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RESEARCH ARTICLE

Variation in antioxidant activity at two ripening stages of wild mango, *Spondias pinnata* (L.f.) Kurz., an underutilized fruit

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ABSTRACT
Antioxidant compounds play a significant role in preventing and scavenging free-radicals by reducing oxidative stress and providing protection to humans against degenerative diseases and infections. Obviously, antioxidant molecules of plant origin are pivotal to combat the oxidative harm in cells. Present work intended to evaluate the antioxidant capacity of the fruits of *Spondias pinnata*. Methanol extracts of the fruits at two stages of maturity were prepared and investigated by various antioxidants analyses such as total antioxidant activity, reducing power and radical scavenging assays (DPPH, nitric oxide and hydrogen peroxide). Its total phenol, flavonoid and tannin contents were also determined. *Spondias pinnata* fruit extracts exhibited effective antioxidant activity and its IC_{50} values of the unripe fruits were 65, 66, 72.23, 83.25, 66.75 µg/ml and ripe fruits were 124.24, 92.50, 97.66, 144.10, 72.25 µg/ml, for total antioxidant activity, reducing power, DPPH radical scavenging, nitric oxide radical scavenging and hydrogen peroxide scavenging assays respectively. The extracts, especially unripe fruit extract had good amounts of total phenolic and flavonoid contents which might contribute the antioxidant activities considerably. It is evident from the study that the fruits of *S. pinnata* possessed potent antioxidant activity and it can be considered as a good dietary choice among the underutilized fruits as well as common fruits. Therefore, wild mango may be a good choice of antioxidants of plant origin for dietary and pharmaceutical uses.

Introduction

Free-radicals are a collection of highly reactive molecules that impair the cellular functions by damaging nucleic acids, proteins and lipids (1, 2). Free radicals of cellular origin or from outside are one of the major agents for various diseases such as cancer, neurodegenerative diseases, cardiovascular diseases, diabetes and to even ageing (3). Antioxidant molecules (natural and synthetic) are considered as possible means for hindering and treating such diseases. Obviously, most of the synthetic molecules are found to be unsafe due to their possible carcinogenicity and toxicity (4). On the other hand, the intake of fruits and vegetables has a strong hindrance in developing such chronic diseases (5). In most cases, vitamins and secondary metabolites particularly polyphenols are considered to be responsible for such antioxidant activity (6). Studies on antioxidant activities of various fruits, vegetables, spices, medicinal plants and even microalgae have shown the presence of rich amounts of natural antioxidants in them (7–9), Hence, the search for plants containing powerful antioxidants remains to be a focus of investigators.

Wild fruits are gaining increasing attention from pharmaceutical industries because of their nutritional value, vitamin and mineral contents and medicinal properties. With reference to their therapeutic characteristics, the antioxidant potential is the most commonly studied benefits (10). India and other tropical countries have an abundance of fruits in the wild and many of them belong to the underutilized category. Wild mango (*Spondias pinnata* (L.f.) Kurz.; Anacardiaceae) is such fruit and is mainly distributed in India, Sri Lanka and other southeast Asian countries (11, 12). It is chiefly found in the Western Ghats and north-eastern states of India. It is a medium-sized tree found in the

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deciduous to semi-evergreen forests and flowers from March to April. The fruits ripen early in November and available up to December (11, 12). Unripe fruits are preserved in brine (pickle) and are usually used in culinary preparations such as curries, jams, sherbet and condiments in places where it grows naturally (13). The nutritional value of this fruit is comparable to that of plum, apricot, cherry, peach and mango (12). Different parts of the plant, especially fruits, stem bark, leaves, and roots have been used in conventional medicinal preparations to treat various ailments such as bronchitis, ulcer, dysentery, diarrhoea, vomiting, skin diseases, dyspepsia and muscular rheumatism (14). The stem bark possesses ample amounts of phenolics and flavonoids and has free radical scavenging activities (15, 16) and anticancer properties (17). An early study (18) points out cytotoxic, antimicrobial and anti-inflammatory activities of essential oils obtained from fruit peel. The leaves and raw fruits have reported for its antibacterial, antioxidant (14), free radical scavenging activity and antitumor properties (12). However, no detailed studies of antioxidant and radical scavenging activities of unripe and ripe fruits of this plant from the Western Ghats are available. Hence, the present study is aimed to evaluate the antioxidant activities and to estimate the phenolic, flavonoid and tannin contents at two ripening stages of fruits of *S. pinnata*.

