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In Vitro Anti-Diabetic Activity of *Syzygium cumini* SEEDS

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ABSTRACT

Alpha- amylase and alpha- glucosidase are played a important role in the carbohydrate digestion. These enzymes digest the carbohydrates from mouth onwards. The inhibition of this enzyme may reduces the glucose concentration in blood stream. The recent researches are find the therapeutic approaches to reduces the glucose level in blood by the inhibition of this enzymes in the in vitro analysis. This can be a major strategy for the management of blood glucose level in the body. The aim of the present study was to investigate the phytochemical analysis of various extract and find out the in vitro anti-diabetic activity of *Syzygium cumini* seeds. The result proves that the presence of phytoconstituents could be responsible for the medicinal properties of this plant including diabetes and the extract exhibit the dose-dependent action by increasing the inhibitory effect on the alpha-amylase enzyme and alpha- glucosidase enzyme.

Keywords: In vitro analysis, anti-diabetic, Syzygium cumini, alpha-amylase, alpha-glucosidase

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INTRODUCTION

Insufficient secretion of insulin or its activity leads the chronic disorder like diabetes mellitus (Kumar and Clark, 2002). The prolonged diabetic condition may influences the metabolic pathways of various biomolecules such as carbohydrate, protein and fat (Lindberg *et al*., 2004). It may causes the various complications such as retinopathy (Bearse *et al*., 2004), nephropathy (Huang *et al*., 2002), cardiovascular complication (Svenson, 2004) and neuropathy (Wallace, 2002). Symptoms like polyurea polydipsia, polyphagia and unexplained weight loss are seen in the patients with diabetes. Diabetes mellitus may be classified into several types type I , type II and other types includes, drug induced infections, uncommon forms of immune mediated diabetes, gestational diabetes etc. Indian Council of Medical Research predicts in India, nearly 40.9 million peoples are affected by diabetes, which is increased up to 69.9 million in the year of 2025. Indian Council of Medical Research indicates 2.1% are from urban population and 1.5% are from the rural population are affected by diabetes (Mohan *et al*., 2007).

Two types of therapy are done in now a day, they are insulin therapy and anti-diabetic drug therapy. The anti-diabetic drugs such as sulfonyl urea, biguanides, meglitinide analogue, thiazolidine, diones α-amylase inhibitors and α-glucosidase inhibitors are commonly used. The treatment of diabetes with synthetic drug available to treat diabetes is complicated because of its adverse effects. Due to this the researchers are try to find the plant used drugs because of its negligible side effects. *Syzygium cumini is a tropical plant in the family of Myrtaceae*. Seed of this plant contains various phytoconstituents such as chlorophyll, fat, resin, gallic acid, resorcinol, dimethyl ether and folic acid. The seeds are fairly rich in the protein, and calcium (Daulatabad *et al*., 1988). The present study aims to find the phytoconstituents present in the seed extracts and analysis the *in vitro* anti-diabetic activity.

MATERIALS AND METHOD

Plant material and extraction

The fresh seeds of *Syzygium cumini* were collected locally and authenticated by the department of Botany, St. Joseph College, Trichy. The shade dried *S. cumini* seeds were powdered mechanically and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvents such as aqueous, ethanol and methanol. About 100 gm of powder was extracted with 600 ml of various extracts. The extracts were concentrated to dryness under controlled temperature 40- 50°C. The extract was preserved in refrigerator till further use. Phytochemical Screening for preliminary phytochemical analysis the freshly prepared crude extracts of seeds were tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, steroids and alkaloids by using standard phytochemical procedures (Evans, W.C and Evans, T. 2003).

In vitro anti-diabetic studies

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 (Malik and Singh, 1980).

Inhibition of alpha-glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm (Krishnaveni *et al*., 1984).

Calculation of 50% Inhibitory Concentration (IC50)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by I % = (Ac-As)/Ac X 100, (Shai *et al*., 2010). where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULTS AND DISCUSSION

The large number phytoconstituents are present in the medicinal plants. It mainly involves the various pharmacological activities (Kaushik *et al*., 2002). Now-a-days, the researchers are trying to find the new bioactive compounds from the plant sources for the discovery of new medicines (Milne *et al*., 1993). The medicinal importance of the plant extract is directly proportional to the presence of phytoconstituents. Since many phytoconstituents are not documented properly. Therefore, the present study aimed to investigate to find the phytoconstituents present in the *S. cumini* seeds of various extracts.

