

## Research Article

### Antioxidant and anti-microbial activity, total phenolic and total flavonoid contents of the peel and seed of *Spondias dulcis* (Amberalla) fruit

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#### Abstract:

**Purpose:** Reactive oxygen species and the free radicals are implicated in many medical disorders as well as in the process of aging. Due to the toxicity of synthetic drugs there is much interest in natural products that could combat diseases. Furthermore side effects and resistance to antibiotics are well documented and as such there is much interest in herbal remedies that have antimicrobial activity. The objective of this research is to investigate the antioxidant and antibacterial potential of the extracts of the seed and peel of *Spondias dulcis* (Amberalla) fruit. **Method:** The percentage radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging assay. The reducing potential of the extracts were determined using Ferric ion reducing assay. The total phenol and total flavonoid contents were obtained from calibrations plots of Gallic acid and Quercetine respectively. The antibacterial assays against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. were carried out using Agar well method. **Results:** The antioxidant activity, total flavonoid and phenolic contents of the ethyl acetate, hexane and dichloromethane extracts of the Sri Lankan variety of *Spondias dulcis* was investigated. Of all extracts the highest activity was shown by the peel extracts of the *S.dulcis* fruit. The dichloromethane extract of the peel of the fruit showed the maximum activity in DPPH radical scavenging assay ( $93.40 \pm 0.02\%$ ) followed by the hexane extract of the peel ( $91.10 \pm 0.01\%$ ) compared to butylated hydroxy toluene (BHT) ( $74.26 \pm 0.01\%$ ) at the same concentration ( $127.5 \mu\text{g ml}^{-1}$ ). In the  $\text{Fe}^{3+}$  reducing power assay the dichloromethane and ethyl acetate extracts of the peel gave values of  $0.35 \pm 0.12\%$  and  $0.30 \pm 0.05\%$  respectively compared to BHT ( $0.71 \pm 0.04\%$ ) at the same concentration ( $3.33 \mu\text{g ml}^{-1}$ ). The highest phenolic content was found in the ethyl acetate extracts of peel ( $463.68 \pm 0.02 \text{ mg (GAE/g)}$ ). The maximum flavonoid content was seen in the hexane extract of the peel ( $1133.77 \pm 0.01 \text{ mg (QE/g)}$ ). However, the antibacterial assay of the peel and the seed extracts did not show a significant activity against the bacterial species; *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. **Conclusion:** Our results show that the non-utilized parts of the *S. dulcis* fruit contains high amount of antioxidant properties and may have the ability to reduce the risk of oxidative stress and defense against degenerative diseases.

**Keywords-** Antioxidant activity, DPPH, Phenolic content, Flavonoid content,



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## Introduction

It is increasingly evident that oxidative stress and the presence of free radicals is the major cause for many diseases.(1) Oxidative byproducts cause extensive damage to DNA, proteins, and lipids which is a major cause to aging and diseases such as cancer, cardiovascular diseases, immune system decline, brain dysfunction and cataracts.(2) Antioxidants such as ascorbate, tocopherol and carotenoids defenses against this damage.(1) Low dietary intake of fruits and vegetables doubles the risk of heart diseases, cancers and cataracts compared to high intake. Radicals like superoxide ( $O_2^-$ ), hydroxyl (OH) and molecules like hydrogen peroxide ( $H_2O_2$ ) are mutagens which are responsible for some of these damages.(1) Lipid peroxidation gives rise to mutagenic lipid epoxides, lipid hydroperoxides, lipid alkoxyl, peroxy radicals, and enals.(1) In addition to the protective mechanisms by enzymes dietary intake of antioxidants appears to be of great importance. Despite the advances in modern medicine, plant based remedies are increasingly popular due to side effects of synthetic counterparts.<sup>3</sup>In recent years multiple drug resistance in human pathogenic microbes has increased due to the indiscriminate use of synthetic antimicrobial drugs. In addition to this multiple drug resistance resulting from the chaotic application of commercial antimicrobial drugs, adverse effects such as hypersensitivity, allergic reaction, immune suppression abdominal pain, anorexia etc has driven the scientists to explore new and effective antimicrobial agents that could act as an alternative of the current regimens.(4) Hence the global interest in the study of antioxidant and antibacterial properties of various plants and their fruits have increased rapidly.(5)