Materials and Methods

Collection of fruits and preparation of extract

The unripe (approximately 60 days after pollination) and ripe (approximately 220 days after pollination) fruits were collected randomly from natural populations of *S. pinnata* growing in the Vellikulam area (altitude 400–700 m) of Kottayam, Kerala, India. A total of 27 unripe and ripe fruits were divided into three replicates (n=3) with nine fruits per replicate. A voucher specimen (RHT65257) has been placed at St. Joseph's College, Tiruchirappalli.

The fruits were brought into the laboratory and washed with distilled water. The pericarp and mesocarp were separated together from seeds of unripe and ripe fruits and subsequently, air-dried for a short time and shade dried at room temperature (29 ± 3 ˚C). The dried pericarp and mesocarp were made into a fine powder and methanolic extract was prepared using Soxhlet apparatus (65 ˚C for 8 hr) from 100 gm powder. The extracts were concentrated using rotary evaporator (Rotavapor® R-210, BUCHI, Flawil, Switzerland) (temperature 40 °C and pressure 337 mbar) and kept at 4 °C for analysis.

Determination of total antioxidant capacity

Phosphomolybdenum technique was used to examine the total antioxidant capacity of unripe and ripe fruits of *S. pinnata* (19). 3 ml of the reagent solution [600 mM H_2SO_4, 28 mM Na_2HPO_4 & 4 mM $(NH_4)_6Mo_7O_{24}$] was transferred to various concentrations (25 µg, 50 µg, 75 µg, 100 µg, 125 µg & 150 µg) of the extract followed by incubation at 95 °C for 90 min. The mixture was allowed to cool and optical density was measured at 695 nm (Shimadzu, UV-150-02, Kyoto). Methanol and ascorbic acid were used as blank and reference compound respectively. The percentage total antioxidant capacity was calculated by using the formula:

$$(A_s/A_m) \times 100. \quad \text{(i)}$$

Here, A_m is maximum absorbance tested, A_s is the absorbance of the sample.

Determination of reducing power

To various concentrations (25 µg, 50 µg, 75 µg, 100 µg, 125 µg & 150 µg) of the extract, 0.2 M sodium phosphate buffer (2.5 ml, pH 6.6) and potassium ferricyanide (5 ml, 1%) were added. Trichloroacetic acid (5 ml, 10%) was transferred to the mixture after incubation (20 min at 50 °C). The reaction mixture was centrifuged and supernatant (5 ml) was mixed with distilled water (5 ml) and ferric chloride (1 m, 1%). The optical density was measured at 700 nm. The reducing power of the samples at different concentrations was calculated by using the above formula (i). This method was slightly modified from the earlier described standard method (20).

Radical scavenging assay (1,1- diphenyl-2-picrylhydrazyl, DPPH)

The free radical scavenging capacity of the samples was analysed using DPPH radical (21). DPPH solution (1 ml, 0.1 mM) in methanol was added to the sample solution (25 µg, 50 µg, 75 µg, 100 µg, 125 µg & 150 µg) in methanol (3 ml). It was mixed thoroughly and kept in the dark (30 min) and the optical density was measured at 517 nm. DPPH radical scavenging efficacy was calculated using the following formula:

$$(\%) = ((A_0 - A_1)/A_0) \times 100 \quad \text{(ii)}$$

where A_0 and A_1 are absorbance values of the control and of the test samples respectively. Ascorbic acid was used as reference compound.

