 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picryl hydrazyl; TBA, thiobarbituric acid; TCA, trichloroacetic acid; EDTA, ethylene diamine tetra-acetic acid; MDA, malondialdehyde.

their possible toxic and carcinogenic effects. This concern has resulted in an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties (Shahidi and Wanasundara, 1992).

Fruits are identified as rich sources of antioxidants and copiously used to overcome oxidative stress. The fact behind the health-beneficial property of fruits is the large number of nutraceutical phytochemicals that they contain viz., polyphenols, carotenoids, sterols, saponins, terpenes and vitamins. Phytochemical components like phenolics, ascorbic acid and carotenoids may have direct influence over the radical-scavenging potential.

Sapota is one such fruit that belongs to Sapotaceae and is one of the major fruit crops in India. The chiku fruit (*Manilkara zapota* L.), also known as the sapodilla, is a small evergreen tree native to Mexico and tropical America and now is well spread throughout the tropics (Mohamed et al., 2003). Unripe chiku, are found to possess extremely high antioxidant capacity that is not attributed to L-ascorbic acid, a constituent that is partly responsible for the antioxidant capacity of many fruits (Shui et al., 2004). In a study by Ma and co-workers, 4-O-galloylchlorogenate and 4-O-galloylchlorogenic acid were reported to be found in chiku extracts (Ma et al., 2003). They also reported that chiku possesses a high antioxidant capacity using the 1,1-diphenyl-2-picrylhydrazyl scavenging assay and showed cytotoxicity in the HCT-116 and SW-480 human colon cell lines. Sapota juice was found to contain moderate amount of sugars and protein and it was also found to be good source of ascorbic acid, carotenoids and phenolics that may have health promoting properties (Anand et al., 2004). Recent reports suggested that sapota had the highest hydrophilic oxygen radical absorbance capacity (H-ORAC) and total phenolic content among 38 different fruits evaluated in Singapore (Isabelle et al., 2010). Though reports are available to suggest the antioxidant potential of sapota pulp extracts, studies pertaining to peel extracts are scanty. Hence, the present study aims to compare the *in vitro* antioxidant activities and phytochemicals (total phenols and flavonoids) in both pulp and peel extracts of sapota.

MATERIALS AND METHODS

All chemicals and solvents were of analytical grade and purchased from Hi Media laboratories, Mumbai. 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was obtained from sigma chemicals, USA. The various other chemicals were DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium nitroprusside, naphthyl ethylene diamine dihydrochloride, sulphanilamide, ferrous sulphate, thiobarbituric acid (TBA), trichloroacetic acid (TCA) and ethylene diamine tetra-acetic acid (EDTA).

The sapota fruits were collected from Coimbatore fruit market. The plant specimen was identified and authenticated for its scientific name by the Scientist 'C' - In - Charge, Department of Fruits and Crops, Horticultural College and Research Institute, TNAU, Coimbatore (No. BSI/SRC/5/23/2010-11/590 dt 5.1.2011). A

voucher specimen of the sample has been deposited in the herbarium of the department.

Preparation of extracts

The peels of sapota fruits were removed from the pulp part and both pulp and peel were shade -dried for about a week and then crushed to make a coarse powder. 10 g of dried pulp and peel powder were extracted exhaustively in 100 ml each in absolute methanol and absolute ethanol in a 250 ml conical flask, kept on a rotary shaker at 190 to 220 rpm for 24 h. After 24 h, the extract was filtered in Whatman No.1 filter paper and the Petri dishes containing the extracts were kept in calcium chloride desiccators for about two days. The resulting solvent extracts were stored and used for further *in vitro* assays. The extraction was done in triplicates.

Preliminary phytochemical screening

The preliminary phytochemicals screening was carried out for various phytoconstituents like carbohydrates, proteins, steroids, starch, cellulose, cholesterol, saponins, alkaloids, flavonoids, terpenoids, catechol, leucoanthocyanidins, volatile oils, tannins, hydrolysable tannins, glycosides and tannins in both pulp and peel extracts of *M. zapota* L using standard procedures.

