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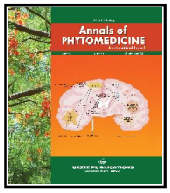

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Proximate composition, phenolic, flavanoid content and antioxidant potential of *Syzygium cumini* (L.) Skeels seed powder

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Abstract

As a source of alternative medicines, plant derived drugs are of great potential and can be used to cure various health related ailments. *Syzygium cumini* (L.) Skeels is commonly known as *Jamun*. *Jamun* seed powder is a very well known antidiabetic agent. Keeping in view the above concerns, the investigation of this plant aims to assess the phytochemical and antioxidant content of the ethyl acetate extract of locally available *S. cumini* seed samples. Seed samples were collected and moisture content was estimated. Various chemicals and phytonutrients like alkaloids, tannins, minerals, crude protein, crude fibre, flavonoids and total phenolics were estimated in shade dried samples. The total phenolic and flavanoid content were 12.05 mg GAE/g and 4.86 mg CE/g, respectively. The antioxidant activity of *S. cumini* seed extract was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and total antioxidant capacity using phospho-molybdenum assay. The results showed that ethyl acetate extract of *Jamun* seed had stronger antioxidant activity.

1. Introduction

Medicinal plants have been playing a vital role on the health and healing of man since down of human civilization (Upadhayaya *et al*., 2011; Aggarwal *et al*., 2022). Nearly 25% of all prescribed medications worldwide come from various plant sources and are used as natural remedies for disease prevention and control. According to a WHO report, about 80% of the world's population relies on plants to provide for their basic medical needs (Ahmdullah and Nayar, 1999; Devi *et al*., 2020; Goel *et al*., 2022). Plant metabolites have long piqued the interest of humans due to their significant pharmacological relevance. Except for allopathy, the majority of medicines practised in China and India, including those practised in naturopathy, homoeopathy, yoga, unani, siddha, and ayurveda, are plant-based (Vaidya and Devasagagam, 2007; Goel *et al*., 2022). Beyond this pharmaceutical approach to using plants, there is a widespread trend to use herbal supplements as dietary additions, primarily with the goal of enhancing quality of life and preventing disease (Joselin and Jeeva, 2014; Kumari *et al*., 2022). Medicinal plants contain various bioactive constituents which are secondary metabolites produced by the plant. The non-nutritive secondary metabolites are known as phytochemicals that have defensive as well as disease preventive properties (Tan *et al*., 2010; Suhas *et al*., 2014; Moond *et al*., 2023). The major secondary metabolites include alkaloids, carbohydrates, flavonoids, tannins, terpenoids and steroids (Edoga *et al*., 2005; Suman *et al*., 2022; Nehra *et al*., 2023).

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The Myrtaceae family includes *Syzygium cumini* (L.) Skeels, also known as *Eugenia jambolana*. It is a traditional Indian medicine plant that goes by the name "*Jamun*". It also occurs in South-East Asia and Eastern Africa in addition to India (Modi *et al*., 2010). All tree parts, especially the seeds, are used to treat a variety of illnesses in connection with its dietary use, the most significant of which is diabetes mellitus (Sagrawat *et al*., 2006). *Jamun* is a potentially important indigenous commercial plant as well as has a wide range of medicinal activities including antioxidant, antibacterial, antifungal, anti-inflammatory, anticancerous, cardioprotective and hepato-protectve (Jagetia, 2017). Its medicinal properties may be due to its ability to synthesize various phytochemicals. The seeds contain a glycoside, jambolin which depress the conversion of starch into sugar, hence are being used primarily in treatment of diabetis mellitus (Parmar *et al*., 2010). *S. cumini* seeds are thought to have antibacterial, anti-inflammatory, antidiabetic, and antidiarrheal properties. According to reports, in the treatment of type 2 diabetes, *S. cumini* seed extract was superior to the oral hypoglacemic or antidiabetic drug glibenclamide when administered to animals at a dose of 5g/kg of body weight. The plant contains various concentrations of acetyl-oleanolic acid, triterpenoids, ellagic acid, isoquercetin, quercetin, kaempferol, and myricetin (Rastogi and Mehrortra, 1990). The current experiment was created to examine the phytochemical components and antioxidant activity of ethyl acetate extract of *S. cumini* seeds in light of the numerous medical applications of *Jamun*.

