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Research Article

EVALUATION OF ALPHA AMYLASE INHIBITORY POTENTIAL OF FOUR TRADITIONAL CULINARY LEAVES

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ABSTRACT

Diabetes mellitus and obesity is considered as one of the leading causes of death in world. Diet has been recognized as a corner stone in the management of diabetes mellitus. Drug, diet and recently spices therapies are the major approaches used for treatment and control of diabetes mellitus. Spices are the common dietary adjuncts that contribute to the taste and flavor of foods. In addition, they are also known for their preservative and medicinal value which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices. Keeping this view, in present study, the ethanol extract of four traditional culinary spices leaves of *Murraya koenigii*, *Coriandrum sativum*, *Trigonella foenumgraecum* and *Tamarindus indica* were tested for their inhibitory potential on α-amylase activity (*in vitro*). Enzyme α-amylase inhibitor acts as an anti-nutrient that obstructs the digestion and absorption of carbohydrate. *M. koenigii*, *T. indica* were found to exhibit interesting inhibitory activity.

Keywords: *Murraya koenigii*; *Coriandrum sativum*; *Trigonella foenumgraecum*; *Tamarindus indica*; *α*-amylase activity

INTRODUCTION

Alpha amylase is a membrane bound enzyme which is located on the brush border of the small intestine and it is required for the breakdown of complex carbohydrates into monosaccharides that can be absorbed. Its inhibitors reduce the glucose peaks that can occur after a meal, slowing the speed of convertion of starch to simple sugars. One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved by delaying the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes in the digestive tract. *α*-glucosidase inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycaemia[1]. Alpha- amylase and glucosidase inhibitors are drug-design targets in the development of compounds for the treatment of diabetes, obesity and hyperlipaemia[2]. Low-cost herbal treatment is recommended due to their lesser side effect for treatment of diabetes[3]**.** Ethnobotanical studies of traditional herbal remedies used for diabetes have identified more than 1,200 species of plants with hypoglycemic activity[4, 5]. The search for new pharmacologically active agents obtained by screening of culinary herbs/spices or their extracts can lead to potent and specific inhibitors for key enzymes linked in diabetes.

Indian cuising's distinctive flavor and aroma is achieved by blend and/or combination of spices including curry leaves, tamarind, coriander, garlic, pepper, cardamom, fenugreek, nutmeg, mustard and turmeric powder. Dietary spices influence various systems in the body such as gastrointestinal, cardiovascular, and reproductive and nervous systems resulting in diverse metabolic and physiologic actions[6]. Drug, diet and recently spices therapies are the major approaches used for treatment and control of diabetes mellitus[7]. Spices are also known to exert several beneficial physiological effects including the antidiabetic influence. In present study, the ethanol extract of four traditional culinary spices leaves of *Murraya koenigii*, *Coriandrum sativum*, *Trigonella foenumgraecum* and *Tamarindus indica* were tested for their inhibitory potential on *α*-amylase activity (*in vitro*).

MATERIALS AND METHODS

Chemical

Acarbose (Bicon Ltd), *α*-amylase (SRL), and potato starch (S.D. Fine-Chem).

Plant

Murraya koenigii L. (Family- Rutaceae, Curry leaves), *Coriandrum sativum* L. (Family- Apiaceae, Coriander leaves**),** *Trigonella foenumgraecum* L. (Family- Leguminosae, Fenugreek), *Tamarindus indica* L. (Family- Caesalpinaceae, Tamarind), the leaves were collected from periphery of Tal. Malkapur. The specimens were submitted to the Herbarium of Pharmacognosy Department, College of Pharmacy, Malkapur and taxonomically identified and authenticated by the experts.

Preparation of Extract

Ethanol extraction was prepared as follows:

The leaves of *Murraya koenigii*, *Coriandrum sativum*, *Trigonella foenumgraecum* and *Tamarindus indica* were shade dried and made into coarse powder and were extracted using ethanol. Dried Powder (75g) of leaves were extracted continously by masseration using ethanol (200 ml, 99.9%) which then evaporated at 45±5 °C to yield dry extract. The extract was preserved in refrigerator till further use.

Phytochemical investigation

Phytochemical assay of all extracts were performed to determine chemical compound content of extracts qualitatively. Based on an established procedure, was conducted to explore the secondary metabolites[8, 9].

Enzyme inhibitory assay

In vitro α-amylase inhibitory assay was performed on extracted leaves. The *α*-amylase inhibition assay was performed using the chromogenic method. Test samples and standard drug were added to buffer solution (pH 6.9) containing *α*-amylase solution and were incubated at 25°C. After these, 1% starch solution (pH 6.9) was added. The reaction mixtures were then incubated at 25°C. The reaction was stopped by adding 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle. Inhibition of starch hydrolysis by α-amylase inhibitor results in a diminished absorbance at 546 nm in comparison with the controls[10].

Calculation of IC_{50}

The concentration of the plant extracts required to inhibit 50% of the enzyme (IC_{50}) was calculated by using the percentage inhibition activities (I %) at different concentrations of the extracts.

I % = (Ac-As)/Ac X 100, where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULTS AND DISCUSSION

Preliminary phytochemical screenings of all ethanol extracts were performed. It gave positive tests for alkaloids, flavonoids, triterpenoids, sterols and phenolic compounds (*M. koenigii*), triterpenoids, flavonoid, tannins, fat and sterols (*C. sativum*), alkaloids, phenolic compounds, flavonoids and glycosides (*T. foenumgraecum*) and phenolic compounds, tannins, flavanoid and glycosides (*T. indica*).

The *α*-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates and potentially useful in control of obesity and diabetes[11, 12]. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faces in the colon [13, 14]. Our finding reveals that Out of four tested spices samples *M. koenigii*, *T. indica* exhibited much *α*-amylase *in vitro* inhibitory activity whereas *C. sativum*, *T. foenumgraecum* exhibited low activity (Table 1). The reaction mechanisms involved in inhibition of *α*-amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein (flavanols) might cause conformational changes in structure[15]. It has been reported that plant phenolic compound modulate the enzymatic breakdown of carbohydrate by inhibiting amylase enzymes [16]. The activity of selected spices might be because of presence of tannin in ethanol extract.

Table 1: *In vitro α*-amylase inhibitory activity of Indian culinary leaves extract[a]

Sr. No	Test material	Percentage Inhibition
1	Reference standard[b]	71.52
2	*Murraya koenigii*[c]	63.28
3	*Coriandrum sativum*[c]	18.77
4	*Trigonella foenumgraecum*[c]	31.32
5	*Tamarindus indica*[c]	67.08

[a]All determination were done in tripilicate, [b]standard *α*-amylase inhibitor- acarbose (Bicon Ltd.), [c]Tested at concentration of 1mg/ml of reaction

CONCLUSION

The selected spices, which are used daily in Indian, were found to exhibit inhibitory activity. From that *M. koenigii*, *T. indica* were found to exhibit interesting inhibitory activity and their active constituents might provide new plant origin *α*-amylase inhibitor with anti-diabetic and anti-obesity activity.

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