In vitro antioxidant capacity assays

Total antioxidant capacity assay

This assay is based on the principle of reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of green phosphate molybdenum complex at acidic pH. The working solutions (10 to 100 µg/ml) of samples were prepared by dissolving the extracts in water. 0.1 ml of the extracts was mixed with 1 ml of reagent solution. The tubes were capped with aluminium foil and incubated at 95°C for 90 min (Uma Maheswari and Chatterjee, 2008). The tubes were then cooled to room temperature and the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as standard. The total antioxidant capacity was expressed as ascorbic acid equivalents.

2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

DPPH scavenging activity was measured by the slightly modified spectrophotometric method of Brand-Williams et al. (1995). 1,1-diphenyl-2-picryl-hydrazyl radical is scavenged by antioxidants through the donation of protons forming reduced DPPH. The electrons become paired off and the solution loses color depending on the number of electrons taken up. The color change from purple to yellow after reduction is quantified by the decrease of absorbance at 517 nm.

The sapota extracts were dissolved in methanol. A solution of DPPH in methanol (0.2 mM) was prepared freshly. 3 ml of this solution was mixed with 1 ml of the samples of varying concentrations (100 to 1000 µg/ml). The solution in the test tubes were mixed well and incubated in dark for 30 min at room temperature and absorbance was measured at 517 nm. The control had equal volume of DPPH in methanol instead of extract. 5 ml of methanol was taken as blank. The percentage inhibition of the radicals due to the antioxidant property of the extract was calculated using the formula:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] * 100.$$

2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) cation radical scavenging assay

The ABTS assay is based on the inhibition of the absorbance of ABTS radical cation, which has a characteristic long wavelength absorption spectrum. The assay was performed by a slightly modified protocol of Re et al. (1999). ABTS radical was produced by reacting ABTS solution (7 mM) with ammonium per sulphate (2.45 mM) and the mixture was allowed to stand in the dark at room temperature for 12 to 16 h to give a dark colored solution. The absorbance was measured at 745 nm. The initial absorbance was found to be around 2.99. This stock solution was diluted with methanol to give a final absorbance value around 0.7 (± 0.2) and equilibrated at 30°C. Different concentrations of the sample (100 to 1000 $\mu\text{g/ml}$) were prepared by dissolving the extracts in water. About 0.3 ml of the sample was mixed with 3 ml of ABTS working standard in a microcuvette. The decrease in the absorbance was measured after mixing the solution in 1 min intervals up to 6 min. The final absorbance was noted. A solution of ABTS working and 0.3 ml of methanol was used as the control. About 3 ml of methanol was used as blank. The percentage inhibition was calculated according to the formula:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] * 100.$$

Nitric oxide radical scavenging assay

Nitric oxide scavenging was measured spectrophotometrically. The nitric oxide generated using sodium nitroprusside is converted into nitrite ions. The chromophore is formed due to the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylene diamine which is measured at 546 nm (Raghavan et al., 2003). Sodium nitroprusside (5 mM) was prepared in phosphate buffer saline. 1 ml of this was mixed with 1 ml of extracts of different concentration (1 to 10 mg/ml) in methanol. The mixture was incubated at 25°C for 30 min. After 30 min, an equal volume of Griess reagent was added to the incubated solution and the absorbance of the chromophore was measured at 546 nm. Control was a solution of reagents devoid of extracts.

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] * 100.$$

Lipid peroxidation inhibition assay

Initiation of lipid peroxidation by ferrous sulphate takes place through the hydroxyl radical formation by Fenton's reaction. These produce malondialdehyde (MDA), which reacts with TBA to form a pink chromogen (Ohkawa et al., 1979). Goat liver was washed thoroughly in cold phosphate buffered saline (pH 7.4). This was then minced in a mortar and pestle with a measured volume of cold buffer in ice. The minced liver was then homogenized in a homogenizer to give a 10% homogenate. The homogenate was filtered using cheese cloth to remove unwanted residues. The filtrate was then centrifuged at 10000 rpm for 10 min in refrigerated centrifuge and the supernatant was used for assay. To 0.5 ml of supernatant, 0.5 ml of extracts of varying concentrations (1 to 10 mg/ml) in water was added. The volume was made up to 1 ml with distilled water.

