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## ***Syzygium Cumini L. Seed A Potent Source of Fiber, Protein and Natural Antioxidants***

*Muhammad Khalid Saeed\**, *Naseem Zahra*, *Asma Saeed*, *Syed Hussain Imam Abidi*,  
*Quratulain Syed*

Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

\*Corresponding Author Email: [rose\\_pcsir@yahoo.com](mailto:rose_pcsir@yahoo.com)

**ABSTRACT:** *Syzygium cumini L. seeds have been documented in traditional medicine in Pakistan. The current research was aimed to assess the physicochemical characteristics, polyphenols and antioxidants of S. cumini seed. The physical characteristics such as the color of S. cumini seed were white to pink, the shapes resembled to oblong and coarse texture. The length, width and weight of fresh S. cumini seed were found to be  $(18.20 \pm 0.81 \text{mm}$ ,  $11.05 \pm 0.41 \text{mm}$  and  $1.80 \pm 0.16 \text{g}$ ), respectively while the color of dried S. cumini seeds were light brown to brown, rhombus in shape with loutish texture. The average length, width and weight of dried S. cumini seeds were  $16.47 \pm 0.45$ ;  $10.14 \pm 0.25 \text{ mm}$  and  $0.75 \pm 0.12 \text{ g}$  respectively. S. cumini seeds powder were evaluated for their chemical composition e.g. carbohydrate, protein, fat, crude fiber, moisture content and ash ( $77.27 \pm 2.50$ ,  $3.62 \pm 0.30$ ,  $6.25 \pm 0.55$ ,  $10.30 \pm 1.20$  and  $1.55 \pm 0.11 \text{ g/100g}$ ), respectively. Quantitative analysis of total phenolic content was performed it was found that the methanolic and water extract had  $52 \pm 1.65$  and  $40 \pm 1.25 \text{ mg GAE/g}$  content. Free radical scavenging activity was also evaluated to estimate the antioxidant property of extract. Among tested extracts maximum % inhibition  $96.61 \pm 1.90\%$  was found in methanol extract and  $69.30 \pm 1.56\%$  in water extract, while BHT has % inhibition  $50.70 \pm 1.32\%$  at concentration  $100 \mu\text{g/ml}$ . Similarly in reducing power activity assay the maximum absorbance  $1.4704 \pm 0.05$  was shown by methanol extract and  $1.2075 \pm 0.03$  in water extract of S. cumini seed powder respectively which was compared with BHT ( $0.9207 \pm 0.02$ ). Therefore, it was concluded that these Syzygium Cumini L. seeds traditional medicinal plants provide a good source of nutrients, namely protein, fiber and natural antioxidants.*

**Key words:** *Syzygium cumini L.*, Fiber, Polyphenols, DPPH, RPA, Antioxidants

## INTRODUCTION

The significance of herbal medicines has increased the demand due to their least toxic side effects unlikely allopathic therapies. An appropriate analysis is crucial to investigate the safety and potency of herbal medicine. In this regard WHO focused to make sure the quality control measures by using modern technologies to apply particular standards and specifications (Chitnis et al., 2012). *Syzygium Cumini* L. is cherished for the colour, flavour and taste of its fruit. It is a very large tropical tree belonging to the *Myrtaceae* family, known by the synonym jambolan or black plum (Jagetia, 2017).

It has chemo-preventive, radio-protective, anti-neoplastic properties and may confer medicinal value (Gajera et al., 2017; Pallavi and Sukumar, 2021). Domestically and industrially *S. cumini* fruit was used abundantly, particularly to produce its juice and as a constituent in wine. Seeds were obtained as a by-product after mass utilization and needs to dispose off (Patil et al., 2012). Seeds make up the 20% weight of fruit and contain high fiber content so utilized as cattle feed. A high amount of polyphenols were reported in seeds so can be a good source of antioxidant agent (Fig. 1).



