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**Research Article**

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# Quantification of bioactive constituents and antioxidant activity of methanolic seed extracts of *Syzygium cumini* (L).

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**ABSTRACT**

*Syzygium cumini L is an evergreen tree belongs to the family Myrtaceae, mainly found in tropical regions. The fruit of the plant is used to treat diabetes. In this present study we have looked for antioxidant activity of methanolic seed extract of Syzygium cumini. Total flavonoid, phenolic and proanthocyanidin content of seed extract were measured. The extract was also checked for antioxidant assays namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, superoxide anion radical scavenging activity and ability of chelating ferrous ions.*

**Keywords**: *Syzygium cumini*, Antioxidant, Free radicals, Phenolics, Flavonoids, Proanthocyanidins.

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## INTRODUCTION

*Syzygium cumini*, an evergreen tropical flowering plant belonging to the Myrtaceae family is dispersed all over India [1]. The plant and its products are found to be rich in alkaloid, isoquercetin, ellagic acid, gallic acid, kaemferol and many other useful biomolecules [2, 3]. The extract obtained from the peel is mainly employed in pharmaceutical and food industries as colorants. The fruit obtained from the tree, commonly known as 'Jamun', has several health benefits like reducing the risk factors for heart disease, diabetes and enteric disorders [1, 4]. The presence of gallic acid is responsible for the mild sour taste of the fruit. The content of calcium, iron, and potassium in the fruit helps in increasing the bone strength. The seeds have been reported to have flavonoids which have a major role in scavenging the toxic free radicals and possess antioxidant activity. The seeds also contain phytochemicals like alkaloids, antimellin and jambosine which reduces the conversion of starch into sugar due to the presence of the ellagic acid [2]. Thus when consumed, the dried extract of the seeds might have the ability to lower the glucose level in blood. The alkaloids help to manage many other human chronic diseases such as neurodegenerative disorders, cancer, liver cirrhosis and cardio vascular diseases [5].

The antioxidant activity of the *Syzygium cumini* has the ability to scavenge the free radical ions and prevent cell damage [4]. Free radicals like Reactive Oxygen Species (ROS) can cause oxidative stress in a cell thus leading to cell toxicity and damage [6, 7]. The extract was also checked for antioxidant assays namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, superoxide anion radical scavenging activity and ability of chelating ferrous ions.

## EXPERIMENTAL SECTION

*Syzygium cumini* fresh fruits were collected from Yercaud in Tamil Nadu, India. Fruits were washed thoroughly and flesh was pleaded off. Known amount of seed (100 g) were kept in an oven at 40◦C for drying to constant weight. The dried seeds were powdered in a mixer-grinder and used for the study

**EXTRACT PREPARATION**

About 4 g of seed powder was added to methanol and kept at room temperature for about 24 hours. After 24 hours the extract obtained was filtered through the 0.45-$\mu$m Whatman filter. The residue was then extracted twice with methanol. The combined extract was concentrated at 40 °C; it was then weighed and stored at 4°C for further use.

**TOTAL PHENOLIC, FLAVONOID, AND PROANTHOCYANIDINCONTENT**

Total phenolic content in the *Syzygium cumini* seed extract was estimated using the modified Folin Ciocalteu method [8]. 0.5ml of seed methanol extract (1 mg/ml) was taken in a test tube and 0.5ml of Folin Ciocalteau reagent was added. The test tube was then gently mixed and undisturbed for 2 minutes. To this 0.5 ml of sodium carbonate (100 mg/mL) was added. The contents were mixed well and allowed to stand for 2 h. The absorbance was read at 765 nm and total phenolic contents were expressed as mg gallic acid equivalent (GAE)/g dry weight.

The flavonoid content in the *Syzygium cumini* sample was measured using the assay described by Zhishen et al. [9]. About 250 µl of methanol extract was added to 1.25 ml of distilled water. To this 75 µl of 5% sodium nitrate was added and left for 5minutes. Then 150 µl of 10% ammonium chloride was added and left for 6 minutes. To this 500 µl of 1M sodium hydroxide was added. The contents were diluted with 275 µl of distilled water and absorbance was read at 510 nm. Total flavonoid contents were expressed as milligrams of quercetin equivalent (QE)/g dry weight. Proanthocyanidin content present in the *Syzygium cumini* seed extract was measured using vanillin assay described by Sun et al. [10]. 0.5 ml of seed extract was mixed with 3 ml of 4% vanillin methanol solution. To this 1.5 ml of HCl was added and mixed. This content was kept at room temperature for 15 minutes and absorbance was read at 500 nm. Total proanthocyanidins content was expressed as milligrams of catechin equivalent (QE)/g dry weight.

