ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 2, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTIOXIDANT ACTIVITY OF SYZYGIUM JAMBOS FRUIT AND ITS SEED

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Received: 01 October 2022, Revised and Accepted: 15 November 2022

ABSTRACT

Objective: This study was designed to investigate the phytochemical screening of different solvent extracts, total phenolic, and flavonoid content and 13 different methods of *in vitro* antioxidant activity of *Syzygium jambos* fruit and its seed.

Methods: The antioxidant activity of the methanolic extract of *S. jambos* fruit and its seed was carried out by 2,2-diphenyl-1-picrylhydrazyl, ABTS radical scavenging activity, site-specific and non-site-specific hydroxyl radical scavenging activity, superoxide radical scavenging, nitric oxide radical scavenging, potassium ferricyanide reducing assay, ferric thiocyanate, total antioxidant capacity, hydrogen peroxide radical scavenging, thiobarbituric acid reactive substances, ferric reducing antioxidant power, and cupric reducing antioxidant Cusing standard procedures.

Results: The preliminary phytochemical screening has revealed the presence of phenolics, flavonoids, alkaloids, tannin, saponin, and carbohydratesexcept for steroids and terpenoids. The total phenolic content and flavonoid content of *S. jambos* fruit and its seed was found to be (*S. jambos* fruit – 127.61 mg of GAE/100 g, *S. jambos* seed – 217.34 mg of GAE/100 g) and (*S. jambos* fruit – 8.64 mg of QE/100 g, *S. jambos* seed – 15.97 mg of QE/100 g), respectively. The fruit and its seed also showed significantly strong antioxidant activity in different *in vitro* methods.

Conclusion: The *Syzygium jambos* fruit and its seed have anadequate quantity of phytochemicals that act as an antioxidant and scavenge free radicals efficiently. Hence, the fruit and its seed may be considered as effective in the prevention and treatment of oxidative stress-induced diseases such as diabetes, cardiovascular diseases, cancer, arthritis, gout, neurodegenerative diseases, and respiratory tract infections.

Keywords: *Syzygium jambos*, Fruit, Seed, Phytochemicals, Antioxidant activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i2.46496. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

All parts of the plant are known to have a considerable quantity of phytochemicals and natural antioxidants. Antioxidants are substances that prevent and stabilize the damage caused by free radicals. It has been proven that the consumption of an antioxidant-enriched diet can lower the risk of several diseases such as cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative, and inflammatory-related diseases caused by free radicals [1].

Medicinal plants have been traditionally used in the treatment of several diseases due to their pharmacological and therapeutic properties. *Syzygium jambos* (L.) Alston is an evergreen tree that belongs to the Myrtaceae family. It is generally found in South-east Asia like Indonesia, Malaysia, and different parts of India. The fruit of this plant is known as "Rose apple," "Malabar plum," "Plum rose," and "Golap-jam" in West Bengal, India. The fruit is yellow and sweet in flavor with crispy and crunchy texture. The fruit also has a unique delicate rose fragrance. In the center of the fruit, there are brown color coated hard seeds that are loosely bound with the inner wall of the fruit [3].

According to folk medicine, *S. jambos* fruit has been used as a tonic for the brain, liver, and as a diuretic. The seeds are used to treat diarrhea, dysentery, and catarrh [4]. It has been reported that *S. jambos* fruit and seed have a significant amount of micronutrients and trace elements [5]. In a recent study, some anti-nutritional factors of *S.jambos* fruit and its seed have been estimated such as oxalate (Fruit – 4.54 mg/100 g, Seed – 9.87 mg/100 g), phytate (Fruit – 5.68 mg/100 g, Seed – 7.34 mg/100 g), alkaloid (Fruit – 3.53 mg/100 g, Seed – 5.38 mg/100 g), tannin (Fruit – 32.43 mg/100 g, Seed – 168.29 mg/100 g), and saponin (Fruit – 386.49 mg/100 g, Seed – 528.55 mg/100 g) [6]. It has been found that the ethanolic extract of *S. jambos* leaves can effectively decrease blood glucose levels [7].

The aims and objectives of this study are –

- To evaluate the detailed antioxidant activity of *S. jambos* fruit and its seed.
- To popularize the health benefits of this underutilized fruit and its seed among people.

Fig. 1: Syzygiumjambos fruit and its seed [2]

MATERIALS AND METHODS

Sample collection and identification

Fruits of *S. jambos* were collected from the market of Baruipur, near Kolkata (West Bengal, India). The sample was identified by the Botanical Survey of India, Shibpur, Howrah. The specimen No. is UC/SD-01, dated on December 30, 2019.