Radical scavenging assay (Nitric oxide, NO)

Griess Illosvoy reaction (22) was done to assess the nitric oxide scavenging capacity of the samples. To various doses (25 µg, 50 µg, 75 µg, 100 µg, 125 µg & 150 µg in 3 ml) of the sample solution in 10 mM sodium nitroprusside (dissolved in 0.5 M phosphate buffer, pH 7.4), Griess reagent (0.1% α-napthyl-ethylenediamine in distilled water and 1% sulphanilamide in 5% H_3PO_4) was added after incubation for 60 min at 37 °C. The pink chromophore generated during the reaction was measured at 540 nm. Nitric oxide scavenging capacity (%) was calculated using the above equation (ii).

Radical scavenging assay (Hydrogen peroxide, H_2O_2)

Hydrogen peroxide radical scavenging was determined according to the standard method described (23). Different concentrations (25 µg, 50 µg, 75 µg, 100 µg, 125 µg & 150 µg) of the sample were mixed with 0.6 ml of 43 mM (in 0.1 M phosphate buffer, pH 7.4) hydrogen peroxide

solution. Optical density of the mixture was detected after 10 min at 230 nm. The inhibition activity (%) was calculated using the equation (ii).

Total phenolic content determination

Phenolic contents of the extracts were estimated using Folin-Ciocalteu method (24). A volume of 800 µl Folin-Ciocalteu reagent and 2 ml sodium carbonate (7.5%) were added to 100 µl of extract. Make the volume (diluted) to 7 ml using distilled water and was kept in dark (2 hr). The optical density was measured at 765 nm. Gallic acid was used as reference molecule and phenolic content was quantified as mg gallic acid equivalent (GAE)/100 gm dry weight of the extract (DW).

Total flavonoid content determination

It was estimated using $AlCl_3$ (25) method and quercetin was the reference molecule. To 0.3 ml distilled water, 100 µl extract and 30 µl $NaNO_2$ (5%) were added. 30 µl of $AlCl_3$ (10%) was added to this after 5 min. Finally, 200 µl of sodium hydroxide (1 mM) was transferred to the mixture after a lapse of 5 min. The test solution was diluted to 1 ml with distilled water before measuring the absorbance at 510 nm. The flavonoid content was quantified as mg quercetin equivalent (QEE)/100 gm DW of the extract.

Total tannin content determination

According to Folin-Denis method (26), 50 µl of extract was diluted to 7.5 ml with distilled water. To this, Folin-Denis reagent (0.5 ml) and sodium carbonate (1 ml) were transferred. Finally, the volume of the mixture was made up to 10 ml by adding distilled water. Optical density was measured at 700 nm. Reference compound used was Tannic acid and tannin content quantified as mg tannic acid equivalent (TAE)/100 g DW of the extract.

Statistical analysis

All analysis was performed in three times for each group and results were given as mean (n=3) ± S.D. and compared by Tukey's HSD tests. The fruit extracts and reference compound providing 50% inhibition (IC_{50}) were calculated from the graph as µg/ml through the dose-response curve. Results were significant if $P \leq 0.05$. SPSS (Version 22.0) software was used for statistical analysis.

Results and Discussion

Total antioxidant activity

The total antioxidant capacity is based on the reduction of Mo (VI) to Mo (V) by metabolites present in the extract and consequently the formation of phosphate/Mo (V) complex at acidic conditions (19). The results of the total antioxidant activity of fruit extracts of *S. pinnata* and reference compound ascorbic acid are depicted in Table 1. The total antioxidant potential of unripe fruits was found to be promising with IC_{50} value of 65 µg/ml (Table 6), which is equal to that of standard ascorbic acid (63.25 µg/ml). The results also show that there was no significant variation in total antioxidant capacity at 50 µg/ml and 100 µg/ml concentrations of unripe fruit extract and ascorbic acid (Table 1). It was found that the ripe fruit extract required a quantity of 124.25 µg/ml for reducing 50% (IC_{50}) of Mo (VI) to Mo (V). At the same time a concentration of 125 µg/ml of unripe fruit extracts reduced 82% of Mo (VI) to Mo (V), i.e. methanol extract of unripe fruits has effective antioxidant potential than that of ripe fruits. The antioxidant activity was given most often by the phenolic compounds in the extracts and this might be reflected in the antioxidant activity of unripe and ripe fruit extracts of *S. pinnata* (27, 28). The unripe fruit extract had good amounts of phenolics (384.24 ± 0.18), which were considerably different from that of mature fruit extract (243.13 ± 0.29) (Table 7). Similar results were reported in *Psidium guajava* L. and *Nypa fruticans* Wurmb (29). In addition to phenolics, flavonoids and tannins have antioxidant potential to a certain extent (30, 31).

