

**IN-VITRO-ANTIDIABETIC ACTIVITY OF N-BUTANOL EXTRACT OF *SESAMUM INDICUM***

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**ABSTRACT**

**Objective:** The present study was aimed to investigate the inhibitory effect of n-Butanol extract of black sesame seeds on the enzymes  $\alpha$ - amylase and  $\alpha$ - glucosidase followed by its Total antioxidant capacity.

**Methods:** The Black sesame seeds were extracted in n-Butanol, powdered and stored for future studies. Phytochemical analysis was performed to detect the presence of many phytochemicals. The extracts were screened Alpha-amylase inhibition assay and Alpha-glucosidase inhibition assay. The total antioxidant capacity of the n-Butanolic extract was evaluated by the phosphomolybdenum method.

**Results:** The obtained results showed the high alpha-glucosidase inhibitory affect than the alpha-amylase inhibition when compared to standard acarbose. Even the Total antioxidant capacity was equivalent when compared with the standard Ascorbic acid values.

**Conclusions:** Based on the results, it can be concluded that *Sesamum indicum* herb has potential phytochemicals which can be used to reduce the postprandial hyperglycemia by inhibiting carbohydrates metabolizing enzymes  $\alpha$ - amylase and  $\alpha$ - glucosidase, and also to combat the free radicals by its anti-oxidant activity.

**Keywords:** Black sesame seeds, n-butanol, Alpha-amylase inhibition assay, Alpha-glucosidase inhibition assay, Phosphomolybdenum reduction.

**INTRODUCTION**

Sesame (*Sesamum indicum* Linn.) seed is one of the most important oilseed crops in the world. Besides providing highly stable oil, sesame seed is also a source of protein-rich meals and is used in sweetmeats and confectionery foods, and has varieties of medicinal properties. Sesame oil plays a prominent role in Indian Ayurvedic and Tibetan Medicines. In the Chinese system of medicine, dried sesame flowers are used in curing alopecia, frostbite, and constipation. Several studies have reported the health-promoting effects of sesame, and this can be attributed to the presence of bioactive compounds including lignans and phenols [1,2].

Recent studies have shown that phenolic phytochemicals have high antioxidant activity and certain therapeutic properties including anti-diabetic [3] and anti-hypertension activity [4]. It has been reported that sesame seeds can improve oxidative stress due to actions of their contents of vitamin E and lignans including sesamin, sesamol, sesamol, and sesame [5].

An earlier report showed the hypoglycemic effect of a hot-water extract from Banaba leaves and To-kai-san in genetically diabetic KK-Ay mice, and animal model for non-insulin dependent (Type 2) diabetes. Based on this, Takeuchi *et al.* [6] showed the effect of hot-water extract from defatted sesame seed on the plasma glucose level in KK-Ay mice, sesame [3].

Complex carbohydrates are degraded to disaccharides and oligosaccharides by alpha-amylase which, in turn, gets converted to monosaccharides by the action of alpha-glucosidase. The final byproduct gets absorbed by the intestine and results in an increase in post-prandial glycemic levels. Inhibition of these enzymes will help in controlling of sugar level by delaying the breakdown of carbohydrates and its absorption [7].

Many studies have been carried out on the anti-oxidant activities of sesame seeds rather than anti-diabetic activity. In this study, we determined the anti-diabetic activity of n-butanol extract of black sesame seeds.

**MATERIALS AND METHODS****Samples preparation**

The Black sesame seeds were purchased from local markets in Chennai, India. The seeds were cleaned physically to removes foreign particles. Then, the seeds were grounded in a mechanical grinder. Pure powder could not be obtained as the oil was present in the seeds. From the ground, the samples 50 g was weighed and mixed with 400 mL of ethyl acetate and 400 mL of n-butanol, respectively. The mixture was kept in shaker for 24 hrs. Next day the solution was filtered using Whatman filter paper No 1. The filtrate was air dried and stored for further studies.

**Phytochemical analysis**

The filtrate was tested for the presence of phytochemicals such as alkaloids, flavanoids, saponins, tannins, phenols, carbohydrates, cardiac glycosides, terpenoids and proteins using standard procedures of Trease and Evans [8], Harborne [9], and Sofowara [10].

**Alpha-amylase inhibition assay**

The method adopted was slightly modified from Apostolidis *et al.* [11]. 250  $\mu$ l of 0.02 M sodium phosphate buffer containing alpha-amylase solution (1 mg/mL) and 500  $\mu$ l of samples of varying concentration were incubated at 37°C for 10 minutes. After pre-incubation, 250  $\mu$ l of 0.5% starch solution in 0.02 M sodium phosphate buffer was added. The reaction was incubated at room temperature for 10 minutes. 1 mL of dinitrosalicylic acid color reagent was added to stop the reaction. The test tubes were then incubated in boiling water bath for 10 minutes and cooled to room temperature. The assay was performed in duplicates. The absorbance was read at 540 nm. The percentage of inhibition was calculated using the below formula:

$$\% \text{ Inhibition} = \frac{([A540 \text{ blank} - A540 \text{ extract}]) * 100}{(A540 \text{ blank})}$$

The IC<sub>50</sub> values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-amylase inhibitor. All tests were performed in duplicate.