**Fig. 1.** *Syzygium cumini* Lam seeds and its powder

The seeds of *S. cumini* are sour, acrid, nourishing, and contain chemical components such as jambolan, isoquercetin, kaempferol corilagin, 3,6-hexahydroxy dibenzoylglucose, glucoside, quercetin, myricetin, 1 - Gallo-glucose,  $\beta$ -glutaryl, 4,6-hexahydroxydibenzoyl-glucose and 3-

gallo-glucose (Swami et al., 2012). Seed alkaloids jambosine and glycoside antimellin have a delaying effect on the conversion of starch to sugars and ellagic acid control blood pressure (Bijauliya et al., 2017). These seeds are reported to be rich in flavonoids, a well-known antioxidant with free radical

scavenging and protective effects (Loganayaki and Manian, 2010; Faria et al., 2011; Mercado et al., 2021). *S. cumini* seed has many pharmacological properties, such as antibacterial effect (Banerjee et al., 2011; Meshram et al., 2011), anti-inflammatory (Kumar et al., 2008; Modi et al., 2010), gastric ulcer (Chaturvedi et al., 2007), it also acts as a liver stimulant, digestive, cooling and blood purifying agent. The seeds are also recognized for their anti-hyperglycemic activity and have been shown to impart a reducing effect in type 2 diabetic patients by lowering fasting glucose levels (Ayya et al., 2015; Sidana et al., 2017), by a Jamboline glycoside, which helps in the maintenance of glucose levels as in the normal limits (Kalse et al., 2016). Several bio-molecules were also reported in seeds such as carbohydrates, vitamins, minerals, and fibers. The main sugar polymers are glucose and fructose was reported (Prakruthi et al., 2021). In pharmaceutical and cosmetic industries *S. cumini* seeds can be a natural and economical source of bioactive components and food-supplemented dietary antioxidants.

The significance of antioxidants is well known in the biosphere, recently becoming more evident with circular economy and recycling methodologies.

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that may damage the cells of organisms. Free radicals are molecules produced when the body breaks down food or when exposed to tobacco smoke or radiation. Natural antioxidants are widely distributed in food and medicinal plants. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis, and anticancer (Raza et al., 2020; Ismat et al., 2022). The effective extraction and proper assessment of antioxidants from food and medicinal plants are crucial to explore the potential antioxidant sources and promoting their application in functional foods, pharmaceuticals, and food additives (Natasa et al., 2021). Several synthetic antioxidants were used such as tert-butyl hydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG), piroxicam and meloxicam (Samra et al., 2022). These synthetic antioxidants have many side effects reported such as liver damage and act as carcinogen (Grice, 1988; Jagesar, 2019), so there is need to isolate natural antioxidants from indigenous sources. Undeniably, in

recent years, many studies emphasized the use of plant based by-products to produce natural and eco-friendly antioxidants which can be effective alternative to synthetic antioxidants (Cama et al., 2010; Krishnaiah et al., 2011; Vayupharap and Laksanalamal, 2012), keeping view this we use *S. Cumini* (jamun) seed which is abundantly available in Pakistan. Therefore the main objective of the present research was to extraction/isolation & determination of natural antioxidants from *S. cumini* seed along with estimation of its fiber, protein and polyphenols.

## **MATERIALS AND METHODS**

### ***Chemicals and instruments required***

Methanol, Folin-ciocalteau reagent, Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), 2,2-Diphenyl picryl hydrazyl (DPPH), and Gallic acid were obtained from Sigma-Aldrich (St. Louis, USA). Instruments used were Spectrophotometer UV-VIS 1700 (Shimadzu, Japan) to measure absorbance, and a centrifuge machine (3000 System) Centurarian Scientific (Germany).

### ***Syzygium cumini L. seed powder preparation***

Ripe, disease-free and healthy *S. cumini* fruit was collected from the PCSIR Labs complex (July 2021 season) Lahore,

Pakistan and identified by botanists. It was cleaned, washed and passed through a pulper to separate the seeds from the pulp. The seeds were then washed in water and dried for 48 hours at 60°C in a hot air dryer. After completely drying it was ground in a grinder to a fine powder with an average particle size of 0.60 mm. The seed powder was stored in an airtight container at -20 °C until further use.

### ***Proximate Composition***

In order to gauge the proximate composition of *S. cumini* seed methods described in AOAC (2016) were used. Analysis was done for crude fat, ash, moisture content, carbohydrates, proteins, and crude fiber.

### ***Total phenols***

Total phenolics were estimated by standard analysis of Singleton *et al.*, (1965) [27]. 0.1 g of the sample was dissolved in 50 ml of methanol and water. 0.2 ml from the above solution was mixed with water to the make volume of 3 ml and wait for three minutes after adding 0.5 ml of Folin reagent then added 2.0 ml of sodium carbonate (20%) solution and incubate the mixture in the dark for 30 min and measure the absorbance by using a spectrophotometer (UV-vis: 1700, Shimadzu, Japan) at 760 nm. From the standard curve of Gallic acid,

concentrations of polyphenols in the extracts were estimated and derived. Results are shown as milligrams of gallic acid equivalents per milligram (mg GAE/g).

