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Research Article

Antidiabetic Activity and Phytochemical Constituents of *Syzygium cumini* Seeds in Puducherry Region, South India

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ABSTRACT

Syzygium cumini is widely used in traditional medicine to treat diabetes in India. The present study was carried out to evaluate the phytochemical bioactive compounds from *Syzygium cumini* seed extract and its *invitro* anti-diabetic activity. The phytochemical screening showed appreciable amount of flavonoid and steroid in the seed extract. The infrared spectral data obtained revealed the presence of characteristic functional groups of alcohol, hydroxyl, aldehydes, alkanes, alkenes, nitro compound and aliphatic amines etc. The extract exhibits the dose-dependent increase in the inhibitory effect on alpha-amylase enzyme upto 95.4%. The result suggested that significant amount of flavonoid in *Syzygium cumini* seed is responsible for antidiabetic properties and it is further confirmed by higher intensity of alpha amylase inhibitory effect.

Keywords: *Syzygium cumini*, Alpha-amylase, Diabetes, Flavanoids, Phytochemical.

INTRODUCTION

Diabetes is a metabolic disorder characterized by chronic hyperglycemia and impaired insulin signaling which generates metabolic changes and an inflammatory status that will eventually affect all body tissues[1]. The basic mechanism of hyperglycemia in Diabetes Mellitus (DM) is an excessive production and decrease utilization of glucose by tissues. Excessive production may be due to enhanced hepatic glycogenolysis and gluconeogenesis. Asian Indians have one of the highest risks of diabetes among all major ethnic groups, and the conversion from pre-diabetes to diabetes occurs more rapidly in this population[2]. The Center for cardio-metabolic Risk Reduction in South Asia study has shown that the overall prevalence of diabetes in 2 major cities of India Asia was Chennai (south India): 22.8% (21.5-24.1%) and Delhi (north India): 25.2% (23.6-26.8%)[3]. The rate of increase in diabetes prevalence has been shown to be higher in men is 3.33/1000/year as compared with women 0.88/1000/year[4]. Due to changes in food habits, prevalence of diabetes has increased significantly in the past four decades. The oral hypoglycaemic agents currently used in clinical practice have characteristic profiles of serious side effects[5]. The adverse effect by continuous use of synthetic drugs has encouraged the use of plant based medicine which could provide maximum healing with minimum or no side effect. Since ancient times, plants and its extracts were used to combat diabetes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among them, 150 species are used commercially on a fairly large scale[6,7]. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect[8].

Syzygium cumini belong to the family of Myrtaceae, known as 'Naaval' in Tamil and Jamun, Jambul and Jambool in India. The original home of *Syzygium cumini* is India. It also found in Thailand, Philippines, Madagascar and some other countries. The plant has been successfully introduced into many other tropical countries such as the West Indies, East and West Africa and some sub tropical regions including Florida, California, Algeria and Israel[9]. The fruits are oblong berries, deep purple or bluish in colour with pinkish pulp, having various medicinal properties and used in Ayurveda as a stomachic, astringent, antiscorbutic, diuretic, antidiabetic, and in chronic diarrhea and enlargement of spleen[10,11]. *S. cumini* seed extract significantly decrease the blood glucose, blood urea, serum cholesterol and serum triglyceride levels in alloxan induced diabetic rats[12]. The fruit of *S. cumini* has a large market for the treatment of chronic diarrhea and other enteric disorders, including its use as an antimicrobial[13]. The leaves are found to reduce radiation induced DNA damage in cultured human peripheral blood lymphocytes[14]. *S. cumini* seeds has been reported to serve various purposes in diabetic patients, such as lowering blood glucose levels and delaying diabetic complications including neuropathy and cataracts[15]. In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many purposes, India is one amongst them. For this reason we have chosen a native plant *S.cumini*, which is widely used traditional system of

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medicine to treat diabetes. The aim of the present study was to evaluate the active ingredients of *S. cumini* and highlight its antidiabetic properties.

MATERIALS AND METHODS

Sample collection

The seeds of *Syzygium cumini* were collected from the premises of Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry. The collected sample was taxonomically identified by plant biologist. The seeds were air dried at room temperature in the Department of Zoology until constant weight was attained. The dried seeds were pulverized into fine powder using electric blender.