Phenolics are important phytoconstituents among various natural antioxidants because of their multiple biologic effects and direct contribution to antioxidant activity (27). According to an earlier study (28), the antioxidant activity of fruits and vegetables are most often correlated with the phenolic constituents. Various studies also demonstrated the wide medicinal applications of plant phenolics (32–34).

Reducing power assay

The methanol extracts of wild mango showed a substantial level of reducing activity. The results of the reducing power of the fruits at two stages of maturity are depicted in Table 2. Reducing capacity of the samples increased with increasing concentrations and significant difference ($P < 0.05$) was observed in reducing power between unripe and ripe fruits with IC_{50} values of 66 µg/ml and 92.5 µg/ml respectively. Reference molecule ascorbic acid exhibited IC_{50} values of 41.75 µg/ml (Table 6). Among the two maturity stages, unripe fruits showed potent reducing power than that of the ripe fruits. The phytochemical analyses confirm that the unripe fruit extract was more abundant in phenolics and flavonoids than ripe fruits. The direct relationship of antioxidant activity with the reducing power of phytoconstituents was well studied on the orange pulp (Newhall variety) (35) and found that immature orange pulp has potent reducing power. Such a reduction in reducing capacity with maturity might be correlated with the reduction of phenolic contents and flavonoids in the fruits (36). Various other studies explain that the antioxidant activity attributed to reducing power could be affected by stages of maturity, geographical origin or cultivar, harvest time, storage time (37), storage conditions, temperature (38, 39) or exogenous usage of chemicals (40).

The reducing power indicates the capacity of a reducing agent to donate electrons and convert free radicals in to more stable form (41). The reducing power of wild mango, especially unripe fruit extract, was found to be higher which give emphasis to its enormous antioxidant potential. The results point out that methanol extract of *S. pinnata* fruits might

Table 1. Total antioxidant activity of methanol extracts of unripe and ripe fruits of *S. pinnata*.

Standard/ sample	Concentration (µg/ml)					
	25	50	75	100	125	150
Ascorbic acid	18.33±0.25[a]	40.86±0.80[a]	58.60±0.10[a]	71.23±0.11[a]	83.60±0.26[a]	92.26±0.30[a]
Unripe fruits	16.94±0.15[b]	39.91±0.16[a]	57.06±0.12[b]	70.51±0.42[a]	81.98±0.23[b]	90.43±0.35[b]
Ripe fruits	9.53±0.45[c]	21.56±0.51[b]	29.83±0.76[c]	40.36±0.55[b]	50.80±0.26[c]	58.83±0.47[c]

Values are mean of triplicate ± S.D. Superscripts with the same letters within each column are not significantly different at $p < 0.05$.

Table 2. Reducing power of methanol extracts of unripe and ripe fruits of *S. pinnata*.

Standard/ sample	Concentration (µg/ml)					
	25	50	75	100	125	150
Ascorbic acid	31.13±0.25[a]	58.90±0.10[a]	74.65±0.13[a]	86.80±0.10[a]	97.18±0.10[a]	100.00±0.00[a]
Unripe fruits	27.10±0.20[b]	42.40±0.21[b]	54.36±0.13[b]	66.56±0.14[b]	78.63±0.20[b]	89.42±0.18[b]
Ripe fruits	16.50±0.43[c]	28.43±0.40[c]	40.46±0.51[c]	54.33±0.32[c]	60.56±0.45[c]	68.30±0.60[c]

Values are mean of triplicate ± S.D. Superscripts with the same letters within each column are not significantly different at $P < 0.05$.

be converted the radical species into stable form and inhibited radical chain reaction. Previous study also showed the direct correlation between antioxidant activities and reducing power (42). Hence, reducing capacity may be taken as an important indicator of potent antioxidant capacity.