To this, 0.05 ml of 0.07 M ferrous sulphate was added. The solution was incubated at room temperature for 30 min. To the incubated solution, 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% TBA (in 1.1% SDS) and 0.05 ml of 20% TCA were added. The tubes were vortexed to ensure appropriate mixing. Then, the tubes were incubated at 100°C for 1 h. The tubes were then cooled to room temperature and the absorbance was read at 532 nm. The control contained phosphate buffered saline (PBS) instead of the sample. The percentage inhibition was calculated:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] * 100.$$

Estimation of total phenols

Total phenolic contents were determined according to the spectrophotometric methods of Kaur and Kapoor (2003). 1 g of the sapota pulp and peel were weighed and ground with 10 ml of 80% aqueous ethanol and methanol in a mortar and pestle. The ground sample was then centrifuged at 10000 rpm for 20 min. The supernatant obtained was poured into separate tubes. The pellets were re-extracted in 50 ml ethanol and methanol. This sample was then again centrifuged at 5000 rpm for 20 min. The pooled supernatants were evaporated to dryness and the residue was then dissolved in 10 ml distilled water. The estimation of total phenol was carried out by taking 0.1 ml of the prepared sample and making it up to 3 ml with distilled water. 0.5 ml of Folin-Ciocalteu reagent was added to the sample. The mixture was incubated for 3 min. 2.0 ml of 20% sodium carbonate was added. The tubes were then boiled in a water bath for 1 min and the blue color developed was read at 650 nm. The results were expressed as mg catechol equivalents.

Estimation of flavonoids

A slightly modified version of the spectrophotometric method was used to determine the flavonoid contents of samples (Karadeniz et al., 2005). 1 g of pulp and peel of sapota sample were weighed and ground with 10 ml of 80% aqueous methanol and ethanol using mortar and pestle. The ground sample was filtered with the help of a Whatmann filter paper no.1. 0.5 ml of the filtrate was taken in a test tube, mixed with 0.15 ml of 10% aluminium chloride and allowed to stand for 5 min. After 6 min, 0.5 ml of 1 M NaOH was added into test tube. The solution in the test tube was then diluted with 0.275 ml of distilled water. The absorbance was read at 510 nm. Flavonoid contents were then calculated using a standard calibration curve, prepared from rutin.

Statistical analysis

The experimental results were expressed as mean \pm SD of three replicates. The data were subjected to two-way analysis of variance (ANOVA) and significance of difference between sample means were calculated by DMRT analysis using IRRISTAT software version 3.1. Difference in mean values were considered significant when $P \leq 0.05$. Linear regression analysis was used to calculate the IC_{50} values

RESULTS

Preliminary phytochemical screening

The phytochemical screening tests performed for both pulp and peel extracts of sapota revealed the presence of carbohydrates, proteins, alkaloids, saponins, terpenoids, flavonoids, tannins, leucoanthocyanidins, anthroquinones, glucosides and catechol and absence of cellulose and hydrolysable tannins in pulp.