Preparation of methanolic extracts

Initially, fruits and seeds were separated and the outer layer of the seeds was removed and cleaned thoroughly. After that, a paste of both the samples was made for freeze-drying using (Laboratory Freeze Dryer, Model-DPRG-01). Then, the methanolic extract of the fruit and its seed was prepared by adding 1 g of each freeze-dried sample powder to the ratio of 80:20 (methanol: water). After that the solutions were stirred on the magnetic stirrer at room temperature for 5 h, it was then centrifuged at 6000 rpm at −4°C for 10 min by using a cold centrifuge (Eppendrof, centrifuge 5430R). Finally, the mixtures were filtered through Whatman No. 1 filter paper. Then, the filtrates were stored at −4°C for further experiments [8].

Chemicals and reagents

All the chemicals and solvents used in the experiments were of analytical grade.

Qualitative phytochemical analysis

Preliminary phytochemical analysis was carried out of different solvent extracts of the *S. jambos* fruit and its seed. The presence of different phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, and tannins was determined by standard procedures [9].

Quantitative analysis of phytochemicals

Estimation of total phenolics content [10]

The total phenolic content of the extracts was determined by the Folin-Ciocalteau reagent. Gallic acid was used as the standard. In brief, 0.5 mL of 50% folin-ciocalteu reagent and 1 mL of 10% sodium carbonate were added to 1 mL of an extract of each sample. The mixture was allowed to stand at room temperature for 60 min in a dark place. Then, the absorbance was measured at a wavelength of 765 nm using a spectrophotometer. The TPC value was calculated from a calibration curve and the results are expressed as mg of gallic acid equivalents/100 g of dry weight.

Estimation of total flavonoids content [10]

Total flavonoid content was determined by the aluminum chloride method using quercetin as a standard. 1 mL of the test sample and 4 mL of water were added to a volumetric flask. Add 0.3 mL of 5% sodium nitrite, and 0.3 mL of 10% aluminum chloride was added after 5 min. After 6 min of incubation at room temperature, 1 mL of 1 (M) sodium hydroxide was added to the reaction mixture. Then, the final volume was made up to 10 mL with distilled water. The absorbance of the sample was measured against the blank at 510 nm. The TFC value was calculated from a calibration curve and the results are expressed as mg of quercetin equivalent/100 g of dry weight.

Determination of *in vitro* antioxidant analysis

The antioxidant activity of methanolic extract of *S. jambos* fruit and *S. jambos* seed was determined by the following different *in vitro* methods (Table 1):

Determination of percentage inhibition

Percentage inhibition is calculated as $[A_0\text{-}A_1/A_0 \times 100]$.

A_0 is the absorbance of the control and A_1 is the absorbance of the test sample/standard. All experiments were performed in triplicates (n=3) and data are expressed as mean±SE. IC_{50} is calculated using the straight line equation of the concentration curve plotted against the percentage inhibition.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening was carried out of different solvent extracts (aqueous, methanol, ethanol, and chloroform) of *S. jambos* fruit and *S. jambos* seed, shown in Table 2. The presence of phenols, flavonoids, tannins, saponins, alkaloids, carbohydrates, protein, and reducing sugar has been found except for steroids and terpenoids.

Table 1: *In vitro* antioxidant activity of methanolic extract of *Syzygium jambos* fruit and its seed

S. No	Method	Standard
1.	Ferric reducing antioxidant power assay (FRAP) by Benzie and Strain [11]	Ascorbic acid
2.	DPPH Free Radical Scavenging Assay by Desmarchelier *et al*. [11]	Gallic acid
3.	ABTS+Free Radical Scavenging Activity by Re *et al*. [11]	Ascorbic acid
4.	Total antioxidant capacity (Phosphomolybdenum Assay) by Prieto *et al*. [11]	Ascorbic acid
5.	Non-site-specific Hydroxyl radical scavenging activity by Aruoma and Halliwell [12]	Gallic acid
6.	Site-specific hydroxyl radical scavenging activity by Hinneburg *et al*. [12]	Gallic acid
7.	Superoxide radical scavenging activity by Liu [13]	Gallic acid
8.	Nitric oxide radical scavenging activity by Marcocci *et al*. [13]	Gallic acid
9.	Hydrogen peroxide Scavenging activity by Ruch *et al*. [13]	Ascorbic acid
10.	Thiobarbituric acid reactive substances assay by Kikuzaki and Nakatani [12]	Ascorbic acid
11.	Cupric ion reducing assay by Apak *et al*. [12]	Gallic acid
12.	Ferric Thiocyanate Method by Kikuzaki and Nakatani [12]	Ascorbic acid
13.	Potassium ferricyanide reducing assay (PFRAP) by Tundis *et al*. [13]	Ascorbic acid