#### ***Antioxidant study by DPPH assay***

Water and methnolic extracts of *S. cumini* seeds were subjected to free radical scavenging activity measured by using the method of Brand-Williams (1995) with slight modifications (Saeed et al., 2021). Methanolic solution (0.1 mM) of 2,2-diphenyl-1-picryl-hydrazine (DPPH) was prepared. In different concentrations (10–100 µg/ ml) of extract 3 ml of DDPH solution was added while Butylated hydroxytoluene (BHT) was used as positive control to compare readings. After 30 minutes of incubation in dark absorbance was taken at 517 nm in triplicates. Scavenging capacity was measured by using following equation:

$$\text{Antioxidant activity (\% Inhibition)} = [(A_c - A_s) / A_c] \times 100$$

Where  $A_c$  is the absorbance of the DPPH' solution (control) and  $A_s$  is the absorbance of sample.

#### ***Reducing power determination***

In order to evaluate the reducing power of *S. cumini* powder extracts a method described by Oyaizu (1986) was used after some modifications. In different concentrations of the sample, 2.5 ml of

phosphate buffer (0.2 M) with pH 6.6 and 1% potassium ferricyanide (2.5 ml) was added. The mixture was heated for 20 min at 50 °C in the water bath. After cooling, the mixture at 22°C -25°C (2.5 ml) of trichloroacetic acid (10%) was added and centrifuged for 10 minutes at 3000 rpm. Supernatants were obtained and mix it with distilled water (2.5 ml) and 0.5 ml of ferric chloride (0.1%) solution. Incubate it for 10 minutes and wait for the reaction completion. Absorbance was taken at 700 nm to measure the reducing capacity of extracts and standard BHT. A higher absorbance value indicates the higher reducing potential of extracts.

#### ***Statistical Analysis***

Except for fiber analysis, data analysis was obtained from triplicate samples. Statistical analysis was performed using the statistical analysis system 9.1.3 software package (Seeram, 2008). A two-sample t-test was used to compare means in mean  $\pm$  standard deviation value were expressed.

### **RESULTS AND DISCUSSION**

#### ***Physical characteristic of dried S. cumini seeds***

Physical characteristics aid in the visual identification of seeds. Therefore, some physical properties were observed mentioned in Table 1. The results

indicated that the color of fresh *S. cumini* seeds were white to pink, oblong in shape and coarse texture, while the color of dried *S. cumini* seeds were light brown to brown, rhombus in shape with loutish texture. The average length, width and weight of fresh *S. cumini* seeds were  $18.20 \pm 0.81$  (mm),  $11.05 \pm 0.41$  (mm) and  $1.80 \pm 0.16$

respectively and the texture was relatively coarse. While the average length, width and weight of dried *S. cumini* seeds were  $16.47 \pm 0.52$  (mm),  $10.14 \pm 0.25$  (mm) and  $0.75 \pm 0.12$  g respectively and the texture was relatively coarse. The results of current study were relatable to another research by Ghosh et al. (2017).

**Table. 1. Physical characteristics of fresh and dried *S. cumini* seed**

Sr. No.	Parameters	Observations	
		Before Drying (Fresh)	After Drying
1	Color	White to pink	Light brown to brown
2	Shape	Oblong	Rhombus
3	Texture	Coarse	Loutish
4	Length (mm)	$18.20 \pm 0.81$	$16.47 \pm 0.52$
5	Width (mm)	$11.05 \pm 0.41$	$10.14 \pm 0.25$
6	Weight (g)	$1.80 \pm 0.16$	$0.75 \pm 0.12$

Data are represented  $\pm$  standard deviation

***Proximate composition of S. cumini seed powder***

Proximate composition of *S. cumini* seed was done to evaluate quantitative analysis of protein, carbohydrate, crude fat, crude fiber, ash and moisture as  $3.62 \pm 0.30$ ,  $77.27 \pm 2.50$ ,  $1.01 \pm 0.06$ ,  $6.25 \pm 0.55$ ,  $1.55 \pm 0.11$  and  $10.30 \pm 0.90$ , respectively (Table 2). These results demonstrate consistent with previous

findings reported by Desai et al. (2018), who described that *S. cumini* seeds consist of 14.31% moisture, 3.01% protein, 1.02% crude fat, 4.21% crude fiber and 2.87% ash. Previous studies have reported that *S. cumini* seeds have moisture content of 5.3% to 16.34%, protein content of 1.97% to 3.84%, ash content of 1.51% to 2.87%, fat content of 0.65% to 1.02%, and crude fiber content of 4.21% % to 7.01% (Akhtar et

al., 2016; Kshirsagar et al., 2019; Pallavi and Sukumar, 2021). Composition of seed in this study was closely related to earlier study except the protein, fiber and ash contents exhibiting varied values with respect to *S. cumini* seed content such as 1.98, 4.27 and 2.46,

respectively (Rafique et al., 2018). These differences may stem from differences in varieties, agricultural practices and climatic conditions (Ahmad et al., 2015; Raman et al., 2020).