Solvent extraction

A portion of the dried seed powder was soaked in the conical flask containing methanol (Analytical grade) and wrapped with aluminum foil for 72 hours with occasional shaking. After 72 hours, the extracts were filtered using Whatman filter paper No: 1. The solvent was removed from the extract by vacuum distillation. The concentrated *S.cumini* seed extract was dried and stored at 4ºC for further photochemical screening study.

Phytochemical screening

The phytochemical screening was the qualitative analyzed in accordance with Harborne[16] to determine the secondary metabolites present in the extract.

Alkaloids

Mayer's test: 1.36gm of mercuric chloride dissolved in 60ml and 5gm of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100ml using distilled water. To 1ml of acidic aqueous solution of samples few drops of reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

Flavonoids

In a test tube containing 0.5ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl and small amount of Zn or Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

Glycosides

A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

Steroids

Salkowski's test: About 100mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring.

Cardiac glycosides

Keller killiani's test: About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid was added. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardenolides.

Saponins

A drop of sodium bicarbonate was added in a test tube containing about 50ml of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins.

Resins

To 2ml of chloroform or ethanolic extract 5 to 10ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5ml of H_2SO_4 was added. Bright purple colour was produced. It indicated the presence of resins.

Phenols

Ferric Chloride Test: To 1ml of alcoholic solution of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

Tannins

Lead acetate test: In a test tube containing about 5ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

Terpenoid

2ml of chloroform and 1ml of conc. H_2SO_4 was added to 1mg of extract and observed for reddish brown colour that indicated the presence of terpenoid.

FTIR Spectroscopic Analysis

Fourier transform infrared Spectrophotometer (Shimadzu, IR prestige-21) was used to identify the characteristic functional groups in the seed extract. A small quantity (5 mg) of the seed extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The sample was scanned for transmittance within 4000 cm^{-1} to 400 cm^{-1} (mid IR region). The IR spectrum was printed with the individual peaks labeled with their corresponding wavelengths.

Inhibition of alpha-amylase enzyme

A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96 mM. Both control and plant extracts were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid. This reaction is detectable at 540 nm[17].

RESULT AND DISCUSSION

The phytochemical constituents of *S.cumini* seed extract were shown in the Table 1. The biochemical test for alkaloids, flavonoids, glycosides, steroids, cardiac glycosides, saponins, resins, Phenols, tannins and terpenoids were positive in the seed extract. Flavonoids

Table 1: Phytochemical constituents of *S.cumini* seeds extract.

Sl. No	Phytochemical constituents	Methanolic extact of *S. cumini* seeds
1	Alkaloids	++
2	Flavonoids	+++
3	Glycosides	++
4	Steroids	+++
5	Cardiac glycosides	+
6	Saponins	+
7	Resins	+
8	Phenols	++
9	Tannins	+
10	Terpenoid	+

+ = present, ++ = moderately present, +++ =Appreciable amount

and steroids are in appreciable amount along with moderate amount of alkaloids, glycosides and phenols in the seed extract.

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms which are used as medications and recreational drugs[18]. Glucoside has the ability to check the conversion of starch into sugar in case of excess production of glucose[12]. Plant phenols are act as primary antioxidant or free radical scavengers[19]. The terpenoids have also been shown to decrease blood sugar level in animal studies[20]. Rusasinghe *et al.*[21] have reported that saponins possess hypochoesterolemic and antidiabetic properties. Flavonoids act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms to attenuate diabetic complications. Flavonoids enhance the effects of Vitamin C and function as antioxidants. Bioflavonoids are responsible for the stimulation of glucose uptake in peripheral tissues and regulation of the activity or expression of the rate-limiting enzymes involved in carbohydrate metabolism[22]. Plant polyphenols and flavonoids are some of the naturally occurring antidiabetic agents which are known to show an inhibitory effect on carbohydrate hydrolyzing enzyme inhibition, by virtue of their capability to bind with proteins[23,24]. In the present investigation, seed extract showed the presence of flavonoids in appreciable amount which accountable for the antidiabetic activities.