Quantitative phytochemical analysis

Dietary polyphenols represent a group of secondary metabolites that are extensively found in fruits, vegetables, wine, tea, extra virgin olive oil, chocolate, and other cocoa products. Phenolic compounds and flavonoids are the largest subgroups under the dietary polyphenols [14].

Total phenolic content

The total phenolic contents of the methanolic extract of *S. jambos* fruit and *S. jambos* seed were determined by the Folin-Ciocalteu method. Gallic acid was used as the standard in this assay. Phenolic compounds have a wide variety of biological activities such as antioxidant, anticarcinogenic and they can modify gene expression [14]. In the present study, the total phenolic content of *S. jambos* fruit and the seed was found to be 127.61 mg of GAE/100 g and 217.34 mg of GAE/100 g, respectively (Table 3 and Fig. 2). These values were determined from the standard graph of gallic acid (y= 0.0102x+0.85, R^2=0.84). Several studies have shown a close relationship between total phenolic content and high antioxidant activity. From this data, it may be concluded that the *S.jambos* seed has more antioxidant capacity than the fruit.

Total flavonoid content

The total flavonoid contents of the methanolic extract of *S.jambos* fruit and its seed were determined by the aluminum chloride method. Quercetin was used as the standard in this assay. Flavonoids have antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, and protein kinase inhibition activity [14]. In this study, the total flavonoid content of *S.jambos* fruit and *S.jambos* seed was found to be 8.64 mg of QE/100g and 15.97 mg of QE/100g, respectively (Table 3 and Fig. 2).These values were determined from the standard graph of quercetin (y=0.0266x+0.424, R^2=0.96).

***In vitro* antioxidant activity**

There are several methods to determine the antioxidant capacity of any substance. These methods vary in terms of their assay principles and experimental conditions to measure the antioxidant activity of

Table 2: Phytochemical screening of different solvent extracts of *Syzygium jambos* fruit and *Syzygium jambos* seed

Phytochemicals	Name of the test	Aqueous		Methanol		Ethanol		Chloroform	
		Fruit	Seed	Fruit	Seed	Fruit	Seed	Fruit	Seed
Phenols	Ferric cyanide test	+++	++	++	++	++	++	++	++
Flavonoids	Lead acetate test	++	++	++	++	++	++	++	++
Alkaloids	Wagner's test	+	+	+	+	+	+	+	+
Tannins	Braymer's test	++	++	++	++	++	++	++	++
Saponin	Foam test	+++	+++	+++	+++	+++	+++	++	++
Terpenoids	Salkowski reaction test	_	_	_	_	_	_	_	_
Carbohydrate	Molisch test	++	++	++	++	++	++	++	++
Protein	Biuret test	+	+	+	+	+	+	+	+
Steroids	Salkowski reaction test	_	_	_	_	_	_	_	_
Reducing sugar	Benedict test	++	+	++	+	++	+	++	+

(+++) strong presence, (++) moderate presence,(+) present, (-) absent

any food item [15]. *S. jambos* fruit and its seed have many different bioactive components with antioxidative potential. That's why 13 *in vitro* methods (2,2-diphenyl-1-picrylhydrazyl [DPPH], ABTS radical scavenging activity, site-specific and non-site-specific hydroxyl radical scavenging activity, superoxide radical scavenging, nitric oxide (NO) radical scavenging, potassium ferricyanide reducing assay [PFRAP], ferric thiocyanate [FTC], total antioxidant capacity, hydrogen peroxide radical scavenging, thiobarbituric acid reactive substances [TBARS], ferric reducing antioxidant power [FRAP], and cupric reducing antioxidant capacity [CUPRAC]) have been used to investigate the antioxidant activity of the fruit and its seed. In this study, different concentrations (100–500 µg/ml) were taken to determine the % inhibition activity of the standard and both the sample extracts. From the % inhibition, IC_{50} value was determined. It was calculated as the concentration at which a sample would inhibit free radicals by 50%. The IC_{50} (half-maximal inhibitory concentration) is a parameter widely used to measure the antioxidant activity of any test sample [15]. This is inversely proportional to the free radical scavenging activity of the sample. Hence, the lower the IC_{50} value signifies the higher antioxidant activity of the sample.