DPPH radical scavenging activity

DPPH radical-scavenging (Table 3) effect of methanol extracts of *S. pinnata* fruits was concentration-dependent mode and had an IC_{50} value of 72.23 and 97.5 µg/ml for the unripe and ripe fruits respectively, and that of ascorbic acid was 41 µg/ml (Table 6). The DPPH radical scavenging analysis clearly showed a significant difference (Table 3) in the inhibition of radicals by unripe and ripe fruit extracts. The increased activity of unripe fruits might be due to the occurrence of several antioxidants and thus indicated its good antioxidant potential (26, 43). Previous studies showed that, the DPPH radical scavenging activity of pomegranate aril was decreased with ripening (44). Lowered radical inhibition ability with ripening might be attributed to reduction in total phenolics, conversion of anthocyanins and other biochemical changes (45).

DPPH radical scavenging is used as a good *in vitro* model to measure the antioxidant activity of phytoconstituents in a short time. Electron donors in the extracts could reduce the radical to a stable diamagnetic molecule and it could be visualized by purple to yellow colour change (2). In the present study, scavenging of DPPH radical by the methanol extract of fruits of *S. pinnata* was lower than that of ascorbic acid. However, the extract exhibited appreciable scavenging activity, especially the unripe fruits and therefore, significant correlation between DPPH radical scavenging capacity and phenolic and tannin contents might be existed (2).

Nitric oxide scavenging activity

The results of this assay, as important as other radical scavenging activities of the extracts, was given in Table 4. Nitric oxide scavenging capacity of the extracts and the reference compound was increased with increasing concentrations. The 50% (IC_{50}) of radicals was scavenged by unripe fruit extract at a concentration of 83.25 µg/ml, while that of the ripe fruit extract was 144 µg/ml (Table 6). Results of the study revealed that the unripe fruit extract found to be a better scavenger of nitric oxide than ripe fruit extract and significant difference ($p < 0.05$) was detected among three experimental conditions (unripe fruit extract, ripe fruit extract, and ascorbic acid). It is well known that continuous production of nitric oxide in mild quantities, lead to vascular collapse, whereas chronic expression leads to various carcinomas and inflammatory condition though it is a signalling molecule (46). Peroxynitrite

Table 3. DPPH radical scavenging activity of methanol extracts of unripe and ripe fruits of *S. pinnata*.

Standard/ sample	Concentration (µg/ml)					
	25	50	75	100	125	150
Ascorbic acid	38.40±0.34[a]	56.43±0.23[a]	69.18±0.16[a]	80.21±0.25[a]	92.58±0.07[a]	100.00±0.00[a]
Unripe fruits	18.18±0.17[b]	34.72±0.14[b]	51.68±0.27[b]	63.99±0.20[b]	76.06±0.22[b]	87.95±0.10[b]
Ripe fruits	11.36±0.50[c]	25.63±0.55[c]	38.53±0.45[c]	51.46±0.41[c]	59.26±0.47[c]	67.53±0.55[c]

Values are mean of triplicate ± S.D. Superscripts with the same letters within each column are not significantly different at $P < 0.05$.

Table 4. Nitric oxide scavenging activity of methanol extracts of unripe and ripe fruits of *S. pinnata*.

Standard/ sample	Concentration (µg/ml)					
	25	50	75	100	125	150
Ascorbic acid	22.13±0.32[a]	38.56±0.20[a]	53.50±0.26[a]	61.36±0.20[a]	70.36±0.20[a]	78.43±0.05[b]
Unripe fruits	20.97±0.10[a]	34.75±0.20[b]	46.20±0.08[b]	58.22±0.25[b]	70.96±0.12[a]	81.56±0.25[a]
Ripe fruits	12.46±0.40[b]	25.86±0.70[c]	33.26±0.47[c]	39.10±0.55[c]	45.53±0.40[b]	51.43±0.40[c]

Values are mean of triplicate ± S.D. Superscripts with the same letters within each column are not significantly different at $P < 0.05$.