**DPPH RADICAL ASSAY**

The DPPH radical scavenging property of *Syzygium cumini* sample was determined using the method of Mensor et al. [11]. Different concentrations of extracts were prepared with methanol (200, 100, 80, 60, 40, 20, 10 and 5 µg/ml). 2ml of extract from each concentration was mixed to 1ml of methanol solution with DPPH radicals. This was shaken vigorously and kept in the dark for 30 minutes. Absorbance was read at 517 nm. The percentage of radical scavenging activity was calculated using the formula:

$$\text{RSA (\%)} = \frac{\text{A control- A sample}}{\text{A control}} \times 100$$

**DETERMINATION OF REDUCING POWER**

The reducing property of the sample was estimated by the methodology described by Oyaizu [12]. 2 ml of methanol extract, 2 ml of 0.2 M (pH 6.6) phosphate buffer and 2 ml of potassium ferricyanide (10 mg/ml) were mixed and incubated at 50°C for 20 minutes. To this 2 ml of trichloroacetic acid (100 mg/ml) was added. 2ml of above mixture was diluted with 2 ml of distilled water and 0.4 ml of 0.1% ferric chloride and content was undisturbed for 10 minutes for reaction to take place. The absorbance was read at 700nm.

**SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY**

The sample was measured for its superoxide radical scavenging activity using the method of Nishikimi et al. [13]. The assay was carried out by mixing 0.5 ml of phosphate buffer (50 mM pH7.6) , 0.3 ml riboflavin (50 µM) , 0.25 ml PMS (20 mM) and 0.1 ml NBT (0.5 mM) in a test tube and to this 1ml of methanol extract solution was added (at different concentrations). The reaction was initiated by illuminating the fluorescent lamp. The absorbance was read at 560nm after an incubation period of 20 minutes. The percentage of scavenging activity was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{A control- A sample}}{\text{A control}} \times 100$$

**ABILITY OF CHELATING FERROUS IONS**

The chelating ability of *Syzygium cumini* seed extract was studied by ferrous ion ferrozine complex method [14]. The extract was dissolved in various dilutions of methanol (10, 8, 6, 4, 2 mg/ml). 0.8 ml of the extract was mixed with 50 µl of 2 mM $FeCl_2$ and 200 µl of 5 mM ferrozine, and was incubated for 10 minutes at 25°C. The absorbance was read at 562 nm. Metal chelating effect of *Syzygium cumini* was estimated using the formula:

$$\text{Activity (\%)} = \frac{\text{A control- A sample}}{\text{A control}} \times 100$$

## RESULTS

### TOTAL PHENOLIC, FLAVONOID, AND PROANTHOCYANIDIN CONTENT

The total phenolic content present in *Syzygium cumini* seed extract was found to be 57.5 GAE/g dry weights. The flavonoid and proanthocyanidin content was found to be 46.25 QE/g and 29.9 mg CE/g, respectively in the methanolic seed extract.

### DPPH RADICAL SCAVENGING ACTIVITY

The *Syzygium cumini* was assessed at varying concentration (5-200 µg/ml). The strongest activity was seen at a concentration of 80 µg/ml with 92.7 % in the methanolic seed extracts. The observed colour change from red to yellow demonstrates the scavenging ability of the seed extract at different concentrations.

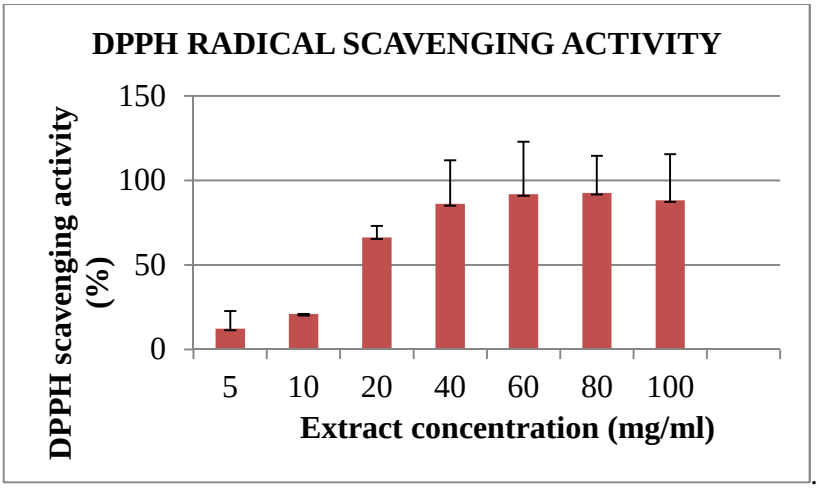


**Figure 1: DPPH Radical scavenging activity of methanolic seed extracts of *Syzygium cumini***

### SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY

The percentage inhibition of superoxide generation was found to have maximum activity 47.72 % at a concentration of 10 mg/ml in the methanolic seed extract of *Syzygium cumini.* Also the scavenging activity of the extract depicted in figure 2 implies that the superoxide anion scavenging activity was dose dependant as the increasing activity can be attributed to the increasing extract concentration.