DPPH radical scavenging activity

The DPPH radical scavenging method is one of the most common methods. It provides an easy, rapid, and convenient way to evaluate antioxidant activity. DPPH is a stable free radical. The hydrogen atom donating ability of the methanolic extract of the *S. jambos* fruit and its seed was determined by the decolorization of the methanol solution of DPPH. The % inhibition at different concentrations (100–500 µg/mL) of gallic acid, *S. jambos* fruit, and seed ranged from 55.8 to 85.67, 39.65 to 90.81, and 48.45 to 98.45 withthe IC_{50} value of 28.71±0.94 µg/mL (y=0.0787x+47.76, R^2=0.91), 160.44±0.81 µg/mL (y=0.1368x+34.74, R^2=0.96), and 111.54±1.03 µg/mL (y=0.1367x+28.186, R^2=0.94), respectively (Table 4 and Fig. 3). Therefore, *S. jambos* seed showed better scavenging activity against DPPH free radicals when compared to *S. jambos* fruit.

Site-specific and non-site-specific hydroxyl radical scavenging activity

The hydroxyl radical trapping potential of any substance is determined by hydroxyl radical-induced deoxyribose degradation. Here, site-specific and non-site-specific hydroxyl radical scavenging activity of the methanolic extract of the samples was evaluated. In the site-specific assay, EDTA is not available. Fe^{3+}can bind directly to the deoxyribose molecule and produce hydroxyl radical [16]. The % inhibition at different concentrations (100–500 µg/mL) of the gallic acid, *S. jambos* fruit and seed ranged from 37.23 to 78.39, 28.6 to 58.5, and 31.63 to 74.98 with the IC_{50} value of 215.14±0.62 µg/mL (y=0.1058x+27.41, R^2=0.98), 376.98±1.29 µg/mL (y=0.0631x+26.26, R^2=0.87), and 327.44±0.78 µg/mL (y=0.0942x+19.22, R^2=0.82), respectively (Table 4 and Fig. 3).

In the non-site-specific assay, hydroxyl radical is generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction) [16]. The % inhibition of the standard, *S. jambos* fruit and seed ranged from 56.38 to 87.45, 32.28 to 79.52, and 27.55 to 82.67 with the IC_{50} of 30.71±0.59 µg/mL (y=0.0811x+47.51, R^2=0.92), 246.18±0.73 µg/mL (y=0.1299x+18.02, R^2=0.96), and 238.98±0.88 µg/mL (y=0.1381x+17.02, R^2=0.97), respectively.

The results suggest that *S. jambos* seed has a better scavenging capacity against hydroxyl radical (site-specific and non-site-specific) than the *S. jambos* fruit. The scavenging capacity of hydroxyl radical is an important antioxidant activity. It has high reactivity which enables it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides. For this reason, removing hydroxyl radicals from the living system is very important for its protection [16].

Superoxide radical scavenging activity

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals. It can change to other harmful reactive oxygen species within the living cells. This assay was based on the capacity of methanolic extract of the samples to inhibit the blue formazan formation by scavenging the superoxide radicals generated in the riboflavin-light-NBT system [17]. According to this study, the % inhibition at different concentrations (100–500 µg/mL) of the gallic acid, *S.jambos* fruit and seed varied between 41.91 and 64.53, 49.34 and 74.32, and 31.74 and 62.84 with the IC_{50} value of 171.94±0.95 µg/ml (y=0.0475x+41.54, R^2=0.80), 131.24±0.82 µg/mL (y=0.0628x+41.75, R^2=0.98) and 290.55±0.48 µg/mL (y=0.0722x+29.08, R^2=0.90), respectively. Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. The superoxide scavenging ability of plant extract might primarily be due to the presence of flavonoids [18]. Here, the *S. jambos* fruit performed the highest scavenging activity against superoxide radical than the standard and *S. jambos* seed.