Table 5. H_2O_2 scavenging activity of methanol extracts of unripe and ripe fruits of *S. pinnata*.

Standard/ sample	Concentration (µg/ml)					
	25	50	75	100	125	150
Ascorbic acid	32.53±0.30[a]	54.49±0.39[a]	68.50±0.20[a]	81.31±0.10[a]	88.23±0.21[a]	96.70±0.20[a]
Unripe fruits	23.33±0.17[b]	38.65±0.08[b]	55.62±0.22[b]	67.41±0.37[b]	78.39±0.22[b]	87.04±0.07[b]
Ripe fruits	18.54±0.33[c]	32.31±0.33[c]	52.38±0.40[c]	65.53±0.40[c]	71.70±0.43[c]	76.43±0.37[c]

Values are mean of triplicate ± S.D. Superscripts with the same letters within each column are not significantly different at $P < 0.05$.

Table 6. The IC_{50} values (µg/ml) of methanol extract of *S. pinnata* fruits and reference compound ascorbic acid.

Assays	Unripe fruits	Ripe fruits	Ascorbic acid
Total antioxidant activity	65[a]	124.25[b]	63.25[a]
Reducing power	66[b]	92.5[c]	41.75[a]
DPPH scavenging	72.23[b]	97.5[c]	41.0[a]
Nitric oxide scavenging	83.25[b]	144.0[c]	68.5[a]
Hydrogen peroxide scavenging	66.75[b]	72.25[c]	45.00[a]

Superscripts with the same letters within each row are not significantly different at $P < 0.05$.

Table 7. Total phenolic, flavonoid and tannin contents of methanol extract of *S. pinnata* fruits.

Extract	Total phenolics (mg GAE/100 g DW)	Total flavonoids (mg QEE/100 g DW)	Total tannins (mg TAE/100 g DW)
Unripe fruits	384.24 ± 0.18	217.46 ± 0.36	72.28 ± 0.23
Ripe fruits	243.13 ± 0.29	183.87 ± 0.64	95.60 ± 0.25

Values are mean of triplicate ± S.D.

($ONOO^-$), a highly reactive species, would be formed when NO reacts with superoxide radical, this in turn increases the toxic effect (47). The present study proved that the fruit extracts of *S. pinnata* have good nitric oxide scavenging effect, especially the unripe fruit extract. Recent studies showed that phenolic compounds have greater NO scavenging capacity in surroundings with acidic pH (48). This may account for the higher nitric oxide radical scavenging activity of the unripe fruit extract.

Hydrogen peroxide scavenging activity

The H_2O_2 scavenging by methanol extracts of fruits of *S. pinnata* and the reference compound ascorbic acid were depicted in Table 5. The IC_{50} values of unripe and ripe fruit extracts were 66.75 and 72.25 µg/ml respectively, and that of ascorbic acid was 45 µg/ml (Table 6). H_2O_2 is an oxidant which is being continuously formed in tissues as by-products of metabolism. As shown in Table 5, *S. pinnata* fruit extracts showed an effective H_2O_2 scavenging in a dose-dependent mode and have significant scavenging potential as reference compound. The scavenging of H_2O_2 by plant extracts may be attributed to their phenolics, which can donate electrons to H_2O_2 and neutralize it to water and the higher H_2O_2 scavenging activity of the unripe fruit extract was also correlated with amounts of total phenolics (49). It has been reported that hydrogen peroxide radical scavenging properties of medicinal fruits such as *Terminalia chebula* Retz. *T. bellirica* (Gaertn.) Roxb. and *Emblica officinalis* Gaertn. were found to be negligible, whereas that of *S. pinnata* showed a promising scavenging effect on H_2O_2 radical (50).

