

***In vitro* studies on antioxidant, α – amylase and α – glucosidase inhibitory activities of ethanol extracts of *Syzygium cumini* seeds**

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ABSTRACT

Introduction and Aim: Diabetes mellitus is a metabolic disorder leads to many secondary complications. The drugs used for treatment causes serious side effects. *Syzygium cumini* is used in traditional medicine for treating many diseases. The aim of the present study is to estimate phytochemical contents, antioxidant activity, α – amylase and α – glucosidase inhibitory activities of ethanol extract of seeds of *S. cumini* by *in vitro* methodology.

Materials and Methods: The powdered seeds were extracted with ethanol. Quantitative analysis of Total alkaloids, Total phenols, Total flavonoids, Total tannins, Total saponins and Total steroids were carried out. DPPH scavenging activity, α – amylase and α – glucosidase inhibitory activities were measured with varying concentration of extract.

Results: Total alkaloids content was observed to be higher followed by Total phenol content. Total saponin was found to be present in lesser amount among the tested phytochemicals. 500 $\mu\text{g/ml}$ and above concentrations of seed extract possess above 90% DPPH scavenging activity; 1000 $\mu\text{g/ml}$ concentration of the extract exhibited 43.20% and 19.80% inhibition activity on α – amylase and α – glucosidase enzymes.

Conclusion: The above results indicate a higher antioxidant activity and appreciable inhibitory activities of enzymes responsible for elevated circulation of glucose. These activities are due to the presence of phytochemicals present in the seeds extract of *S. cumini* and can be utilized for the management of Diabetes.

Keywords: *Syzygium cumini*; ethanol extract; phytochemicals; antioxidant; α – amylase; α – glucosidase.

INTRODUCTION

Diabetes mellitus is one of the most common chronic endocrinal metabolic disorders. Diabetes mellitus is associated with insufficient production or utilization of insulin in the body resulting elevated level of circulating blood glucose known as hyperglycemia. In India, it is expected that by the year 2025 there would be around 57.2 million people affected with diabetes (1). Oral hypoglycemic agents such as sulfonylureas, glibenclamides and biguanides and insulin are used for the treatment of diabetes. The oral hypoglycemic agents in practice were found to cause unwanted serious side effects (2). Therapeutically it is necessary to reduce the postprandial hyperglycemia to manage diabetes mellitus (3). In carbohydrate metabolism, α –

amylase and α – glucosidase are the important enzymes involved in the breakdown and digestion of carbohydrates. Inhibitors of α – amylase and α – glucosidase are potential compounds for the treatment of diabetes (4).

Hence it is desirable to search for a hypoglycemic agent especially plant based having therapeutic efficacy and without any deleterious side effects. One such a plant with edible fruits is *Syzygium cumini* Skeels tree belongs to Myrtaceae family, commonly called as Jamun and it is also known as black plum, Java plum and Indian blackberry (5). In Tamil language, it is called as Naaval tree. The fruit is called as Naaval Pazham. Traditional healers use *S. cumini* for treating diabetes and related complications (5). Vaish *et al.*, (6) reported the therapeutic effect of jamun seeds in alloxan induced diabetes.

The barks, seeds and leaves of *S. cumini* was reported to possess anti diarrheal activity (7). Seed extract of *S. cumini* was found to enhance insulin secretion in the isolated pancreatic islet cells of normal and diabetic animals. The extract was also found to inhibit the insulinase activity in liver and kidney (8, 9). It was reported that the presence of alkaloids, glycoside jambolin and jambosine in seeds of *S. cumini* cease the breakdown of starch to sugar (5). The present study was focused to estimate the phytochemical content, antioxidant and antidiabetic activity of *S. cumini* seeds extract by *in vitro* method.

MATERIALS AND METHODS

The fruits of *S. cumini* was collected from in and around the villages of Orathanadu Taluk, Thanjavur District. The pulp of the fruits was removed and the seeds were washed with tap water followed by distilled water and shade dried. Dried seeds were powdered using a pulverizer. The powdered seed was extracted with ethanol. After 24 h, the insoluble content present were removed by centrifugation at 3000rpm for 15 minutes. The supernatant was rotary vacuum evaporated at 60°C and lyophilized. The dried extract was stored and used for the analysis.

Quantitative analysis of phytochemicals

Quantitative analysis of total alkaloids (10), Total phenols (11), Total flavonoids (12), Total tannins (13), Total saponins (14) and Total steroids (15) were carried out.

2, 2 – diphenyl – 2- picryl hydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was carried out by the method of Mensor *et al.*, (16). 1.0 ml of 0.3 mM DPPH ethanol solution was added to 2.5 ml of varied concentrations (100 - 1000µg/ml) of ethanol extract of *S. cumini* seeds and the reaction was allowed to take place at room temperature for 30 minutes and the absorbance was measured at 518nm where as 1mM Morin was used as positive

control. The results were expressed as % of scavenging activity.

α- Amylase inhibitory assay

The α-amylase inhibition assay was carried out by 3, 5-dinitrosalicylic acid (DNSA) method (17). 100 to 1000 µg/ml concentration of seed extract was prepared by dissolving in 10% DMSO and phosphate buffer and sodium chloride at pH 6.9. Equal volumes (200µl) of α-amylase and plant extract was mixed and incubated for 10 minutes at 30°C. Then 200µl of 1% starch solution was added, after 3 minutes incubation, the reaction was terminated by DNSA reagent and the reaction mixture was boiled in a water bath for 10 minutes at 85 - 90°C. After cooling the mixture the absorbance was measured at 540 nm in a spectrophotometer. Acarbose was used as a positive control. α- amylase inhibition activity was expressed as % of inhibition.