2. Materials and Methods

2.1 Plant material

Syzygium cumini (L.) Skeels (*Jamun*) seed samples were acquired from the campus of Chaudhary Charan Singh Haryana Agricultural University, Hisar. The proposed study was conducted in Department

of Chemistry, CCS HAU, Hisar. The plant materials were brought and before processing, the materials were kept under the shade at room temperature.

2.2 Prepartion of plant extract

Extraction plays an important role as it helps in the recovery of desired medicinally bioactive constituents from plants by using selective solvents and leaving out those non-desired with an aid of the solvents (Dhanani *et al*., 2017). The powdered sample of *Jamun* seed was percolated by using conventional Soxhlet apparatus using ethyl acetate as solvent. The extracts was collected and kept for the further studies.

2.3 Determination of moisture content

2 g of powdered seed sample of *Jamun* in triplicate was taken and method of AOAC (1995) was used to calculate the percentage of moisture content.

$$\text{Moisture content (\%)} = \frac{\text{Powdered wt. (before drying)} - \text{Powdered wt. (after drying)}}{\text{Powdered wt. (before drying)}} \times 100$$

2.4 Determination of ash content

A 2 g sample of powdered *Jamun* seed was weighed in triplicates and added to a crucible that had already been lit and weighed; the crucible was then placed in a muffle furnace for 2 h. The crucibles containing the samples were immediately moved from the furnace into a desiccator, allowed to cool, and their weight was recorded.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.5 Determination of crude fat content

A thimble was used to collect 2 g of the dried, powdered *Jamun* seed sample, which was then put in a Soxhlet extractor. The Soxhlet assembly was coupled with a 250 ml round-bottomed flask that had been dried and pre-weighted. Following that, petroleum ether was added up to 1.5 syphons, or 150-175 ml. The assembly was heated, and an 8 h extraction process was carried out. Following extraction, the round bottomed flask's petroleum ether was evaporated, and the weight of the round bottomed flask containing the extract was once again calculated. The crude fat content (%) was determined as follows:

$$\text{Crude fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.6 Determination of crude fibre content

A sample of 3 g of powdered *Jamun* seed, free of moisture and fat, was collected. Later, the modified Maynard method was used to calculate the percentage of crude fibre content (1970).

2.7 Determination of crude protein content

Micro-Kjeldahl method (AOAC, 1990) was used for the determination of nitrogen content. By multiplying % of nitrogen with 6.25, crude protein was calculated.

2.8 Determination of total carbohydrates content

Total carbohydrates content was calculated by difference as follows:

Total carbohydrates content (%)

= 100 – [Moisture (%) + Ash (%) + Crudefat (%) + Crude fibre (%) + Crude protein (%)]

2.9 Mineral analysis

The mineral content of *Jamun* seed samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) after Microwave-assisted acid digestion.

2.10 Quantitative determination of tannin content

Vanillin-HCl method of Burns (1971) was used for estimation of tannin content as catechin equivalent.

2.11 Quantitative determination of alkaloid content

Method of Harborne (1973) was used for estimation of alkaloid content in *Jamun* seed sample.

2.12 Quantitative determination of total phenolics

Analysis of total phenolics were done by the Folin-Ciocalteu method (Singleton and Rossi, 1965) and expressed as milligrams of gallic acid equivalent per gram (mg GAE/g). 0.2 ml of extract was taken in a test tube and to adjust the optical density with in calibration limits were diluted with respective solvents. Each test tube received 1.0 ml of 1mol/l Folin-Ciocalteu reagent and 2.0 ml of Na_2CO_3 (20%, w/v), which were mixed before the final volume of water, 10.0 ml, was added. After centrifuged, the reaction mixture at 6000 rpm for 10 min, the mixture was incubated for 8 min using UV-VIS spectrophotometer (Model UV 1900, Shimadzu) the absorbance of supernatant solution was taken at 730 nm after 8 min against a blank prepared in the same way having respective solvent instead of extracts. The standard curve was used to calculate the total phenolic content in each extract, and the results are given as milligrams of gallic acid equivalent per gram (mg GAE/g).