NO radical scavenging activity

The NO assay is based on that sodium nitroprusside in an aqueous solution at physiological pH can produce NO. Then, it acts with oxygen to produce nitrite ions. In this study, the % inhibition at different concentrations (100–500 µg/mL) of gallic acid, *S. jambos* fruit, and seed ranged from 38.32 to 75.12, 36.34 to 72.14, and 32.76 to 69.63 with the IC_{50} value of157.66±1.03 µg/mL (y=0.0832x+37.19, R^2=0.81), 217.53±0.39 µg/mL (y=0.1002x+28.25, R^2=0.87), and 184.23±0.77 µg/mL (y =0.0797x+31.48, R^2=0.83), respectively (Table 4 and Fig. 3). NO is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. It is involved in the regulation of various physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation, and regulation of cell-mediated toxicity. An excess concentration of NO is implicated in the cytotoxic effects observed in various disorders such as AIDS, cancer, Alzheimer's, and arthritis [19]. This study indicates that the *S. jambos* seed has a better scavenging capacity against NO than comparing the fruit.

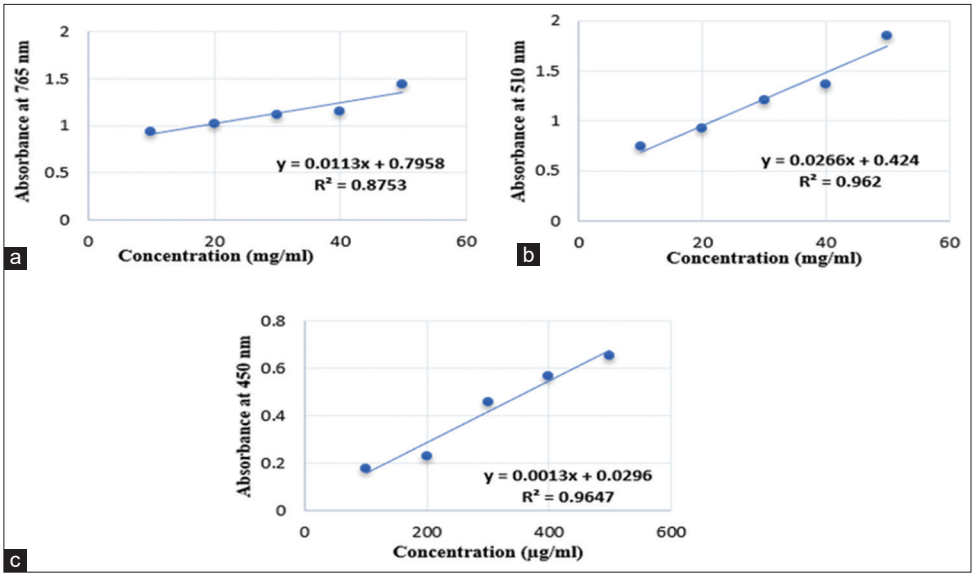

Fig. 2: (a) Calibration curve for TPC using gallic acid as standard (b) Calibration curve for TFC using quercetin as standard (c) Calibration curve for CUPRAC using gallic acid as standard