The genus *Spondias* consists of 17 species.(4) Out of them seven are native to neotropics and about 10 are native to Asia. About 10 species of *Spondias dulcis* bear edible fruits. *S. dulcis* is a fast growing equatorial tree which is popular in Bangladesh in the name Amra while its English name is Hog plum or Golden Apple.(4) Basically it is used as a food source but the other parts of the plant are also used in various medical treatments. Its astringent bark is used as a remedy for diarrhea and it is also used in eyesight enhancement and eye infections. The fruit is also used to cure itchiness, internal ulceration, sore throat and inflammation of skin.(4) The fruit is also an antidote. Furthermore the polysaccharides identified from the fruit pulp have electrical activity on peritoneal macrophages and the leaves of the plant show anti diabetic activity.(4)

In this study the hexane, dichloromethane and ethyl acetate extracts of seeds and peel of *S. dulcis* fruit was studied for their antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and  $Fe^{3+}$  reducing activity), total phenolic content, total flavonoid content and antibacterial potential against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

### ***In vitro* antioxidant assay**

#### **DPPH radical scavenging assay**

This assay is based on the measurement of scavenging ability of antioxidants towards the stable radical  $DPPH^+$ .(6) A dilution series of each extract was prepared. DPPH reagent prepared in methanol (5mg/100ml, 2.0ml)

was added to each test sample (1.5ml) and mixed with 0.5ml of methanol. The mixture was allowed to stand for 10 min in the dark and absorbance was measured at 517 nm.(6) Butylated hydroxy toluene (BHT) was used as reference standard. The antioxidant capacity (%) was estimated using the following formula.

$$\text{Antioxidant effect (\%)} = (\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance}) \times 100$$

### Ferric ion reducing assay

The reducing power was based on  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  transformation.  $\text{Fe}^{2+}$  can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. 0.25 ml of different concentrations of the sample or standard (BHT) was mixed with 1ml of the phosphate buffer (0.2M  $\text{Na}_2\text{HPO}_4$ , 0.2M  $\text{KH}_2\text{PO}_4$ , pH 6.6) and 1ml of 1% potassium ferricyanide. The mixture was incubated at  $50^\circ\text{C}$  for 20 minutes followed by addition of 10% trichloroacetic acid (1.25ml).<sup>6</sup>To this mixture 0.25 ml of 1%  $\text{FeCl}_3$  was added and kept for 20 minutes.<sup>6</sup>The absorbance was measured at 700 nm. Higher absorbance in the reaction mixture indicates a higher reducing power. The reducing power (%) was calculated based on the following formula.

$$\text{Reducing power (\%)} = (\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance}) \times 100$$

### Antibacterial assay

Agar well diffusion method was carried out to screen anti-bacterial properties.(7) The crude extracts were tested against the following species

- *Staphylococcus aureus*
- *Escherichia coli*

- *Bacillus subtilis*

All the equipment and materials used were sterilized in the autoclave for 20 minutes at  $121^\circ\text{C}$ . Nutrient broth was measured and transferred into a conical flask containing 100 ml of distilled water. The solution was sterilized in an autoclave for 15 minutes at  $121^\circ\text{C}$ . Then the mixture was allowed to cool and then the bacteria species were carefully introduced under sterile conditions. The 4 mixtures were incubated overnight. Nutrient agar was weighed, transferred to 2 conical flasks containing 150 ml of distilled water. The solution mixture was sterilized in an autoclave at  $121^\circ\text{C}$  for 15 minutes. The solution were then taken out and allowed to cool for about 15 minutes and poured into sterile petri dishes.(7) Then the bacteria suspension in nutrient broth was diluted with distilled water up to 0.01% in a test tube. Using a micropipette 100 $\mu\text{l}$  of bacteria suspension was evenly spread on the agar medium. About 6 mm diameter wells were made on agar. Azithromycin was used as the positive control, while dimethyl sulfoxide (DMSO) alone was used as the negative control. Azithromycin was prepared at a concentration of 5 mg/ml and the crude extract was prepared by dissolving 2 mg of each extract in 0.3 ml of DMSO. The dishes were sealed with parafilm and were incubated overnight in upright position at  $30^\circ\text{C}$ . Then the inhibition zones were measured after 18 hours.(7)