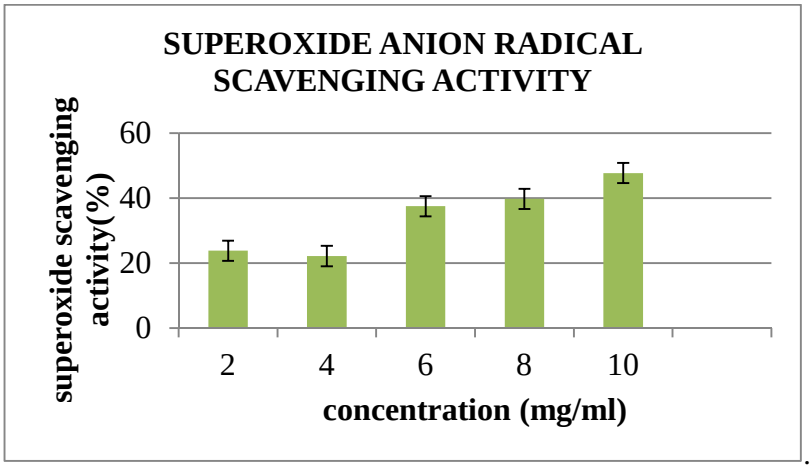


**Figure 2: Superoxide anion radical scavenging activity of methanolic seed extracts of *Syzygium cumini***

### ABILITY OF CHELATING FERROUS IONS

Although the sample showed 34.73 % of chelating ability at a concentration of 2mg/ml, the chelating ability of the methanolic seed extract obtained from *Syzygium cumini* was found to be dose dependant. The figure 3 shows that the chelating ability decreases with increase in concentration of the seed extract.

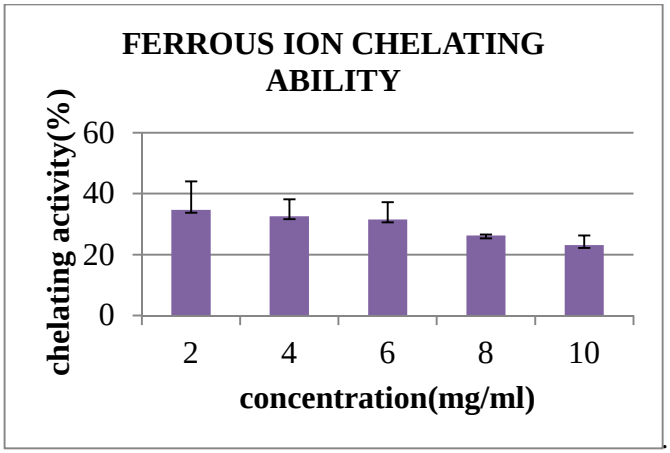


**Figure 3: Ferrous in chelating ability of methanolic seed extracts of *Syzygium cumini***

## DISCUSSION

Free radicals present in cells can cause oxidative damage as the valence shells of free radicals are having unpaired electrons which make it highly reactive and unstable. For the cell stability, the biomolecules become electron donor or recipient to scavenge the toxic free radicals [7, 15]. The DPPH assay employed in this study measures the radical scavenging activity of the biomolecules present in the methanolic seed extract based on the DPPH reducing ability of the extract. If any compound has the ability to reduce DPPH, then it can be claimed to possess radical scavenging and antioxidant activity depending on the colour change observed. The hydroxyl group in the flavonoids and Phenolics is responsible for the redox reaction which in turn leads to scavenging of the free radicals [16]. Therefore, the presence of high total phenolics indicates significant antioxidant property and cell protective effect. The antioxidant capacity of a compound can also be attributed to the metal ion chelating ability as the concentration of the ferrous ions is reduced. The fruit of this plant has ancient history of medical use in treatment of digestive ailments and chronic diarrhoea. Apart from this, several experimental and clinical studies have been suggesting that different parts of *Syzygium cumini* possess promising activity against diabetes mellitus [1, 3]. Based on above results, we conclude that the methanolic seed extract results of the present study reassures the same. Hence more investigation is required to establish therapeutic significance of this plant and its products.

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