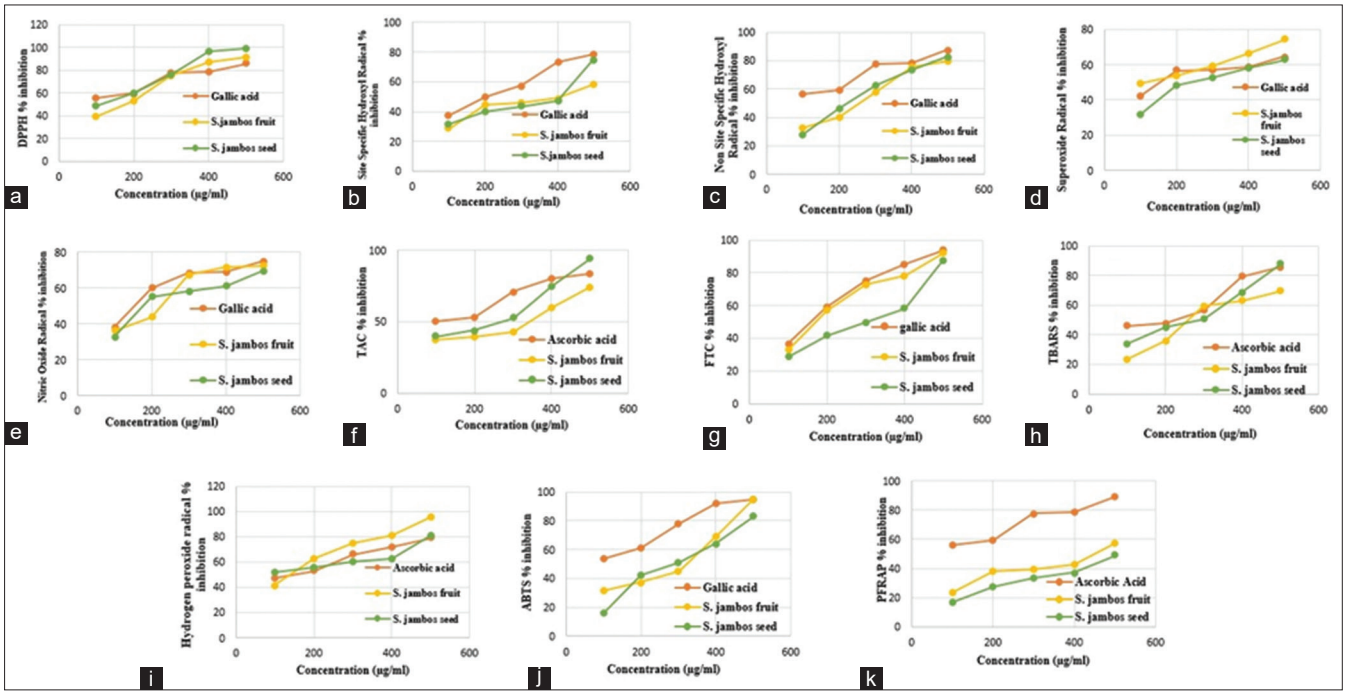

Fig. 3: (a) DPPH radical scavenging activity, (b) site specific hydroxyl radical scavenging activity, (c) non site-specific hydroxyl radical scavenging activity, (d) superoxide radical scavenging activity, (e) nitric oxide radical scavenging activity, (f) TAC scavenging activity, (g) FTC scavenging activity, (h) TBARS scavenging activity, (i) hydrogen peroxide radical scavenging activity, (j) ABTS radical scavenging activity, and (k) PFRAP scavenging activity

Table 3: Total phenol content and total flavonoid content of *Syzygium jambos* fruit and *Syzygium jambos* seed

Phytochemical assay	*Syzygium jambos* fruit	*Syzygium jambos* seed
Total phenolic content in mg/100g equivalent of Gallic acid	127.61±0.96	217.34±1.38
Total flavonoid content in mg/100g equivalent of Quercetin	8.64±0.73	15.97±0.87

Values are expressed as Mean±SE (n=3)

FTC method and TBARS assay

The antioxidant activity of the extracts against lipid peroxidation was determined using FTC and TBARS methods. The FTC method was used to evaluate the peroxides at the initiation of lipid peroxidation, and the TBARS method was used to analyze free radicals after the oxidation of peroxides [20]. In the FTC assay, the % inhibition at different concentrations (100–500µg/ml) ofAscorbic acid, *S. jambos* fruit, and seed ranged from 36.74 to 93.76, 32.8 to 91.76, and 28.6 to 87.43 with the IC_{50} value of 157.49±0.81µg/ml (y=0.14x+28.02, R^2=0.95), 182.89±0.57 µg/mL (y=0.1389x+24.76, R^2=0.94), and 277.16±0.69 µg/

mL (y =0.1342x+12.86, R^2=0.92), respectively. According to the TBARS assay, the % inhibition of ascorbic acid, *S.jambos* fruit, and seed varied between 45.98 and 85.74, 23.68 and 69.62, 33.82 and 87.84 with the IC_{50} of 185.45±0.82 µg/mL (y=0.1112x+29.71, R^2=0.91), 296.22±0.75 µg/mL (y=0.1187x+14.83, R^2=0.92), and 245.87±0.79 µg/mL (y=0.1318x+17.76, R^2=0.95), respectively. In the FTC method, *S.jambos* fruit documented strong antioxidant activity than the seed, whereas, *S. jambos* seed showed better activity in the TBARS assay (Table 4 and Fig. 3). The FTC method measures the amount of peroxide produced during the initial stages of lipid oxidation. Subsequently, at a later stage of lipid oxidation, peroxide decomposes to form carbonyl compounds that are measured by the TBA method. Oxidative stress in cells and tissues can be best monitored by its lipid peroxidation assay, a well-established mechanism both in plants and animals [20].