2.13 Quantitative determination of total flavonoids

We used catechin as the reference standard to calculate the total amount of flavonoids present. According to Marinova *et al*. (2005), the colorimetric aluminium chloride assay was used to determine the total flavonoids (2005). In test tubes containing 4.0 ml of double distilled water and 0.3 ml of $NaNO_2$ (5%, w/v), 1.0 ml of ethyl acetate extract was added. 0.3 ml of 10% AlCl3 was added after 5 min. After adding 2.0 ml of 1M NaOH, the volume was immediately increased to 10.0 ml with double-distilled water. After thoroughly blending the solution, the optical density at 510 nm was measured using a UV-VIS spectrophotometer (UV 1900, Shimadzu) in comparison to a blank that was made in the same way but contained the appropriate solvent in place of the extracts. The amount of total flavanoids in various extracts was determined using the standard curve, and the results are expressed as mg of catechin equivalents per gram (mg CE/g).

2.14 Evaluation of DPPH free radical scavenging activity

Method of Hatano *et al.* (1988) with slight modifications was used for the evaluation of DPPH free radical scavenging activity.

2.15 Evaluation of total antioxidant capacity

Modified phosphomolybdenum method by Prieto *et al*. (1999) was used for the evaluation of total antioxidant capacity of *Jamun* seed powder.

2.16 Statistical analysis

Each sample was taken in triplicates for statistical analysis, and the results are expressed as mean ± standard error (SE). In online statistical analysis, a one-way analysis of variance (ANOVA) was performed to determine whether there were any significant variations in sample mean values (OPSTAT). Regression analysis was used in Microsoft Excel to determine the IC_{50} values of the DPPH free radical scavenging activity. The rest of the measurements were made using Excel 2016 by Microsoft.

3. Results

3.1 Proximate composition, mineral and chemical analysis

In proximate composition, the seed part of *Jamun* consist the moisture content (9.53 %), crude fibre content (5.67%), ash content (2.45%), crude protein content (5.31%), crude fat (1.56) and total carbohydrates (75.48%).

The minerals (Fe, Mn, Zn and Cu) content was estimated and data is presented in Table 1. The mineral content in seed part of *Jamun*, Fe (32.15 ppm), Mn (1.05 ppm), Zn (74.72 ppm) and Cu (2.18 ppm). In chemical analysis, seed part of *Jamun* contains the tannin content (0.55 mg CE/g) and alkaloid content (3.78%).

Table 1: Proximate composition, mineral content and chemical analysis of *S. cumini* seed

Proximate composition	Moisture content (%)	9.53
	Crude fibre content (%)	5.67
	Ash content (%)	2.45
	Crude protein content (%)	5.31
	Crude fat content (%)	1.56
	Carbohydrate (%)	75.48
Mineral analysis	Fe (ppm)	32.15
	Mn (ppm)	1.05
	Zn (ppm)	74.72
	Cu (ppm)	2.18
Chemical analysis	Tannin content (mg CE/g)	0.55
	Alkaloid content (%)	3.78

3.2 Phytochemical content

In phytochemical parameters, the content of total phenolics and total flavonoids were estimated. Due to their antioxidant activity, phenols are crucial plant constituents. The total phenolic and total flavonoid contents of plant extracts are frequently used to explain the antioxidant activities of those extracts. Figure 1 shows the standard curve for determining total phenol content that was obtained using gallic acid. The total flavonoid content in *Jamun* seed was calculated using the standard curve of catechin as shown in Figure 2. The total phenolics in the ethyl acetate extract of *Jamun* seed was 12.05 mg GAE/g and total flavanoids was 4.86 mg CE/g in the *Jamun* seed.

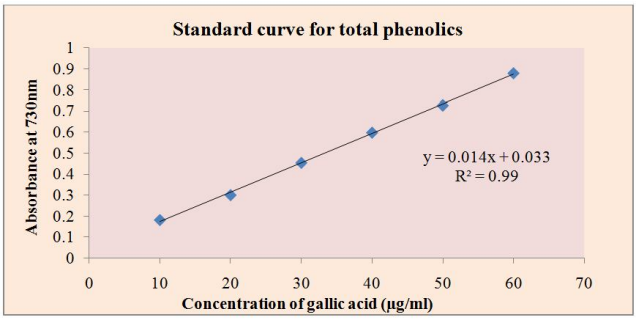

Figure 1: Standard curve of total phenolics using gallic acid as a standard.