The FTIR spectrums of *S.cumini* seed extract exhibited absorption in the range from 3402.48 cm^{-1} to 668.79 cm^{-1} (Figure 1). The spectrum exhibited a broad band around 3402.48 cm^{-1} assigned to Alcohol and hydroxyl group (O-H) stretching. This may also indicated the presence of phenol and flavonoid. The sharp peak observed at 2926.47 cm^{-1} indicates the presence of alkanes group (C-H). Another sharp peak at 2360.43 and 2343.81 cm^{-1} assigned to C=O stretching vibrations in carbonyl groups which clearly confirms the presence of aldehydes. A long and sharp peak at 1720.41 cm^{-1} attributed to aldehydes and saturated aliphatic (H-C=O). A sharp peak at 1618.03 cm^{-1} attributed to primary amines (N-H). The sharp peak at 1546.11 cm^{-1} indicated the presence of nitro compound (N-O). The peak at 1449.51 and 1351.50 cm^{-1} shows the presence of alkanes (C-H). Sharp peak observed at 1217.02 and 1039.21 cm^{-1} indicates the presence of aliphatic amines (C-N). The peaks observed at 920.97, 757.14 and 668.79 cm^{-1} represents C-H stretch of alkenes. This FTIR spectrum analysis result of *S.cumini* seeds shows the presence of characteristic functional groups of alcohol, hydroxyl, alkanes, aldehydes, nitro compound, primary amines and aliphatic amines.

Alpha-amylase is a digestive enzyme found in the secretions of the intestinal mucosa, pancreas, and the saliva. It is responsible for the breakdown of α-1, 4-glycosidic bonds in starch. Thus, the catalytic activities of the enzyme (especially in the small intestine), increase the availability of glucose in the blood, since the pH of the intestine is around 6.9, α–amylase has access to starch at this pH and catalyses the breakdown of this polysaccharide into monosaccharide and disaccharide. In this study *S.cumini* extract revealed a significant inhibitory action of alpha-amylase enzyme. The percentage inhibition at 0.2-1.0 ml concentrations of *S. cumini* extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from 38.6% - 95.4% for lowest concentration to the highest concentration. There was a dose dependent increase in percentage inhibitory activity against α–amylase enzyme as shown in figure 2.

The ethanol extract of *S.cumini* seed fed orally at various doses significantly decreased blood sugar level in alloxan induced diabetes. The blood sugar level was not elevated, even after discontinuing the extract for 15 days[25]. Blood and urine glucose levels of streptozotocin induced diabetic rats were decreased upon 30 days treatment with ethanolic extract of *S.cumini* seed at doses of 100mg/kg/day[26]. In addition to blood glucose lowering effect, the seed extract was also shown to recover peripheral glucose tolerance in streptozotocin induced diabetic rats[27]. The present investigation revealed that seed has proven potent α-amylase inhibitor. Bhat *et al.*[28] also reported that the chloroform, methanol and aqueous extracts of *S.cumini* seeds have shown to possess significant alpha amylase inhibitory activity. The α-amylase inhibitors are among the drugs that reduce hyperpostprandial blood glucose by inhibiting the hydrolysis of the starch[29]. Inhibition of α-amylase contributes to improve symptoms of type 2 diabetes by delaying or interrupting glucose absorption as a result of slowing starch digestion. Although the main purpose of α-amylase inhibition is to slow down maltose and glucose production, it can also slow α-glucosidase function by eliminating the substrate of this enzyme[30].