Total antioxidant capacity (phosphomolybdenum assay)

The assay is based on the reduction of Mo (VI)-Mo (V) by the samples and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH [21]. The % inhibition at different concentrations (100–500 µg/mL) of the ascorbic acid, *S.jambos* fruit, and seed ranged from 50.41 to 83.74, 37.71 to 73.9, and 39.7 to 93.81 with the IC_{50} value of 110.88±1.32 µg/mL (y=0.0937x+39.61, R^2=0.93), 294.34±0.86µg/ml (y=0.0926x+22.92, R^2=0.87), and 222.46±0.59 µg/mL (y=0.1387x+19.32, R^2=0.92), respectively. The use of phosphomolybdenum assay has been reported as an effective method to evaluate the total antioxidant potentials of various plant extracts. Recent studies have shown that flavonoids and polyphenols contribute significantly to the phosphomolybdate scavenging activity [18]. In the present study, *S. jambos* seed showed greater antioxidant activity comparing the *S. jambos* fruit extract (Table 4, Fig. 3).

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide (H_2O_2) is a byproduct of respiration and is made in all living cells. It is harmful and must be removed as soon as it is produced in the cell. In this assay, *S. jambos* fruit and seed were tested against hydrogen peroxide radicals. The % inhibition at different concentrations of the ascorbic acid, *S.jambos* fruit, and seed varied between 47.12 and 79.11, 41.59 and 95.39, and 51.7 and 81.43 with the IC_{50} value of 139.51±0.55 µg/mL (y=0.0826x+38.56, R^2=0.98), 132.53±0.67 µg/mL (y=0.1264x+33.03, R^2=0.96), and 111.21±0.63 µg/mL (y=0.0661x+42.68, R^2=0.84), respectively. Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, and food. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (•OH) that can initiate lipid peroxidation and cause DNA damage [18]. Hydrogen peroxide scavenging activity depends on the phenolic content of the extracts, which can donate electrons to H_2O_2 and thereby neutralizing it into water. Here, the *S. jambos* seed performed better scavenging activity against H_2O_2 radical than the *S. jambos* fruit (Table 4 and Fig. 3).

ABTS radical scavenging activity

ABTS radical is a blue chromophore produced by the reaction of ABTS and potassium persulphate. The $ABTS^{\bullet+}$ is reactive toward most antioxidants including phenols, thiols, and Vitamin C. The total antioxidant activity of the methanolic extract of the standard and the samples was calculated from the decolorization of $ABTS^{\bullet\pm}$. According to this assay, the % inhibition at different concentrations (100–500 µg/mL) of ascorbic acid, *S. jambos* fruit and seed varied between 53.87 and 94.64, 31.56 and 92.61, and 16.05 and 82.8 with the IC_{50} value of 84.63±1.12 µg/mL (y=0.1854x+34.30, R^2=0.97), 266.45±1.28 µg/mL (y=0.1578x+8.127, R^2=0.91), and 293.35±0.87 µg/mL (y=0.1556x+4.535, R^2=0.97), respectively. Hence, this study suggests that *S. jambos* fruit has slightly more antioxidant activity against ABTS free radicals than comparing the *S. jambos* seed.

PFRAP

The scavenging activity of the samples was also determined using the PFRAP method. Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The % inhibition at different concentrations (100–500 µg/mL) of the ascorbic acid, *S.jambos* fruit and seed varied between 55.81 and 89.32, 37.71 and 79.34, and 27.23 and 74.28 with the IC_{50} of 46.88±0.76 µg/mL (y=0.086x+46.30, R^2=0.93), 275.35±0.63 µg/mL (y=0.1085x+20.24, R^2=0.86), and 313.82±0.48 µg/mL (y=0.129x+9.58, R^2=0.88), respectively. The reducing power of any substance is closely associated with its antioxidant activity. In this assay, ascorbic acid was used as standard. Among the methanolic extract of the samples, *S. jambos* fruit showed better reducing activity against free radicals than the *S. jambos* seed extract.

FRAP assay

FRAP is one of the most popular methods used for the determination of total antioxidant activity. This assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe2+-TPTZ)[21]. In this study, ascorbic acid was used as the standard. The FRAP value of *S.jambos* fruit and seed was found to be (44.12 µM Fe^{2+}/g dry extract and 140.62 µM Fe^{2+}/g dry extract), respectively. The ferric reducing antioxidant power of a plant extract depends on its total phenolic content and bioactive phytoconstituents present in it [22]. In the present study, the *S.jambos* seed was found to have strong free radicals scavenging capacity than the *S. jambos* fruit (Table 5).