**Table 2. Nutritional facts of dried *S. cumini* seed powder**

Sr. No.	Parameters	Values (g/100g)
1	Moisture (%)	10.30 ± 0.90
2	Ash (%)	1.55 ± 0.11
3	Crude fat (%)	1.01 ± 0.06
4	Crude fiber (%)	6.25 ± 0.55
5	Crude protein (%)	3.62 ± 0.30
6	Carbohydrate (%)	77.27 ± 1.5
7	Energy (Kcal/100g)	332.65 ± 2.7

± standard deviation

***Total polyphenols***

Available total phenolic content (TPC) values in (mg GAE/g) for *S. cumini* seeds include: 52±1.65 for the methanolic seed extract and 40±1.25 for the water extract, which seem to be a better source of total phenols. TPC was higher in the results of Kapor et al. (2015), who described hot-air dried *S. cumini* seeds had 3.67±0.49 g GAE/100, while Balyan and Sarkar (2017) had a lower content. Indicates high total polyphenol content i.e. 415 mg gallic acid equivalents/g *S. cumini* seed dry extract. These results are in line with

Sheikh et al. (2011), Rydlewski et al. (2017) and Arivalagan et al. (2021). Polyphenols were bioactive component of plants and reported for their antioxidant activity as they demonstrate reducing potency by acting as a reducing agent. Those bioactive biomolecules were act as hydrogen donors and exhibit antioxidant activity (Phuyal et al., 2020). Due to the presence of polyphenols in *S. cumini* seed have shown higher antioxidant capacities (Singh et al., 2016; Swami and Kalse, 2020). Finding of current study supported the beneficial aspect of

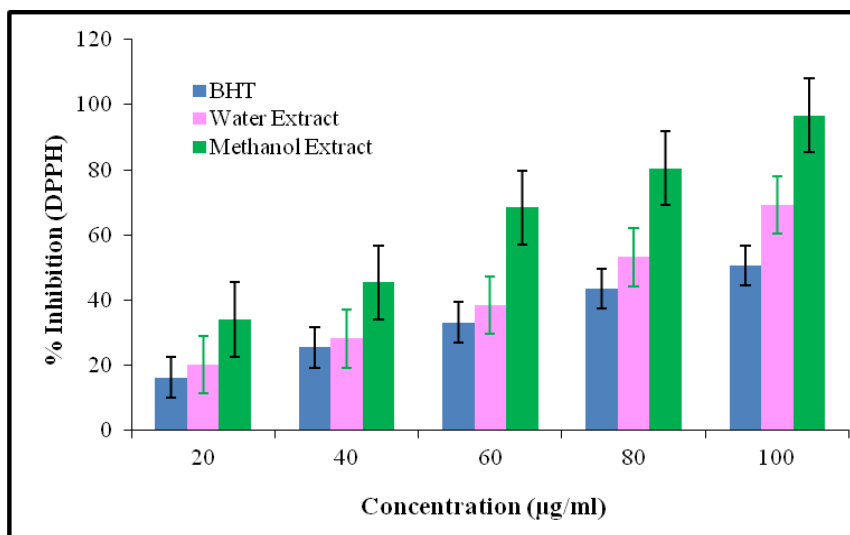
*S. cumini* seeds due to the presence of polyphenols.

## Antioxidant Activity

### I. DPPH Assay

The antioxidant activities of methanol and water extracts of *S. cumini* seeds were evaluated by the scavenging antioxidant (DPPH) method. At different concentrations of extract average, scavenging activity was seen as shown Fig. 2. Percentage inhibition (% I) values ranged from 28.40 to 92.60 % for methanol seed extract, 20.50 to 72.80 % for water extract, and 16.32 to 50.70 % for BHT and followed the order of effectiveness: methanol extract > water extract > BHT at same concentration. Present data is consistent with that of Hammam et al. (2019), who investigated the free radical scavenging activity of water, methanolic and acetone extract of *S. cumini* seeds and