### Total phenolic content

The total phenolic content of extracts of peel and seed was determined by Folin Ciocalteu method with slight modifications.(8) The sample (0.5 ml) was mixed with 0.5 ml of Folin Ciocalteu reagent (1:1). After 5 min, 0.5 ml of  $\text{Na}_2\text{CO}_3$  (6% w/v) followed by 2ml of distilled water was added to the mixture.

The mixture was kept in the dark for 60 min, after which its absorbance was read at 765 nm. Gallic acid (GA) standard curve ( $R^2=0.99$ ) was used to measure the phenolic content and total phenolic content was expressed as mg GAE/g of dry weight of extract.

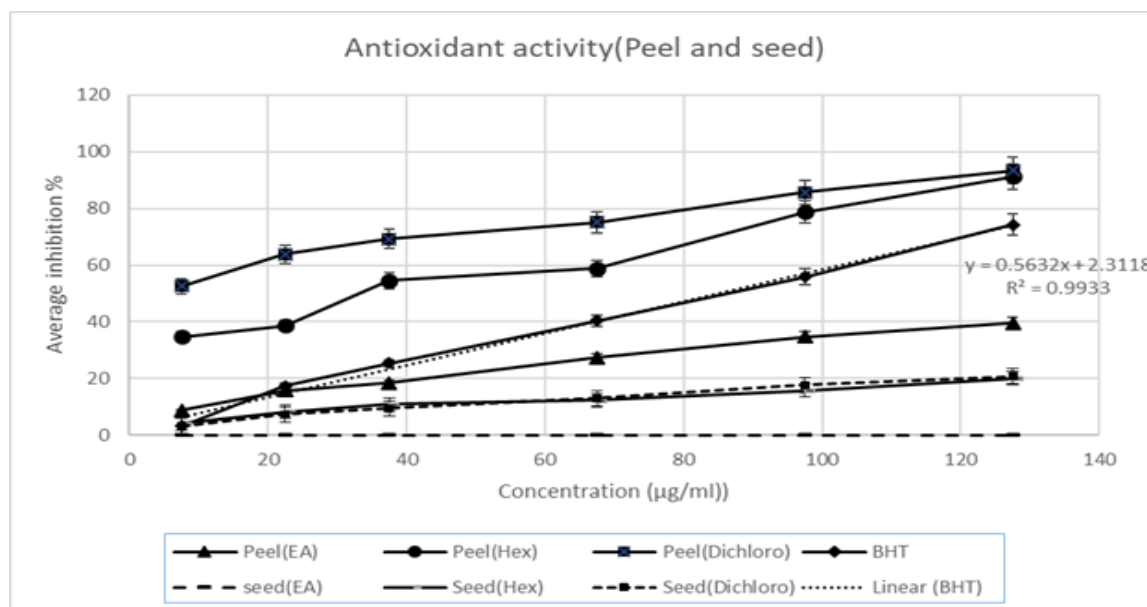
### Total flavonoid content

Total flavonoid content was measured by the aluminum chloride colorimetric assay. (8) In a 0.5 ml of the sample/ standard, 2 ml of distilled water was added followed by 0.15 ml of 5%  $\text{NaNO}_2$ . The mixture was kept in the dark for 5 minutes. After that 0.15 ml of 10%  $\text{AlCl}_3$  was added and again

kept in the dark for 6 minutes. After 6 minutes 1 ml of  $1\text{mol dm}^{-3}$  NaOH and 1 ml of distilled water was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve ( $R^2=0.99$ ) was constructed using Quercetine (QE) and total flavonoid content was expressed as mg QE/g of dry weight of the extract.