### Alpha-glucosidase inhibition assay

The assay procedure is modified from Pistia-Brueggeman and Hollingsworth [12]. Alpha-glucosidase (20  $\mu$ l, 1.25 u/mL) was premixed with 200  $\mu$ l of samples of various concentrations and made up in a 50 mM phosphate buffer at pH 6.8 and incubated at 37°C for 10 minutes. 1 mM pNPG dissolved in phosphate buffer was added to initiate the reaction and incubated at room temperature for 20 minutes. The reaction was terminated by adding 20 $\mu$ l of 1M Na<sub>2</sub>CO<sub>3</sub> and final volume was made up to 1 mL with phosphate buffer. The absorbance was read at 405 nm. The assay was done in duplicates. The IC<sub>50</sub> values were calculated. The percentage of inhibition was calculated using the below formula:

$$\% \text{ Inhibition} = [(A405 \text{ blank} - A405 \text{ extract})] * 100 / (A405 \text{ blank})$$

The IC<sub>50</sub> values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-glucosidase inhibitor. All tests were performed in duplicate.

### Total anti-oxidant activity (TAC)

TAC of the n-butanolic extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.*, [13]. An aliquot of 500  $\mu$ l of extracts with varying concentrations was combined with 4.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and boiled at 95°C for 90 minutes, and the absorbance was measured at 695 nm. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The values were expressed as equivalents of ascorbic acid in  $\mu$ g per mg of extracts.

## RESULT AND DISCUSSION

The preliminary phytochemical analysis of n-butanolic extract of black sesame seeds (Table 1) showed the presence of a limited number of phytochemicals. Any of these phytochemicals, either singly or in a combination with each other could be responsible for the in-vitro antidiabetic activity of the plant.

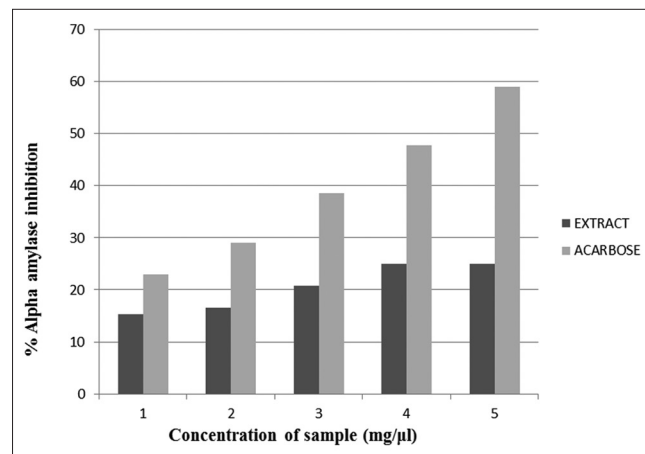
$\alpha$ -Amylases are endoglucanases, which hydrolyse the internal  $\alpha$  1,4 glucosidic linkages in starch.  $\alpha$ -Glucosidase is one of the glucosidases located in the brush border surface membrane of intestinal cells and is a key enzyme for carbohydrate digestion. These enzymes have been recognized as therapeutic targets for modulation of postprandial hyperglycemia.  $\alpha$ -Amylase and  $\alpha$ -glucosidase inhibitors are known to reduce postprandial hyperglycemia by partially inhibiting the enzymatic hydrolysis of complex carbohydrates and hence may delay the absorption of glucose. Acarbose, voglibose and miglitol are synthetic inhibitors used either alone or in combination with insulin secretagogues for patients with Type 2 diabetes. However, these inhibitors are reported to cause several side effects. Several other safer natural inhibitors are reported from plant resources [14].

**Table 1: Phytochemical analysis of different extracts of *S. indicum***

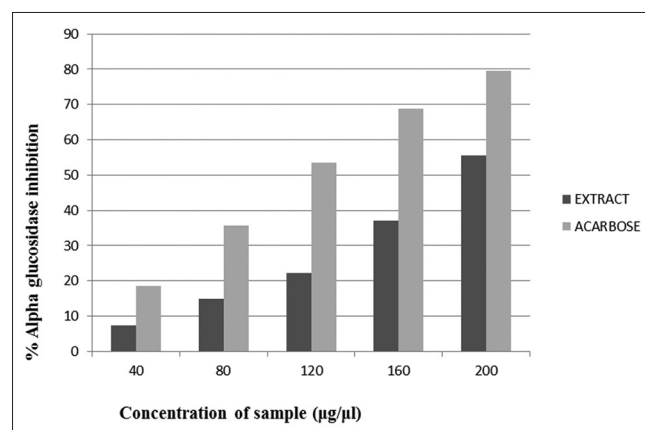
S. No	Phytochemicals	Butanol extract
1	Alkaloids	-
2	Carbohydrates	-
3	Flavonoids	-
4	Glycosides	+
5	Saponins	+
6	Tannins	-
7	Terpenoids	+
8	Phenols	-
9	Proteins	-
10	Steroids	+
11	Amino acids	-