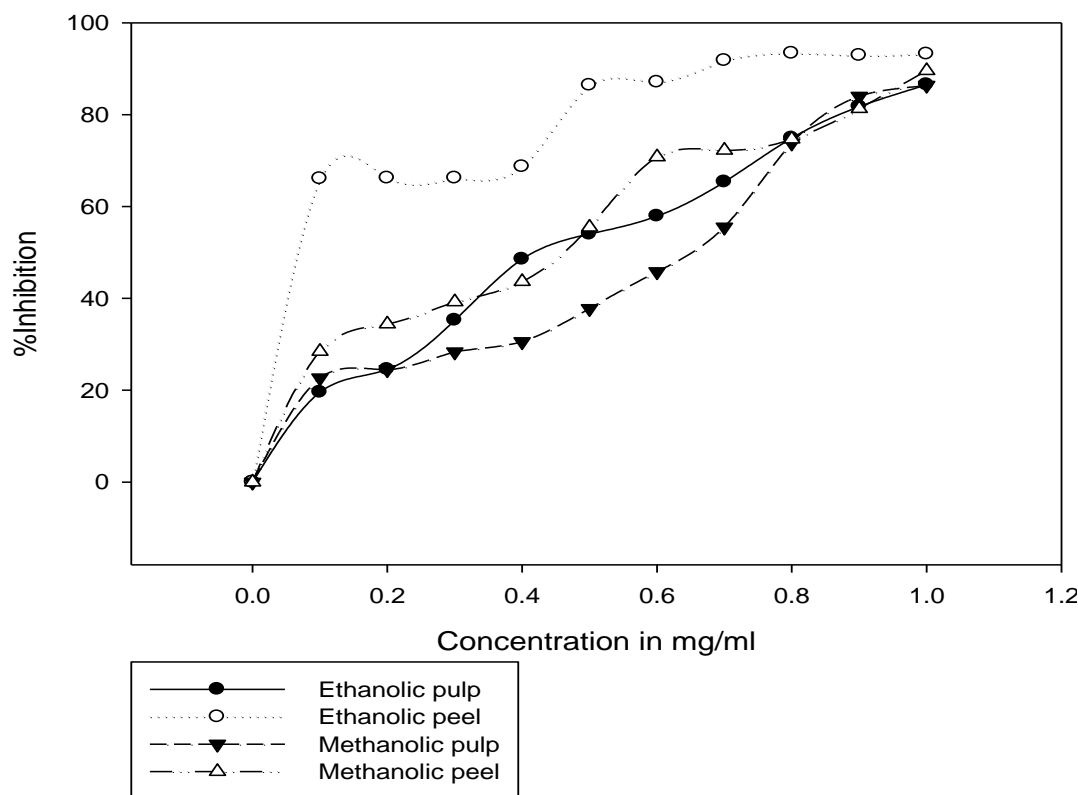
Total antioxidant activity, total phenols and flavonoids

The results of total antioxidant capacity assay (Table 1)

Table 1. Estimation of antioxidant capacities in pulp and peel extracts of *Manilkara zapota*.

Plant Extract	TAC	TPC	TFC
EtOH ext.pulp	31.40 ^a ± 1.77	11.43 ^a ± 1.09	5.05 ^b ± 0.17
EtOH ext.peel	31.74 ^a ± 1.91	21.17 ^c ± 0.45	6.36 ^d ± 0.11
MeOH ext.pulp	29.75 ^a ± 0.74	14.97 ^b ± 2.72	4.05 ^a ± 0.20
MeOH ext peel	63.31 ^b ± 0.48	20.85 ^c ± 0.44	5.89 ^c ± 0.08

Values represent mean ± SD of three replicates. TAC -Total antioxidant capacity was expressed as μM ascorbic acid equivalents g^{-1} ; TPC, total phenol content was expressed as mg catechol equivalent g^{-1} fresh tissue; TFC, total flavonoids content was expressed as mg rutin equivalents g^{-1} fresh tissue. Means followed by a common letter are not significantly different at the 5% level by DMRT.

**Figure 1.** Scavenging activity (%) on DPPH radicals by ethanolic and methanolic extracts of pulp and peel of *Manilkara zapota*.

obtained shows that methanolic extracts of sapota peel has highest antioxidant activity in the range of 63.31 $\mu\text{M}/\text{g}$ and the least activity was shown by methanolic extract of pulp in the range of 29.75 $\mu\text{M}/\text{g}$. The contents of total phenols and flavonoids in pulp and peel of sapota are depicted in Table 1. Among the ethanolic and methanolic extracts of both pulp and peel, ethanolic extract of sapota peel exhibited highest phenol (21.17 mg /g) and flavonoid content (6.36 mg/ g).

DPPH radical scavenging activity

The DPPH scavenging abilities of ethanolic and methanolic extract of pulp and peel extracts of sapota are

depicted in Figure 1. At 0.1 to 1 mg/ml, scavenging abilities of ethanolic extracts of pulp and peel of sapota exhibited 19.53 to 86.53 and 66.08 to 93.20%, whereas methanolic extracts of pulp and peel exhibited 22.67 to 86.38 and 28.44 to 89.57%, respectively. However, at 1 mg/ml, ethanolic extract of peel exhibited highest DPPH radical scavenging activity and methanolic extract of pulp showed least DPPH radical scavenging with respect to IC_{50} . An IC_{50} value is the concentration of the sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%. Among the crude extracts, ethanolic extract of sapota peel exhibited highest potential for DPPH radical scavenging activity as it had the least IC_{50} value of 0.13 mg/ml (Table 2).