### Results

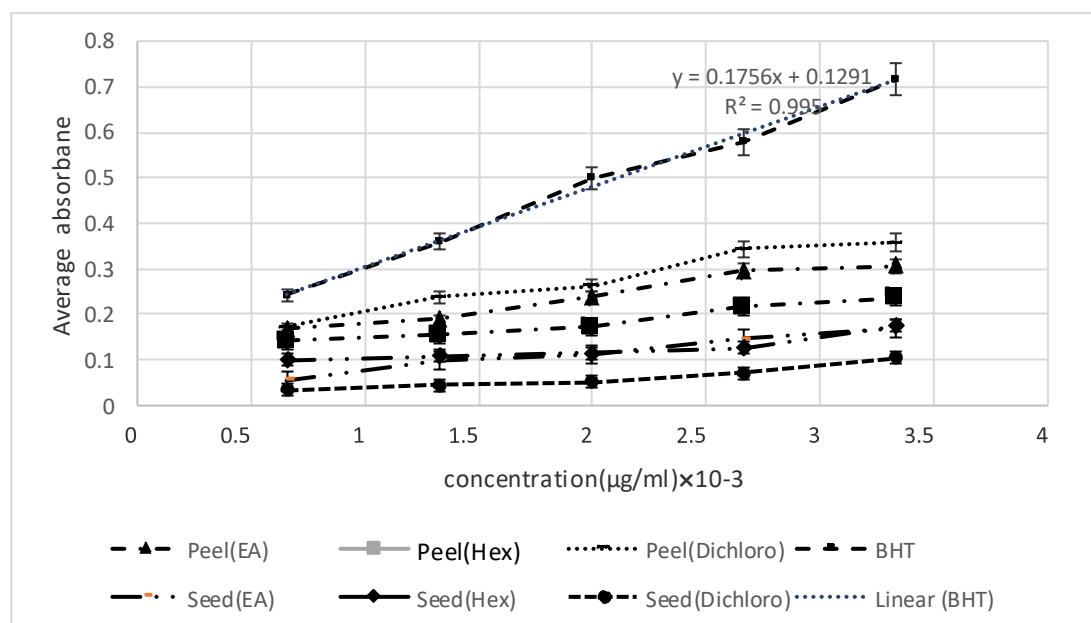
Figure 1 shows that the DPPH radical scavenging activity of extracts of the peel and seeds of *S. dulcis* progressively increases with increase in concentration.



**Figure 1: DPPH radical scavenging activity of different extracts of the peel and seed of *S. dulcis* fruit**

All six extracts shows the radical scavenging activity at all concentrations. The inhibition values are directly related to the radical scavenging ability of the sample. Dichloromethane and hexane extracts of the peel shows the highest activity at all concentrations and it is higher than that of BHT. The lowest activity is shown by the extracts of the seed.

Figure 2 shows that the change of ferric ion reducing power of the different extracts with the concentration. In here also the absorption values are directly related to the reducing ability of the plant extracts. All the extracts show increase in activity with increase in concentration. The maximum activity was given by the dichloromethane extract of the peel. The positive control BHT is also plotted.