leaves and suggested that methanolic extracts have higher antioxidant activity. Similar results were obtained by Elizabeth Margaret et al. (2015) and Sehwal and Madhusweta, (2016). Antioxidants interact with DPPH by transferring electrons or hydrogen atoms to DPPH, thereby neutralizing free radical species (Rohadi et al., 2017; Wasswa et al., 2019). The level of free radicals (DPPH) neutralized is reflected by the % inhibition. Due to this neutralization, the color of the reaction mixture changed from purple to yellow which confirmed by decrease in absorbance at 517 nm (Mercado et al., 2021). In a similar study it was found that antioxidant activity was maximum in *Centella asiatica* (in aqueous) and *Spheranthus indicus* (in methanol) extracts (Yasmin et al., 2020).

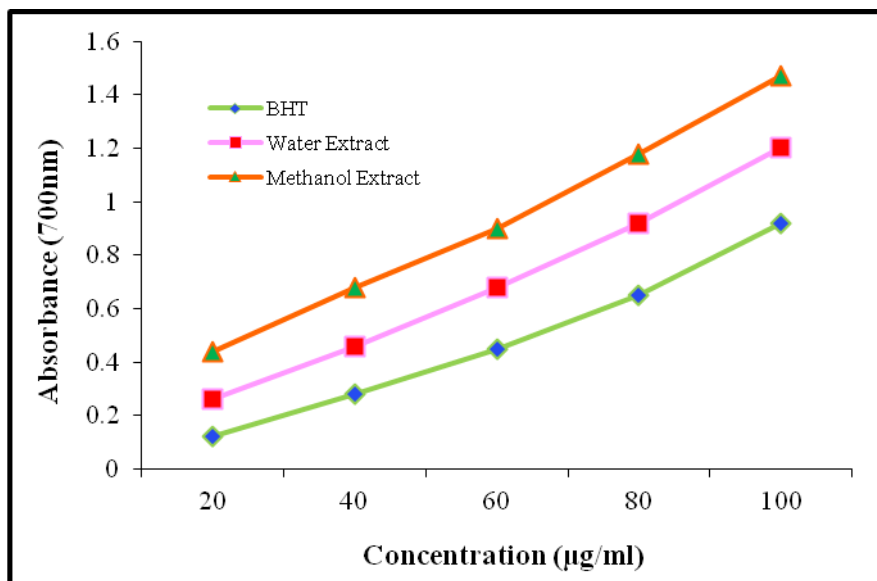


**Fig. 2.** % Inhibition of water, methanolic extract of *S. cumini* seed and BHT by DPPH assay

## **II. Reduce Power Assay**

Higher reducing potency of the methanolic extract of *S. cumini* seed at a concentration of 100  $\mu\text{g/ml}$  was observed than its water extract. The reducing activity of the methanol extract of *S. cumini* seed with an absorbance of  $1.6704 \pm 0.05$  was the highest and the reducing activity of water extract with an absorbance of  $1.2075 \pm 0.02$  was lower. The results showed that a greater degree of inhibition could be achieved with increasing concentrations of both extracts and thus demonstrate reducing activity in a concentration-dependent manner (Fig. 3). Reducing activity of standard BHT ( $1.3426 \pm 0.03$  at  $100 \mu\text{g/ml}$ ) was not as much effective as compared to our experimental sample. During the determination of the

reducing ability of the extract,  $\text{Fe}^{3+}$  was converted to  $\text{Fe}^{2+}$ , which then reacted with  $\text{FeCl}_2$  and formed complex ferrous iron (Wajiha and Qureshi 2021). As in the current study, Ahmad et al. (2020) reported that the reducing power of the methanol/water (80:20, v/v) extract of *S. cumini* seeds was enhanced with increasing extract concentration. Numerous reports have demonstrated a relative direct correlation between the reducing potency and antioxidant potential of various plant extracts (Munira et al., 2018; Pavai et al., 2019; Yadav et al., 2020). Our present results suggested that *S. cumini* seed extract can terminate the free radical chain reaction by donating electrons to free radical species and converting them to neutralized stable form.



**Fig. 3.** Reducing potential of methanol and water extract of *S. cumini* seed and BHT

## CONCLUSION

It is concluded that, *S. cumini* seeds contained a higher amount of fiber, protein and natural antioxidants. Methanolic extracts of *S. cumini* seeds had significantly ( $p < 0.05$ ) more polyphenols and antioxidant capacity in both assays, compared to water extracts. Therefore, this study showed that commonly discarded seeds can be used as natural antioxidants as an alternative to artificial ones.

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