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α-GLUCOSIDASE AND α -AMYLASE INHIBITORY ACTIVITIES OF *RAPHANUS SATIVUS* LINN.

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ABSTRACT

Herbal medicine has been used for many years by different cultures around the world for the treatment of diabetes. There has been an enormous interest in the development of alternative medicines for type 2 diabetes, specifically screening for phytochemicals with the ability to delay or prevent glucose absorption. The goal of the present study is to evaluate the *invitro* antidiabetic activity of *Raphanus sativus* ethanolic extract and fractions by α-glucosidase and α -amylase inhibitory activity. *Raphanus sativus* ethanolic extract and fractions showed dose dependent inhibition of α-glucosidase and α -amylase enzyme and exhibited lower inhibitory activity than acarbose. The study revealed the antidiabetic potential and could be helpful to develop medicinal preparations and nutraceuticals and function foods for diabetes.

INTRODUCTION: Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves [1].

One of the therapeutic approaches is to decrease the postprandial hyperglycaemia by retarding absorption of glucose by inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidases [2]. From this point of view, many efforts have been made to search for more effective and safe inhibitors of α-glucosidase and α-amylase from natural materials to develop physiological functional food to treat diabetes [3].

Many traditional plants treatments for diabetes have been reported in India but only a small number of these have received scientific and medical evaluation to assess their efficacy.

On the basis of ethno medical/tribal information *Raphanus sativus* is used to treat and prevent diabetes.

However, the studies on anti-diabetic effects of *Raphanus sativus* were not focused on the enzyme inhibitory activity of the extract and fractions.

The present study is designed study the *invitro* antidiabetic activity of *Raphanus sativus ethanolic* extract and to understand how the extract and fractions acts against α-glucosidase and α-amylase.

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MATERIALS AND METHODS:

Materials: The entire plant of *Mukia maderspatana* was collected from the forests of Doddabetta in Nilgiris. The plant species was identified and authenticated by Botanist, Government Botanical garden, Ooty.The voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, JSS College of Pharmacy, Ooty . Porcine pancreatic amylase, α glucosidase from Bakers yeast, p-nitrophenyl-a-d-glucopyranoside and dinitrosalicylic acid were purchased from Sigma chemicals.

Preparation of crude extract and fractions: 100 g powdered sample of various extracts were weighed and soaked in 250 ml of 95% ethanol in a separating funnel for 24 hours, with intermittent shaking. The plant extract were then collected and filtered through Whatman No. 1 filter paper. The extract were concentrated at 50^0C using vaccum rotatory evaporator and then air-dried. The dried powder was stored at 40 ^{0}C in an airtight bottle. The extract was fractioned with chloroform and n-butanol and all were used for invitro antidiabetic studies.

α- Amylase Inhibition Activity: The α -amylase inhibitory activity of the *Raphanus sativus* ethanolic extract and fractions (chloroform and n-butanol) were determined [4]. A total of 250 µl of sample and 125 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) containing α - amylase solution (0.5 mg/ml) was incubated at 25^{o}C for 10 min. After preincubation, 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25^{o}C for 10 min. The reaction was stopped with 0.5 ml of dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 5 ml of dis-tilled water, and absorbance was measured at 540 nm. Acarbose was used as the positive control. The α -amylase inhibitory activity was calculated as follows:

$$\text{Inhibition (\%)} = (1- A_{sample} / A_{cont}) \times 100$$

Where, A_{samp} and A_{cont} were defined as absorbance of the sample and the control respectively.

α -Glucosidase Inhibitory Activity: The α -glucosidase inhibitory of activity *Raphanus sativus* ethanolic extracts and fractions (chloroform and n-butanol) were determined[5]. A mixture of 50 µl of sample and 100 µl of 0.1 M phosphate buffer (pH 6.9) containing α -glucosidase solution (1 U/ml) was incubated in 96 well plates at 25 ◦C for 10 min. After preincubation, 50 µl of 5 mM pNPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 ◦C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by microplate reader. Acarbose was used as the positive control. The α -glucosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

$$\textbf{Inhibition (\%)} = (1- A_{sample} / A_{cont}) \times 100$$

where A_{samp} and A_{cont} were defined as absorbance of the sample and the control respectively.

RESULTS: Pancreatic α- amylase is a key enzyme in the digestive system and catalyzes the initial step in the hydrolysis of starch, which is a principal source of glucose in the diet. The *Raphanus sativus* ethanolic extract and fractions (chloroform and n-butanol) showed dose dependent inhibition of the α- amylase enzyme (IC_{50} =35-48µg/ml). As observed, the extract and fractions exhibited lower α-amylase inhibitory activity, compared with that of acarbose, which showed potent inhibition of α –amylase.

α-Glucosidase, a key enzyme for carbohydrate digestion, has been recognized as a therapeutic target for modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality to occur in type 2 DM. The *Raphanus sativus* ethanolic extract and fractions (chloroform and n-butanol) showed dose dependent inhibition of the α -glucosidase enzyme (IC_{50} = 30-34µg/ml). As observed, the extract and fractions exhibited lower α -glucosidase inhibitory activity, compared with that of acarbose, which showed potent inhibition of α –glucosidase.

The inhibitory activities of extract and fractions of *Raphanus sativus* on enzyme inhibitory activity is given in **Table 1**

TABLE 1: α–AMYLASE AND α–GLUCOSIDASE INHIBITORY ACTIVITY OF *RAPHANUS SATIVUS*

Groups	Treatment	α –amylase (IC 50) µg/ml	α –Glucosidase (IC 50) µg/ml
1	EERS	35.52±1.21	30.88±0.68
2	CFRS	35.28±0.38	39.65±2.61
3	BFRS	48.84±0.54	34.07±0.25
4	ACARBOSE	11.84±0.19	13.39±0.11

Values are expressed as Mean ± SEM (n=3). EEMM – Ethanolic extract of *Raphanus sativus.* CFMM – Chloroform fraction of *Raphanus sativus.* BFMM – n- butanol fraction of *Raphanus sativus*

DISCUSSION: The treatment goal of diabetes patients is to maintain near normal levels of glycemic control, in both the fasting and post-prandial states. Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine [6]. α-Amylase catalyses the hydrolysis of a-1, 4-glucosidic linkages of starch, glycogen and various oligosaccharides and α–glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes [7]. Therefore, effective and nontoxic inhibitors of a-amylase and a-glucosidase have long been sought.

In this study, we have investigated the anti-diabetic potential of the *Raphanus sativus*, which is used in traditional ayurvedic medicine for the treatment of several diseases. This valuable herb was not previously investigated for its *invitro* anti-diabetic activity. However, our study clearly established the anti-diabetic potential of *Raphanus sativus*, and revealed that the active principles responsible may be flavonoids, terpenes and phenolic compounds .

Flavonoids, like anti-oxidants, may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes [8].

Although, in the present study, the enzyme inhibitory activity of these extract and fractions were assayed *invitro*, the results from this work should be relevant to the human body. In addition to α-amylase and α-glucosidase inhibitory activities, these phyto-constituents are also reported to have several other biological activities including anti-bacterial, anti-oxidative, anti-cancer etc.[9]. This supportive evidence further increases the medicinal importance of this *Raphanus sativus* indicating that this herb is not only beneficial for diabetes but also may be useful to a number of other human health complications.

CONCLUSION: This study investigated the potential anti-diabetic activity of the *Raphanus sativus*, focusing on the inhibitory effects on α-glucosidase and α -amylase. Further isolation of active principles would be helpful to explain the pharmacological mechanism and also to develop medicinal preparations, nutraceuticals or functional foods for diabetes and related symptoms.

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