CUPRAC assay

CUPRAC method has been widely used to measure the antioxidant capacity in food, plants, human serum, biological samples, dietary polyphenols, and Vitamins C and E. It is a simple, reliable, and cost-effective method. This method is based on the simple redox reaction between antioxidants and the free radicals, where the antioxidant activity can be measured by the reduction of cupric ions to cuprous

Table 4: IC_{50} value of *in vitro* antioxidant activity of methanolic extract of *Syzygium jambos* fruit and *Syzygium jambos* seed

Antioxidant assay	Standard	IC_{50} (µg/ml)		
		Standard	*Syzygium jambos* Fruit	*Syzygium jambos* Seed
DPPH Free Radical Scavenging Assay	Gallic acid	28.71±0.94	160.44±0.81	111.54±1.03
Site-specific hydroxyl radical scavenging activity		215.14±0.62	376.98±1.29	327.44±0.78
Non-site-specific Hydroxyl radical scavenging activity		30.70±0.59	246.18±0.73	238.98±0.88
Superoxide radical scavenging activity		171.94±0.95	131.24±0.82	290.55±0.48
Nitric oxide radical scavenging activity		157.66±1.03	217.53±0.39	184.23±0.77
Ferric Thiocyanate Method (FTC)	Ascorbic acid	162.47±0.81	182.89±0.57	277.16±0.69
Total Antioxidant Capacity (Phosphomolybdenum Assay)		110.88±1.32	294.38±0.86	225.85±0.51
Hydrogen peroxide scavenging activity		139.51±0.55	132.53±0.67	111.21±0.63
Thiobarbituric acid reactive substances Assay (TBARS)		185.45±0.82	296.22±0.75	245.87±0.79
ABTS+Free Radical Scavenging Activity		84.63±1.12	266.45±1.28	293.35±0.87
Reducing power assay (PFRAP)		46.88±0.76	275.35±0.63	313.82±0.48

Values are expressed as Mean±SEM (n=3)

Table 5: FRAP and CUPRAC Activity of *Syzygiumjambos* Fruit and *Syzygiumjambos* Seed

Antioxidant assay	Standard	Result	*Syzygium jambos* fruit	*Syzygium jambos* seed
Ferric Reducing antioxidant power assay (FRAP)	Ascorbic acid	(μM Fe^{2+}/g dry extract)	44.12±0.72	140.62±1.07
Cupric Reducing Antioxidant Capacity (CUPRAC)	Gallic acid	(μg GAE/g dry extract)	587±0.84	394±0.59

Values are expressed as Mean±SE (n=3)

ions by antioxidants [22]. In this study, gallic acid was used as the standard. The CUPRAC value of *S.jambos* fruit and its seed was found to be (587 μg GAE/g dry extract, and 394 μg GAE/g dry extract), respectively. The CUPRAC value was determined from the standard graph (y=0.0013x+0.029, R^2=0.96). Here, the methanolic extract of *S. jambos* fruit performed a slightly better reducing capacity of cupric ion to cuprous than the seed extract (Table 5).

CONCLUSION

An antioxidant is a substance that has gained importance in recent years due to its ability to neutralize free radicals. In this study, the phytochemical screening of different solvent (aqueous, ethanol, methanol, and chloroform) extracts of *S. jambos* fruit and its seed has indicated the presence of bioactive compounds such as flavonoids, phenols, alkaloids, tannins, and saponins in an extensive quantity that exhibits antioxidantactivity. To ensure it, 13 different *in vitro* antioxidantstudies (DPPH, ABTS radical scavenging activity, site-specific, and non-site-specific hydroxyl radical scavenging activity, superoxide radical scavenging, NO radical scavenging, PFRAP, FTC, total antioxidant capacity, hydrogen peroxide radical scavenging, TBARS, FRAP, and CUPRAC) were performed. The findings confirm that *S. jambos* fruit and its seed have strong antioxidant activity against free radicals. They may have the potential to reduce and scavenge ROS which is produced due to oxidative stress. Therefore, the fruit and its seed may be helpful in the prevention and treatment of oxidative stress-induced diseases such as diabetes, cardiovascular diseases, cancer, arthritis, gout, neurodegenerative diseases, and respiratory tract infections. Further *in vivo* study is needed to know about the therapeutic properties of *S. jambos* fruit and its seed.

ACKNOWLEDGMENT

The experiment has been financially supported by the University Grant Commission. The Botanical Survey of India, Shibpur, Howrah has helped in the identification of the sample.

AUTHORS CONTRIBUTION

SuchandraDutta: Conceptualization, investigation, and prepare the manuscript. Shreyasi Halder: Investigation and validation. Kazi Layla Khaled: Supervision.

CONFLICTS OF INTEREST

None.

AUTHORS FUNDING

This experiment was funded by the University Grant Commission (UGC), New Delhi, India.

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