Table 2. Comparison of IC₅₀ values of the various *in vitro* assays.

Crude extract	DPPH	ABTS+	Nitric oxide	LPO
EtOH- pulp	0.50	0.96	3.41	6.63
EtOH- peel	0.13	0.51	1.31	1.92
MeOH- pulp	0.95	0.67	3.61	6.62
MeOH- peel	0.45	0.39	2.30	1.98

DPPH, Diphenyl picryl hydrazyl; ABTS, 2,2 -azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); LPO, lipid peroxidation.

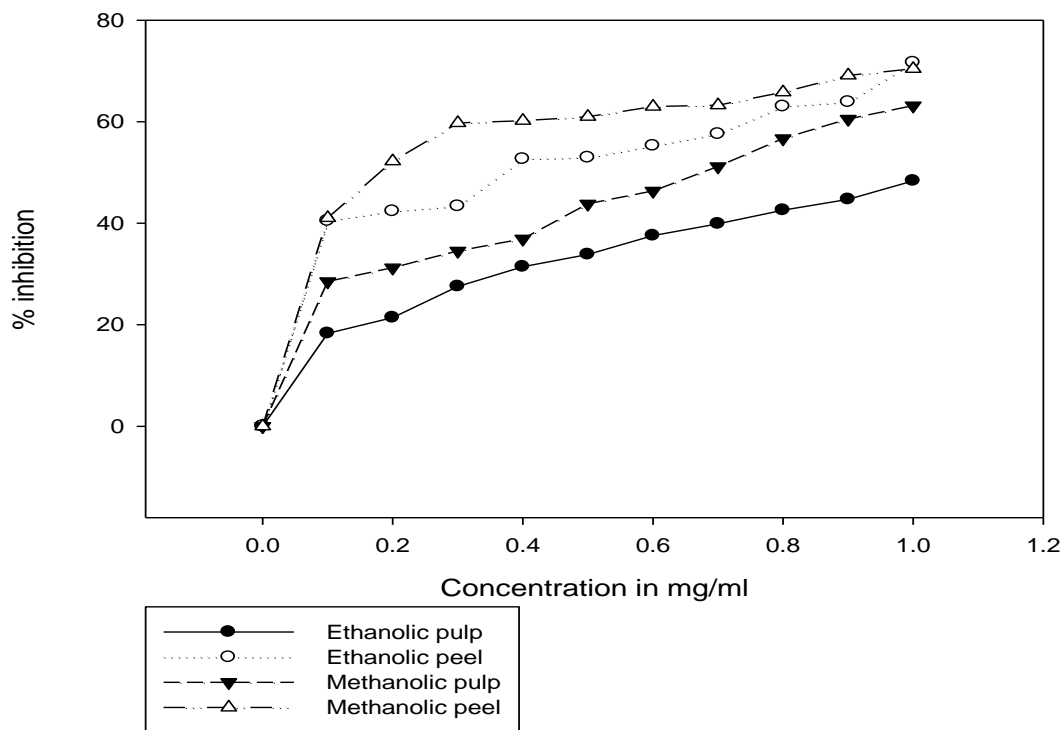


Figure 2. Scavenging activity (%) on ABTS cation radicals by ethanolic and methanolic extracts of pulp and peel of *Manilkara zapota*.

ABTS radical scavenging activity

The percentage inhibition of the ABTS radical by both ethanolic and methanolic extracts of pulp and peel of sapota increases with increase in concentration as shown in Figure 2. At 0.1 to 1 mg/ml, scavenging abilities of ethanolic extracts of pulp and peel extracts of sapota showed inhibition of 18.3 to 48.35 and 40.49 to 71.65%, while methanolic extracts of pulp and peel showed percentage inhibition of 28.52 to 63.13 and 41.03 to 70.42%, respectively. However, at 1 mg/ml, ethanolic extract of peel showed the highest ABTS radical scavenging activity and ethanolic extract of pulp showed least ABTS scavenging activity. Among the crude extracts, methanolic extract of peel showed the highest potential for ABTS radical scavenging activity as it exhibited least IC₅₀ value of 0.39 mg/ml (Table 2).