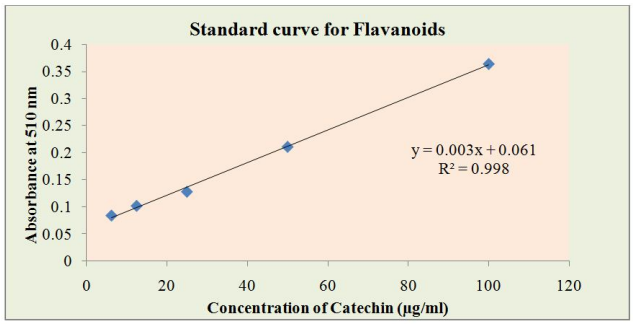

Figure 2: Standard curve of total flavanoids using catechin as a standard.

3.3 Antioxidant activity

The DPPH free radical is a stable free radical that is frequently used to gauge the antioxidants' capacity to scavenge free radicals.The percentage of DPPH free radical scavenging activity kept on increasing when the concentration of ethyl acetate extracts of *Jamun* seed was increased (Table 2).The theory behind the phospho-molybdenum assay, which estimates total antioxidant capacity, is that antioxidants in the sample convert Mo (VI) to Mo. (V). In an acidic medium, Mo (V) reacts with the sodium phosphate group to form a green-colored complex called the Mo (V) phosphate complex (phospho molybdenum complex), which can be detected using a UV-Vis spectrophotometer. IC_{50} value for DPPH scavenging and phospho-molybdenum assay is 31.67 and 29.58 µg/ml, respectively. The value suggests that ethyl acetate extract of *Jamun* seed had appreciable antioxidant activity.

Table 2: Antioxidant activity of ethyl acetate extract of *S. cumini* seed

Concentration of extract (µg/ml)	% DPPH free radical scavenging activity	Total antioxidant capacity (%)
5	9.2	8.7
15	37.4	29.2
25	43.5	47.4
35	64.1	58.1
45	73.04	69.0
55	78	76.0
IC_{50} Value	**31.67**	**29.58**

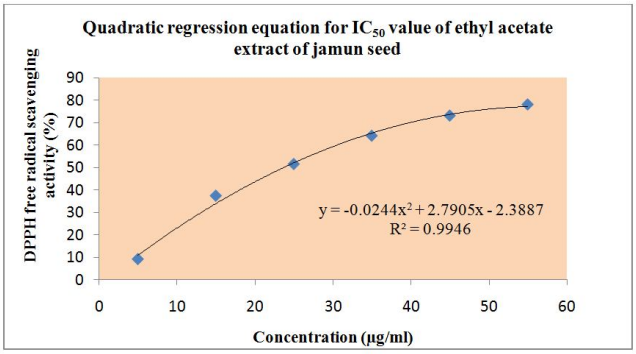

Figure 3: Quadratic regression equations for IC_{50} (µg/ml) value of DPPH free radical scvenging assay.

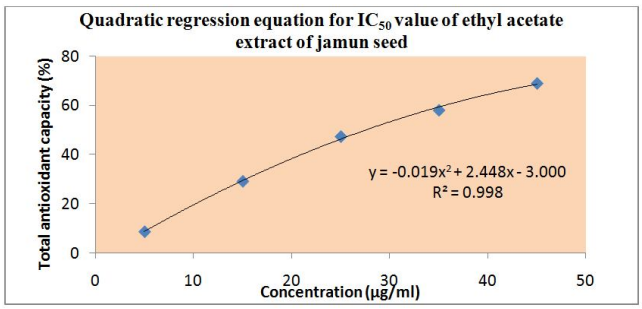

Figure 4: Quadratic regression equations for IC_{50} (µg/ml) value of total antioxidant capacity (%).