Total phenolic, flavonoid and tannin contents

Antioxidant activity is a fundamental property vital for life. A direct relationship exists between many of the biological processes, such as antiaging, anticarcinogenicity and antimutagenicity and the presence of natural antioxidants (28, 51, 52) of either exogenous or endogenous origin. The phytoconstituents such as phenolics, flavonoids and tannins contribute much to the antioxidant activity of fruits and vegetables (28, 52–55), which are good source of natural antioxidants. The phenolic compounds are the principal antioxidants, which scavenge free radicals, are widely distributed in plant kingdom (56).

Total phenolic content (Table 7) of wild mango was 384.24 ± 0.18 and 243.13 ± 0.29 mg GAE/100 gm DW of the unripe and ripe fruit extracts respectively. A significant difference in phenolic contents was found in ripe and unripe fruits of *S. pinnata* in the present study. This might be associated with the increased polyphenol oxidase activity at mature stages (57, 58), thereby an apparent decrease was observed at the ripe fruit extract. The polymerization of leucoanthocyanidins and hydrolysis of the arabinose ester of hexahydrodiphenic acid also contribute much to the reduction in phenolic contents at later stages of fruit development (59). The fruit phenolic content can be affected not only by ripeness but also by other factors such as species, variety, harvesting time, geography, cultivation, climate and storage conditions (60–62).

The results of the occurrence of flavonoids and tannins in relation to the fruit ripening is presented in Table 7. Flavonoids are benzopyrone derivatives, abundant in plants and shows antioxidant activity. The flavonoid contents of unripe and ripe fruits of *S. pinnata* was 217.46 ± 0.36 and 183.87 ± 0.64 mg QE/100 gm DW of the extracts respectively. Flavonoids are good for health because of their antioxidant and anti-inflammatory activities. These molecules hinder the low-density lipoprotein (LDL), the oxidation and facilitate cardioprotection (63). Present study indicates that the ample amounts of flavonoids in *S. pinnata* fruits substantiates its use as natural antioxidants. Additionally, the total tannin content of the unripe and ripe fruit extracts was 72.28 ± 0.23 and 95.60 ± 0.25 mg TAE/100 g DW mg of the extracts respectively (Table 7).

Flavonoids and tannins have found effective antioxidant activity through multiple actions such as scavenging of reactive oxygen species (ROS), hindering the pathways of ROS generation, chelating trace metals and reduce oxidizing radicals by donating hydrogen and their effects on human nutrition and health (51, 64). In the present study, the highest flavonoid contents were found in unripe fruit extract and highest tannin contents were observed in ripe fruit extracts. Earlier reports showed that various flavonoids converted to complex molecules such as tannins and lignins as the fruits became ripen (65). Consequently, due to change in phenolics, flavonoids and tannins with maturity, the ripe fruits of *S. pinnata* possessed

relatively lower quantities of flavonoids and phenolics as well as greater amount of tannins than unripe fruits.

In the present study, the highest total antioxidant activity, reducing power and different radical scavenging activities were displayed by unripe fruit extract. Therefore, the study revealed that there was a significant relationship between antioxidant capacity and phenolics and flavonoids contents as it was higher in the unripe fruit extract of *S. pinnata*.

Conclusion

The extracts of *Spondias pinnata* fruits, especially unripe extracts showed antioxidant potential which are as good as to that of reference compound ascorbic acid. From the present study, it could be recommended that ripening stage of *S. pinnata* fruits had effects on the amount of total phenolics, flavonoids and tannin contents which may directly be correlated with the antioxidant activity. In addition to phenolics, flavonoids and tannins, the raw fruits of *S. pinnata* (both ripe and unripe) may contain good amount of vitamin C (ascorbic acid) and hence, the actual antioxidant potential of these fruits may be higher than that obtained in the present study. Finally, we could suggest that *S. pinnata* fruits at any stage of maturity, especially the unripe fruits, be a good source of natural antioxidants and initiatives would be taken to procure the nutritional and medicinal benefits of this fruit.

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Authors' contributions

Both the authors have contributed equally to work.

Conflict of interests

The authors declare that we have no conflict of interest.

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