α- Glucosidase inhibitory assay

It was assayed by the method of Kim *et al.*, (18). 100µl of α glucosidase (1 U/ml) was incubated with 50µl of different concentrations of seed extract of *S. cumini* for 10 minutes. Then 50 µl of substrate p – nitrophenyl glucopyranoside (3.0 mM) was added to initiate the reaction, and the reaction mixture was incubated for 20 minutes at 37°C and terminated by the addition of 2.0ml of 0.1 M sodium carbonate solution and the activity was measured at 405 nm. The results were expressed as % of inhibition of α - glucosidase activity. Acarbose was used as a positive control.

RESULTS

One hundred grams of *S. cumini* seed powder was extracted with ethanol and the dry weight of the seed extract was 1.89g. Table 1 presented the Total alkaloids, Total phenol, Total flavonoids, Total tannins, Total saponin and Total steroids. Total alkaloids content was higher in the extract followed by Total phenol content. Total saponin was found to be in lower amount among the tested phytochemicals.

Table 1. Quantitative analysis of phytochemicals in ethanol extract of seeds of *S. cumini*

Phytochemicals	Quantity (mg/g)
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Total alkaloids	159.69 ± 8.2
Total Phenol	141.5 ± 8.0
Total flavonoids	94.26 ± 6.1
Total tannin	65.35 ± 4.3
Total saponin	14.53 ± 1.0
Total steroids	67.6 ± 5.1

(Values are expressed as Mean ± SD of triplicates)

Table 2 represented the DPPH scavenging activity, α – amylase inhibition and α –glucosidase inhibition activity of ethanol seed extract of *S. cumini*. 100 – 1000 μ g/ml concentration of ethanol seed extract of *S. cumini* was tested. 500 μ g/ml concentration of ethanol extract exhibited more than 90% scavenging activity (98.11%). All the higher concentrations above 500 μ g/ml expressed 99% scavenging activity. 1000 μ g/ml

concentration of seeds extract exhibited 43.20% of α – amylase inhibition activity and 19.80% of α – glucosidase inhibition activity whereas positive control acarbose exhibited 99.30% and 93.70% inhibition activity respectively. There was found to be a gradual increase in the inhibition of enzyme activity with the increasing concentration of the seeds extract.

Table 2. DPPH scavenging activity, α – amylase inhibition and α –glucosidase inhibition activity of ethanol extracts of seeds of *S. cumini*.

Extract Concentration (μ g/ml)	% of DPPH scavenging activity	% of α –amylase inhibition activity	% of α –glucosidase inhibition activity
100	49 ± 0.98	3.70 ± 0.20	0.40 ± 0.10
200	61.45 ± 1.22	8.00 ± 0.30	2.00 ± 0.10
300	75.78 ± 1.67	13.10 ± 1.1	3.90 ± 0.20
400	88.61 ± 1.88	17.80 ± 1.4	6.20 ± 0.20
500	98.11 ± 2.32	22.40 ± 2.10	8.60 ± 0.30
600	99.65 ± 2.21	28.20 ± 2.30	11.10 ± 0.60
700	99.61 ± 1.34	33.70 ± 2.90	13.50 ± 0.70
800	99.59 ± 1.31	36.90 ± 3.0	15.90 ± 0.90
900	99.80 ± 0.26	36.90 ± 3.1	17.40 ± 1.0
1000	99.79 ± 0.31	43.20 ± 3.5	19.80 ± 1.1
Positive control	99 ± 0.98	99.30 ± 1.1	93.70 ± 2.5

(Values are expressed as Mean ± SD of triplicates)

DISCUSSION

Phytochemical analysis plays a greater role to identify a compound of therapeutic efficiency. Earlier qualitative studies in our laboratory on the seeds extract of *S. cumini* revealed the presence of alkaloid, phenol, flavonoid, tannin, saponin, Steroids, cardiac glycosides, anthraquinone glycosides, oils and fats, lignin, terpenoids, phlobatannins, coumarin, quinine, sugar and amino acids. In diabetic condition an imbalance between free radicals and antioxidant scavenging system occurs result in elevated lipid peroxidation and complications (19, 20). It was reported that the

phytochemicals like flavonoids and total phenols present in the seed extract were found to serve as an antioxidant and account for the scavenging effect on free radicals (21, 22). Reddy *et al* (23) have reported that polyphenol rich plant foods have insulin like effect and also acts as inhibitors of enzymes like α - amylase and α - glucosidase. α - amylase is an enzyme that functions by cleaving the carbohydrates into smaller units of saccharides and α -glucosidase breaks the 1,4- α -bonds of starch and disaccharides to glucose(24). α -glucosidase found in the small intestine brush border cell lining. Drugs serves as Inhibitors of α -glucosidase inhibit the breakdown of

carbohydrates as a result slows down the absorption of glucose and decrease the level of postprandial hyperglycemia (3, 4). Alpha-glucosidase inhibitors act by inhibiting the enzyme. The drugs which are for treating type II Diabetes includes enzyme inhibitors produces side effects like diarrhea, abdominal bloating and flatulence (25). The observations in the present study have shown 43.20% inhibition of α -amylase and 19.80% inhibition of α -glucosidase enzymes by the ethanol extracts of seeds of *S. cumini*. The synthetic drugs may be effectively replaced by seeds of *S. cumini* in the management of diabetes.

CONCLUSION

The results of the present work indicated the antioxidant potential and hypoglycemic activity of seeds of *Syzygium cumini* by inhibiting the activity of key enzymes in digestion of carbohydrates and thereby support the traditional use of this plant for the treatment and management of diabetes mellitus.

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