4. Discussion

Medicinal plants have been acknowledged as an important source of natural antioxidants throughout the world. Plants have medicinal value because of their phytochemicals, which have definite physiological effects on people. Alkaloids, tannins, flavanoids, and phenolic compounds are the most significant of these bioactive components found in plants. Phenolic compounds are essential in scavenging free radicals because of their high antioxidant potential. Plant metabolites known as flavanoids are thought to have positive effects on health because of their role in cell signalling and as antioxidants.Thus, the current study involved the estimation of proximate composition and mineral analysis alongwith phytochemical parameters in ethyl acetate extract of *Jamun* seed powder and evaluation of antioxidant activity using DPPH scavenging assay and phospho-molybedate assay.

The moisture content (9.53 %), ash content (2.45%), crude fibre content (5.67%), crude protein content (5.31%), crude fat (1.56) and total carbohydrates (75.48%) in seed powder of *S. cumini* were measured in current study. Prasad *et al*. (2010) estimated the moisture content in *Jamun* seed powder as 9.43 % which is very close to our present findings. Ash content (2.18%) was estimated by Raza e*t al.* (2015) in *Jamun* seed samples which were similar to the present findings. Kshirsagar *et al*. (2019) estimated ash content in *Jamun* seed powder which was found to be 1.51 g/100g. As a result, the information discovered regarding the amount of ash in seeds is comparable to that found by other researchers. The determined data are, therefore in agreement. Yadav and Singh (2020) evaluated five advanced germplasm of *Jamun* for nutritional value and reported 3.87 to 4.18 mg/100 g of crude fiber content in *Jamun* seed. Crude protein content and crude fat content in *Jamun* seed powder was found to be 3.84 g/100 g and 1.02 g/100 g, respectively, by Kshirsagar *et al*. (2019). Our data closely match the estimates from other studies.

In the current study, Fe (32.15 ppm), Mn (1.05 ppm), Zn (74.72 ppm), Cu (2.18 ppm), tannin content (0.55 mg CE/g) and alkaloid content (3.78%) were estimated in *Jamun* seed powder. Ghosh *et al*. (2017) analyze the nutritional parameters of *Jamun* seed powder and reported 2.13 mg/100 g, of Cu content, 0.46 mg/100 g of Zn content and 0.4 mg/100g of Mn content and 4.2 mg/100 g of Fe content in *Jamun* seed. Ranjan *et al*. (2011) reported 6 to 19 % of tannin content in *Jamun* seed samples. The information obtained in present study is consistent with that of other researchers.

In our current findings, the total phenolics in ethyl acetate extract of *Jamun* seed was (12.05 mg GAE/g) and the total flavanoid content (4.86 mg CE/g) was estimated. Primary redox properties of plant phenolics such as free radical scavenging, hydrogen donating and singlet oxygen quenching are mainly responsible for making them as one of the major groups of phytochemicals acting as a primary antioxidants. Antioxidant activities of plant extracts are often explained by their total phenolic and flavanoid content. Priya *et al*. (2017) estimated the total phenolic and flavanoid content in three different variety of *S. cumini* seed in various extracts (hexane, ethyl acetate, methanol, 70 % methanol and water) and reported that highest phenolic and flavanoid content was exhibited by 70% methanolic fraction for all the three variants (from 808.5 to 906 mg GAE/g phenolic content and 33.8 mg CE/g dry weight basis flavanoid content).

Current studies show that the amount of DPPH free radical scavenging activity increases with extract concentration. The DPPH free radical scavenging activity of ethyl acetate extract is highest at 55 µg/ml (78%), followed by 73.04 to 9.2% at 45 to 5 µg/ml. Similarly for phospho-molybdenum assay, ethyl acetate extract has highest total antioxidant capacity at 55 µg/ml (76%), followed by 69.0 to 8.7 % at 45 to 5 µg/ml. Other scientists have also evaluated the antioxidant activity of various extracts of *Jamun* seed. Priya *et al*. (2017) evaluated antioxidant activity of aqueous (WE), ethyl acetate (EA), hexane (HE), methanol (ME) and 70% methanol extracts of *Jamun* seed by DPPH radical scavenging activity method. As a result, it appears that the data collected agrees with that of other researchers.

5. Conclusion

There are many herbal plants in the world but the *S. cumini* is considered to be the queen of herbs due to its great medicinal values. The current findings are very significant for the industries of pharmaceutical firms, drugs, and dietary supplements.The present study suggests that *Jamun* seed powder has a potential source of antioxidants, phenols and flavanoids in the shade dried sample.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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