Nitric oxide radical scavenging activity

The nitric oxide scavenging potential of both ethanolic and methanolic extract of sapota pulp and peel is represented in Figure 3. At 1 to 10 mg/ml, scavenging abilities of ethanol extracts of pulp and peel of sapota on nitric oxide radicals were 51.38 to 70.25 and 61.48 to 83.91% and methanolic extracts of sapota pulp and peel were 44.48 to 75.31 and 60.26 to 76.96%. Among the crude extracts of pulp and peel of sapota, both ethanolic extracts of peel exhibited a highest potential for nitric oxide scavenging activity with a IC₅₀ value of 1.31 mg/ml (Table 2).

Lipid peroxidation inhibition activity

The lipid peroxidation inhibition capacity of ethanolic and

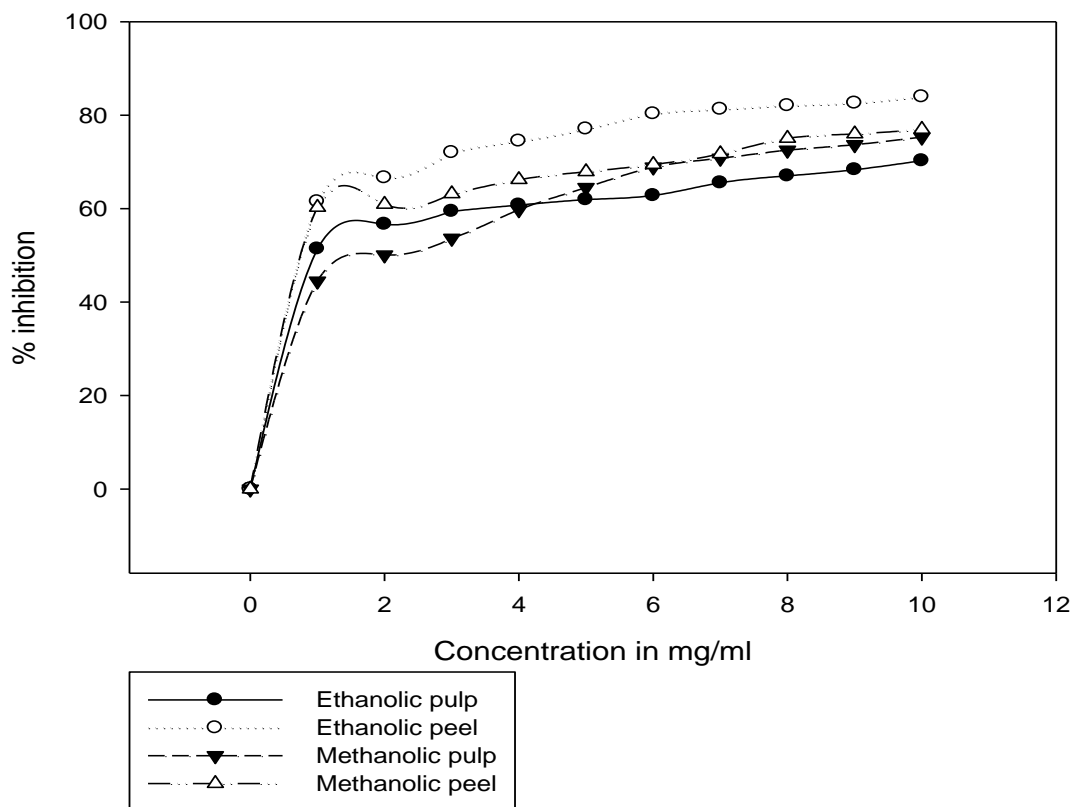


Figure 3. Scavenging activity (%) on nitric oxide radicals by ethanolic and methanolic extracts of pulp and peel of *Manilkara zapota*.

methanolic extracts of pulp and peel of sapota is displayed in Figure 4. At 1 to 10 mg/ml, lipid peroxidation inhibition of ethanolic extracts of pulp and peel were 12.64 to 68.94 and 57.22 to 95.85%, whereas methanolic extracts of pulp and peel were 12.43 to 71.80 and 23.10 to 72.51%, respectively. However, at 10 mg/ml, ethanolic extract of peel showed highest inhibition and ethanolic pulp showed least inhibition. Among the crude extracts, ethanolic extract of peel exhibited a highest potential for lipid peroxidation inhibition activity as it has least IC_{50} value 1.92 mg/ml (Table 2).