**Figure 2: Ferric ion reducing power on different extracts of the peel and seed of *S.dulcis* fruit**

**Table 1: Total flavonoid and phenolic contents of the different extracts of *S.dulcis* fruit**

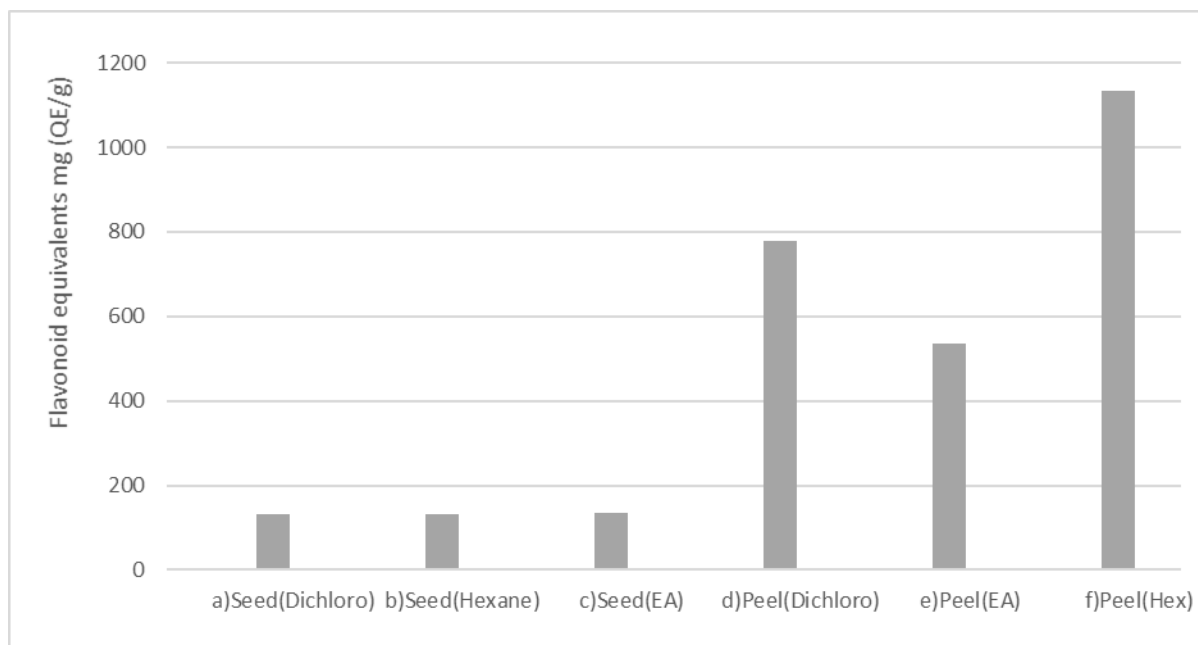
Sample	Flavonoid equivalents mg (QE/g)	Phenolic equivalents mg (GAE/g)
a) Seed(Dichloro)	131.22±0.06	55.07±0.01
b) Seed(Hexane)	130.98±0.06	86.43±0.01
c) Seed(EA)	134.45±0.07	205.61±0.06
d) Peel(Dichloro)	778.05±0.01	157.46±0.01
e) Peel(EA)	534.56±0.02	463.67±0.02
f) Peel(Hex)	1133.77±0.01	84.34±0.01

Total phenolic contents and flavonoid contents of the *S. dulcis* fruit extracts are given Table 1. The maximum flavonoid content was seen in the hexane extract of the peel which is amounted to (1133.76±0.01) (Figure 3).

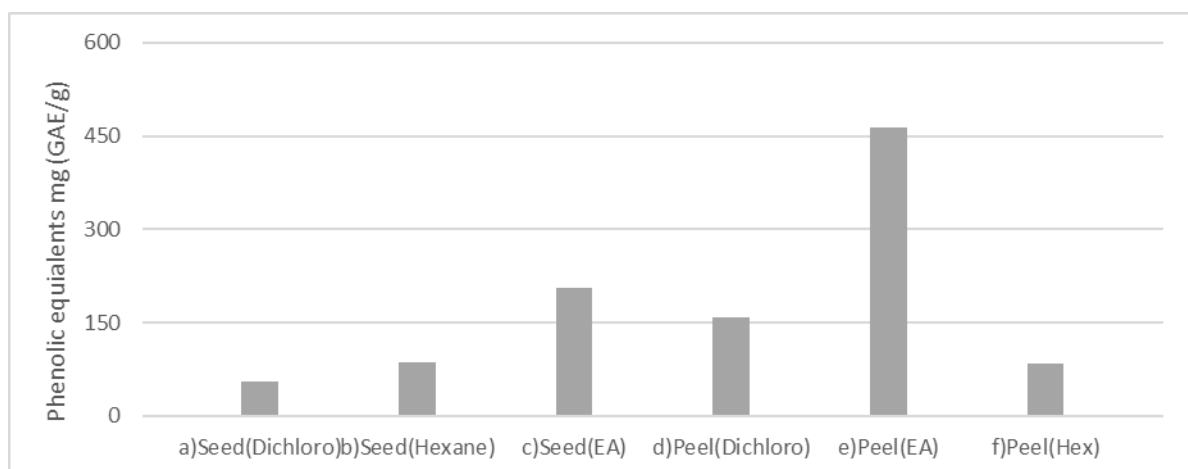
The highest phenolic contents were found in the ethyl acetate extracts of peel and the seed which is recorded as