CONCLUSION

The present study demonstrated that the methanolic extract of *S.cumini* seeds possesses phytochemicals such as alkaloids, flavonoids, glycosides, steroids, cardiac glycosides, saponins, resins, phenols, tannins and terpenoids etc., which are of high therapeutic value. The infrared characterization revealed the presence of aliphatic as well as aromatic compounds. This study also demonstrated that the extract has potent α-amylase inhibitor with a higher degree of inhibition. The results suggested that the *S.cumini* seeds possess significant

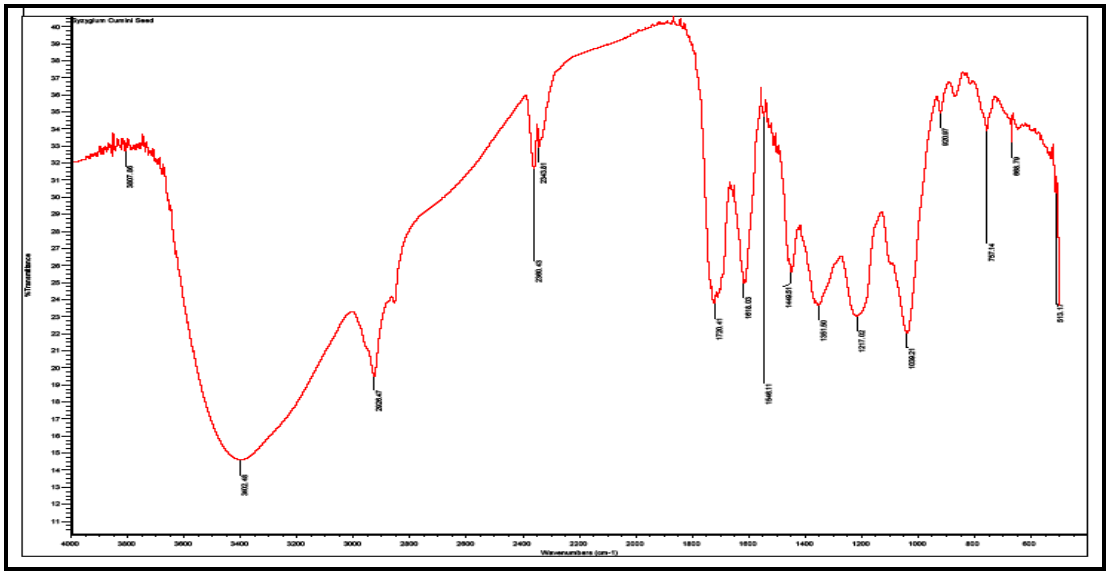

Figure 1: FTIR Spectroscopic analysis *Syzygium cumini* seed extract.

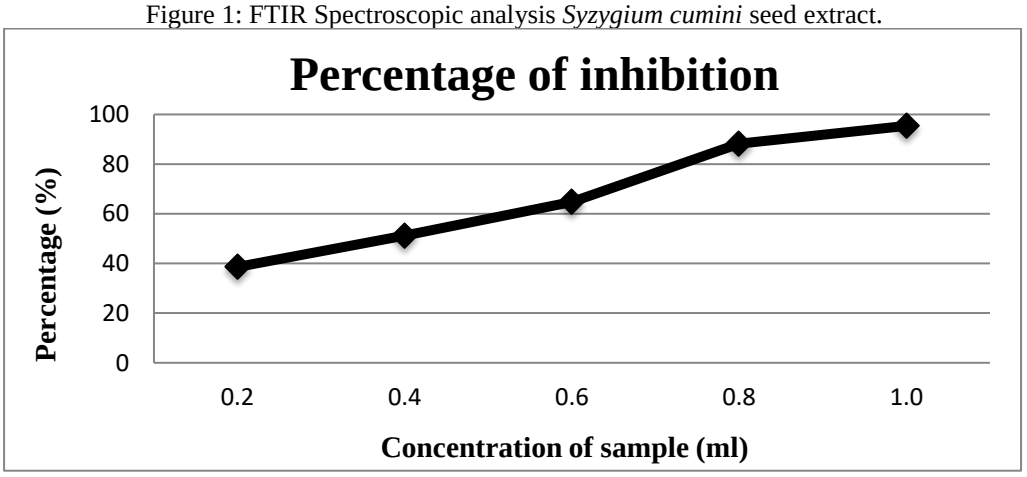

Figure 2: *In vitro* antidiabetic activity of alpha-amylase.

antidiabetic activity. This study helps to determine the regional pharmaceutical value of *S.cumini* seeds as a traditional medicinal utilization for the management of diabetes. Further, isolation of the active principle can leads to development of promising pharmacological active drug candidates.

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