DISCUSSION

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer. The defensive effects of natural antioxidants in fruits and vegetables is attributed to three major groups: vitamins, phenolics and carotenoids. Sapota is reported to contain polyphenols, leucoanthocyanidins, tannins and triterpenoids that contribute for its antioxidant properties (Ma et al., 2003; Anand et al., 2004). The preliminary screening tests may

be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development. Similar observations for phytochemicals like alkaloids, phenols, tannins, flavonoids, saponins, steroids, quinines, cellulose were reported in *Acalypha indica*, *Vitex negundo* and *Coriandrum sativum*.

Total antioxidant activity

The total antioxidant assay gives an estimate of the overall antioxidant potential of the plant. There is a formation of phosphomolybdenum complex, the intensity of which indicates the potential of the sapota as a scavenger of free radicals. The results obtained in the present investigation revealed that methanolic extract of sapota peel showed highest antioxidant activity. Studies by Shui et al. (2004) depicted that unripe chiku are found to possess extremely high antioxidant capacity that is not attributed to L-ascorbic acid, a constituent that is partly responsible for the antioxidant capacity of many fruits.

Flavonoids and total phenols

Flavonoids are large compounds occurring ubiquitously in food plants. They occur as glycosides and contain seven

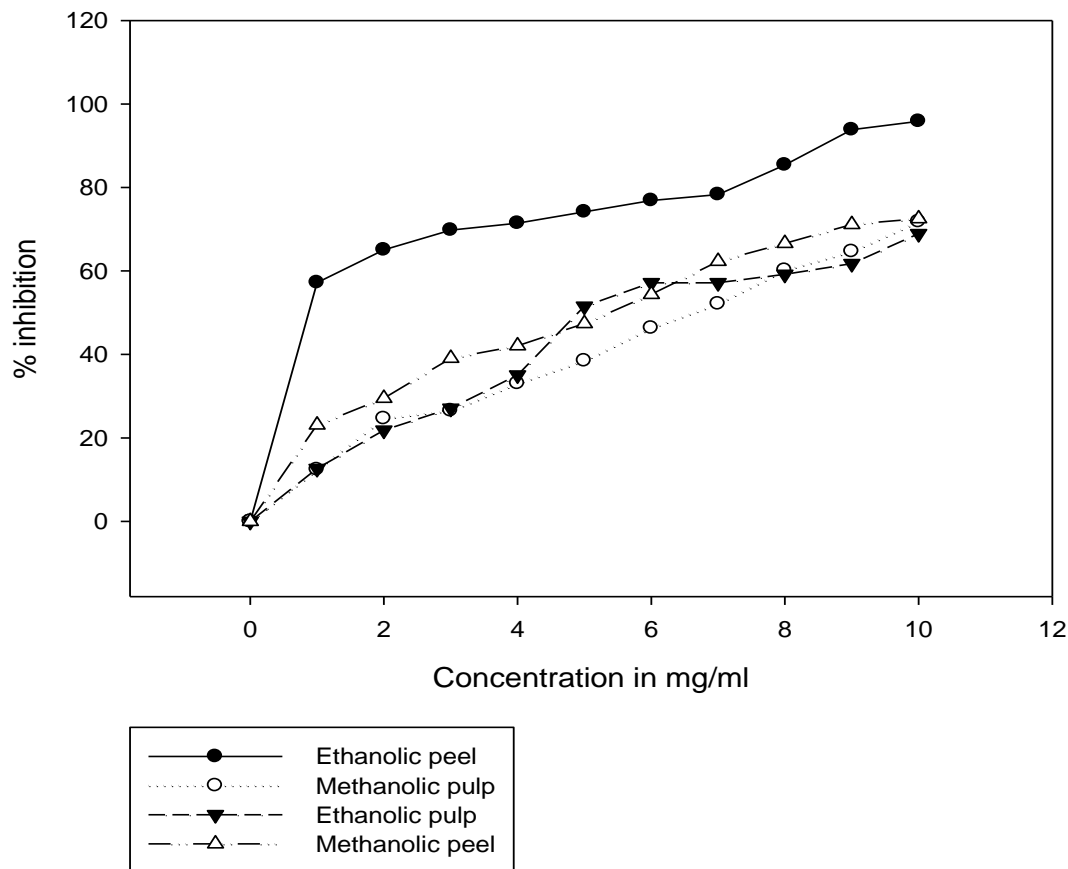


Figure 4. Lipid peroxidation inhibition (%) in goat liver homogenate by ethanolic and methanolic extracts of pulp and peel of *Manilkara zapota*.

ral phenolic hydroxyl groups on their structure. Many flavonoids are found to be strong antioxidants capable of effectively scavenging reactive oxygen species because of their phenolic hydroxyl groups (Cao et al., 1997). Phenols are secondary metabolites in plants and are known to possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and also decrease cardiovascular complications (Yen et al., 1993). Among the ethanolic and methanolic extracts of both pulp and peel extracts of sapota, ethanolic extract of peel showed the highest phenol and flavonoid content.

DPPH radical scavenging activity

DPPH scavenging activity was measured by the slightly modified spectrophotometric method. 1,1-diphenyl-2-picryl-hydrazyl radical is scavenged by antioxidants through the donation of protons forming reduced DPPH. The electrons become paired off and the solution loses color depending on the number of electrons taken up. The color change from purple to yellow after reduction is quantified by the decrease of absorbance at 517 nm (Shimada et al., 1992). The extent of DPPH radical