(463.67±0.02) and (205.61±0.06) GAE/g of the dry weight respectively (Figure 4).

In the antibacterial assays none of the plant extracts showed any significant inhibition zones against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* compared to the positive control azithromycin (inhibition zones, *Bacillus subtilis* 3.4 cm, *Escherichia coli* 2.5 cm, *Staphylococcus aureus* 3.5 cm)



**Figure 3: Total flavonoid contents of the different extracts of the peel and seed of *S.dulcis* fruit.**



**Figure 4: Total phenolic contents of the different extracts of the peel and seed of *S.dulcis* fruit.**

## Discussion

The main objective of this research was to determine the antioxidant activity, antibacterial activity total flavonoid and phenolic contents of the peel and seed of *S. dulcis* fruit. The DPPH assay is based on the ability of antioxidant compounds to lose hydrogen and become stable. The DPPH

free radical, which is having a maximum absorption at 517 nm, can easily accept electrons or hydrogen radical from antioxidant molecules to become a stable diamagnetic molecule. The deep purple color of the DPPH radical is reduced due to the activity of radical scavengers in the test sample.(9)

Reducing power is a measure of ability of extract to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ . Compounds which are having the ability to react with potassium ferricyanide ( $\text{Fe}^{3+}$ ) to form potassium ferrocyanide ( $\text{Fe}^{2+}$ ) finally reacts with ferric chloride to form ferric-ferrous complex.(9) This results the yellow color of the test sample to turn into blue.

Our results indicate that the dichloromethane extract of the peel of *S. dulcis* fruit shows the highest activity with respect to radical scavenging activity and the reducing power. Phenols and flavonoids are considered as the important class of phytochemicals which can exert antioxidant activity.(10) The total phenolic content in the six extracts were estimated by Folin-Ciocalteu method using Gallic acid as the standard.(11) The reagent is formed from a mixture of phosphotungstic acid and phosphomolybdic acid. Phenols reduce the phosphomolybdic acid present in the Folin-Ciocalteu reagent from yellow to green.(11) Of all extracts ethyl acetate extract of the peel showed the highest phenolic content. Hexane extracts of both peel and seed showed more or less the same phenolic content.

The total flavonoid content for hexane, ethyl acetate and dichloromethane extracts were measured with the aluminum chloride colorimetric assay using Quercetin as the standard. Aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxide groups of flavones and flavonols.<sup>12</sup> In addition to that it forms labile complexes with *ortho* dihydroxide groups in A/B rings of flavonoids.(12) Maximum flavonoid content was observed in hexane extract of the peel.

It is noteworthy that all extracts peel and seed of *S. dulcis* fruit showed very significant DPPH radical scavenging activity, ferric ion reducing activity, high phenolic content and high flavonoid content. Therefore this research study proves that the consumption of peel and seeds of *S. dulcis* fruit may combat diseases and disorders that arise due to the oxidative stress.

## Conclusion

In addition to the endogenous enzymatic antioxidant defenses, consumption of dietary antioxidants are of great importance. Fruits and vegetables are the main sources of antioxidants. Our results shows that the non-utilized parts of the *Spondias dulcis* fruit contains high amount of antioxidant properties and may have the ability to reduces the risk of oxidative stress and defense against degenerative diseases.

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