The seeds of *S. cumini* were collected, dried and then crushed extracted by using various solvent such as aqueous, methanol and ethanol. The various extracts of *S. cumini* are subjected to find the presence of the various compounds. **Table 1** shows the presence of the various phytoconstituents in the various extract of *S. cumini*. In the present study, the alkaloids quinone and flavonoids are present in the all the extracts of *S. cumini* seeds. Phenol is present in the

aqueous and ethanolic extract of *S. cumini* seeds. The biological activity of the plant source or not depending on the single phytoconstituents, but it is may be due to the synergic effect of various phytoconstituents such as tannins, phenol, terpenoids etc. Based on the results, further studies are carried out only with ethanolic extract of *S. cumini* seeds.

Table 1: Phytochemical constituents of various extracts of S. cumini seeds

S.No	Phytoconstituents	Aqueous	Methanol	Ethanol
1	Alkaloids	+	+	+
2	Carbohydrates	-	+	+
3	Tannin	-	-	+
4	Terpenoids	-	-	+
5	Quinones	+	+	+
6	Total protein	-	+	+
7	Flavonoids	+	+	+
8	Phenols	+	-	+

IN VITRO ANTI-DIABETIC ACTIVITY OF *S. cumini* SEEDS

The present study to find the *in vitro* anti-diabetic activities of various concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) of methanolic extracts of *S. cumini* seeds with the standard drug acarbose by an inhibitory activity of α-amylase and α-glucosidase enzymes. The percentage of inhibition of α-amylase was increased in higher concentration (1 mg/ml) of *S. cumini* seeds extract and standard drug acarbose. The inhibition of ethanol extract and acarbose of α-amylase were 14.8% and 18.4% at 0.2 mg/ml and 81.9% and 90.1% at 1 mg/ml and the IC50 values were 0.72 mg/ml and 0.58 mg/ml **(Table 2**).

Table 3 shows the in vitro anti-diabetic activity of α-glucosidase on *S. cumini* seeds. In this assay, the maximum inhibition was noted at 1 mg/ml and minimum inhibition was noted at 0.2 mg/ml. The minimum percentage of inhibition of ethanolic and acarbose was 11.3%and 17.9% at 0.2 mg/ml and the maximum percentage of inhibition was 78.1%and 91.6% at 1 mg/ml. IC50 values are 0.74mg/ml and 0.56 mg/ml of ethanol and acarbose respectively.

α-amylase and α-glucosidase are the main carbohydrate enzyme, it mainly involved in the digestion of polysaccharides to monosaccharides (Cheng and Fantus, 2005). The inhibition of these enzymes which may reduces the glucose concentration in blood stream. The number of synthetic drugs are available, but it causes various side effects like vomiting, diarrhea etc (Chakrabati and Rajagopalan, 2002). The previous studies reports that the inhibition of this enzymes by the plant extract is directly proportional to the anti-diabetic activity of the plant in the in vitro studies. Manikandan *et al*, (2017) proves that the methanolic extract of leaves of *Psidium guajava* strongly inhibits this enzymes. This may prove in the present study also.

Table 2: *In Vitro* Anti-diabetic Activity of ethanolic extract of *S. cumini* seeds by Alpha-Amylase Method

S.No	Concentration of sample (mg/ml)	% of inhibition of ethanolic extract of *S. cumini*	% of inhibition of acarbose
1	0.2	14.8	18.4
2	0.4	29.1	34.3
3	0.6	45.7	52.6
4	0.8	62.5	71.2
5	1.0	81.9	90.1
	IC_{50}	0.72	0.58

Table 3: *In Vitro* Anti-diabetic Activity of ethanolic extract of *S. cumini* seeds by Alpha-Glucosidase Method

S.No	Concentration of sample (mg/ml)	% of inhibition of ethanolic extract of *S. cumini*	% of inhibition of acarbose
1	0.2	11.3	17.9
2	0.4	26.7	33.7
3	0.6	44.9	54.8
4	0.8	58.3	72.9
5	1.0	78.1	91.6
	IC_{50}	0.74	0.56

CONCLUSION

In this present study, find out the various phytoconstituent present in the aqueous, ethanolic and methanolic extract of S. cumini. Among these the ethanolic extract which contains large number of phytoconstituents. In vitro alpha amylase and alpha glucosidase activity of crude ethanol extract of *S. cuminin* seeds. The plant showed significant inhibition activity, so further studies are warranted.

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