scavenged was determined by the decrease in intensity of purple color in the form of IC_{50} values. Lower IC_{50} value represents higher antioxidant activity. In the present study, ethanolic extract of peel exhibited highest DPPH radical scavenging activity among the crude

extracts. Studies by Ma et al. (2003) showed the high antioxidant activity in DPPH assay in the ethyl acetate soluble fraction of the fruit.

ABTS radical scavenging activity

ABTS assay measures the relative antioxidant ability to scavenge the radical ABTS and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and chain breaking antioxidants (Miller et al., 1997). Among the crude extracts, methanolic extract of peel showed highest potential for ABTS radical scavenging activity. The antioxidant activity of sapodilla fruit has been reported to be very high in the ABTS assay (Leong and Shui, 2002).

Nitric oxide radical scavenging activity

Nitric oxide is a free radical produced in mammalian cells and is involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases (Ialenti et al., 1993). Oxygen reacts with excess NO to generate nitrite and peroxynitrites anions, which act as free radicals (Cotran et al., 1999). The extent of nitric oxide radical scavenged was determined by the decrease in intensity of pink colored chromophore at 546 nm in the form of IC₅₀ values. With respect to the present study, ethanolic extract of the peel displayed highest nitric oxide scavenging potential compared to other extracts.

Lipid peroxidation inhibition activity

Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids (PUFA) and involves free radicals. This is a basic membrane damage process and results in deleterious effects. Initiation of lipid peroxidation was carried out by the addition of ferrous sulphate. This occurs by the formation of hydroxyl radicals by Fenton's reaction (Braugler et al., 1987). These produce MDA which reacts with TBA to form a pink chromogen. This inhibition could be caused by the absorbance of ferryl-per ferryl complex or by scavenging the hydroxyl or the superoxide radicals or by changing the Fe³⁺/Fe²⁺ ratio or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself. Among the crude extracts, ethanolic extract of sapota peel exhibited a highest potential for lipid peroxidation inhibition activity.

Conclusion

The peel extracts of sapota showed highest radical scavenging potential and high antioxidant activity compared to pulp extracts. Among the ethanolic and methanolic extracts, ethanolic peel extracts exhibited least IC₅₀ which indicates high potential for multiple radical scavenging activity, speculating the fact that such fruit peels could be utilized in food industries for their value added compounds. Further trials are underway to optimize extraction parameters for the isolation of bioactive components responsible for its antioxidant activity.

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