*S. indicum*: *Sesamum indicum*

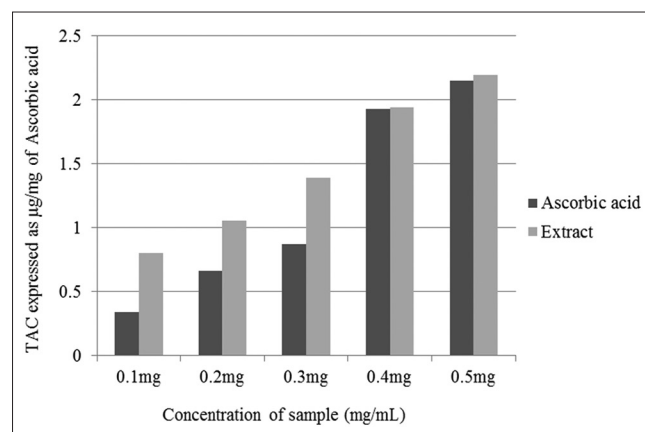
In our study, we checked the  $\alpha$ -glucosidase inhibitory activities of n-Butanolic extract of *S. indicum* and the value obtained were compared with standard acarbose (Fig. 2). There was a dose-dependent increase in the percentage inhibition activity against alpha-glucosidase enzyme. At a concentration of 200  $\mu$ g/mL sesame extract had 58% of inhibition activity while acarbose had 80%. It can be seen that n-butanolic sesame seed extract has high alpha-glucosidase inhibitory activity than alpha-



**Fig. 1: % inhibition of alpha-amylase enzyme by n-butanolic extracts of *Sesamum indicum* and reference alpha-amylase inhibitor, acarbose**



**Fig. 2: % inhibition of alpha-glucosidase enzyme by n-butanolic extract of *Sesamum indicum* and reference alpha-glucosidase inhibitor, acarbose**



**Fig. 3: Total antioxidant capacity assay of n-butanolic extract of *Sesamum indicum* and reference antioxidant, ascorbic acid**

amylase inhibition. There are recent studies showing the association of antioxidant activity and  $\alpha$ -glucosidase inhibition. Thus, the inhibition of enzyme in this study can be related to the presence of antioxidants in the n-butanolic extract of *S. indicum*.

On investigating the  $\alpha$ -amylase inhibitory activities of methanol extract of DSM, we found that the extract showed only low inhibitory activity against  $\alpha$ -amylase. The maximum inhibition shown was only 24.95% and the concentration was 5 mg/mL (Fig. 1). On further increasing the concentration the extract showed a constant inhibitory activity. Mild inhibition of pancreatic  $\alpha$ -amylase and strong inhibition of intestinal  $\alpha$ -glucosidase is considered as an effective strategy for Type 2 diabetic management [15].

The significantly equal absorbance values of n-butanol sesame seed (Fig. 3) extract with ascorbic acid indicates the presence of total anti-oxidant activity (TAC) (expressed as ascorbic acid equivalent) suggesting that the extract, exhibits considerable amount of antioxidant activity. The TAC was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. It evaluates both water-soluble and fat-soluble antioxidants (TAC).

The goal of this study was to provide *in vitro* evidence for  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition, which is less reported in sesame. As observed, extracts with higher antioxidant capacity also had higher antidiabetic inducing effect. TAC observed in this study can also be attributed to the presence of glycosides, saponins, terpenoids and steroids. Thus, this study also points out the possibility of exploring the utilization of sesame seeds not only as a potential source of protein, but also as a source of health-protective bioactive for developing functional food.

## CONCLUSION

In this study, *in-vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase activity of crude n-butanol extract of *S. indicum* seeds were evaluated. The result of this study shows that the butanolic extract of black sesame seeds has high  $\alpha$ -glucosidase inhibitory activity than  $\alpha$ -amylase inhibitory activity. Moreover, it also exhibits TAC when compared to standard acarbose. The phytochemical analysis showed the presence of limited number of bioactive compounds. Hence, these bioactive compounds are expected to have the *in-vitro* anti-diabetic activity. Further research is required to carry out the screening of these bioactive compounds which possess the anti-diabetic activity.

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