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Phytochemical Analysis and Evaluation of *In-vitro* Alpha-amylase Inhibition Activity of Rhizome Extract of *Curcuma longa* (Turmeric)

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A B S T R A C T

One of the major public health issues of the twenty-first century is the prevalence of type 2 diabetes which is rising in both developed and developing nations. It has been established that postprandial hyperglycemia strongly depends on the absorbed monosaccharides and the velocity of absorption in the small intestine and it is mediated by carbohydrates hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase. One of the therapeutic approaches for decreasing of blood glucose rise after a meal is to retard the absorption of glucose by inhibition of alpha-amylase and alpha-glucosidase. Owing to side effects synthetic drugs used to control postprandial hyperglycemia, research for new group of agents from natural resources especially from traditional medicines became an alternative approach for the treatment of postprandial hyperglycemia. Hence the current study was conducted with the main purpose of the phytochemical screening and determination of *in-vitro* alpha-amylase inhibition activity of rhizome extract of *Curcuma longa.* Results delineated that major phytochemicals found in aq. extract of rhizome of *C. longa* were found to be alkaloids, flavonoids, saponins, phenolic compounds, and tannins. Furthermore, results of quantitative estimation of aq. extract of rhizome of *C. longa* delineated that total phenolic quantity was found to highest (36.34 GAE) followed by total flavonoids (21.46 GAE), and quantities of tannins (4.82 GAE). The IC_{50} value exhibited by standard (Acarbose) and aq. extract of rhizome of *C. longa* was found to be31.23 µg/ml and 51.28 µg/ml respectively. In conclusion, results of the present study clearly demonstrated that aq. extract of rhizome of *C. longa* exhibited alpha-amylase inhibition with IC_{50} value 51. 28 µg/ml which was comparable with that of the IC_{50} value of standard Acarbose. Therefore, the present study demarcated that aq. extract of rhizome of *C. longa* would prove to be a probable drug molecule for the treatment of postprandial hyperglycemia.

Introduction

Diabetes mellitus is a chronic metabolic disorder and is characterized by high blood glucose level which results from defects in both insulin secretion and/or insulin action (Holman *et al.,* 1999). Diabetes mellitus is associated with reduced quality of life because of its complication and increased risk for mortality and morbidity. Long-term hyperglycemia is an important factor in the development and progression of micro- and macro-vascular complications which include neuropathy, nephropathy, cardiovascular, and cerebrovascular diseases (Altan, 2003). Type 2 diabetes is one of the global public health concerns in the 21st century (Ginter and Simko, 2012), in both developed and the developing countries are experiencing increasing rates of diabetes. By 2030, 50% of the adult populations of economically advanced countries are predicted to be diagnosed with type II diabetes, mainly due to the contributions of increasing urbanization, aging populations, obesity and sedentary lifestyles (Ginter and Simko, 2013).

Insulin, an endocrine peptide hormone, produced in the beta cells of pancreas, is responsible for the glucose uptake and its utilization by the body tissue rendering hypoglycemic effects and the autoimmune destruction of these cells leads to insulin deficiency causing type I diabetes or insulin abnormalities which results in resistance to insulin action, leading to type II diabetes (DeFronzo *et al.,* 2015; Kahn, 1985). It has been established that postprandial hyperglycemia strongly depends on the absorbed monosaccharides and the velocity of absorption in the small intestine and it is mediated by carbohydrates hydrolyzing enzymes such as pancreatic alpha-amylase and intestinal alpha-glucosidase; two members of exo-acting glycoside hydrolase enzymes. Postprandial glucose levels can be regulated through inhibition of these enzymes which delay and in some cases halt carbohydrate digestion thus prolonging overall carbohydrate digestion time causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise (Hanefeld *et al.,* 2004).

One of the therapeutic approaches for decreasing of blood glucose rise after a meal is to retard the absorption of glucose by inhibition of carbohydrate hydrolysing enzymes such as alpha-amylase and alpha-glucosidase (DeFronzo, 1999; Chiasson *et al.,* 2002). The use of synthetic drugs *viz.* acarbose, miglitol and voglibose are the regular practices in control postprandial hyperglycemia despite its gastrointestinal adverse effects (Neuser, 2005).

Hence, the research for new group of agents from natural resources especially from traditional medicines became an alternative approach for the treatment of postprandial hyperglycemia. These traditional medicines are relied upon for healthcare in many parts of the world (WHO, 1980). Several medicinal plants species have been used to control diabetes in the traditional medicinal systems of many cultures worldwide (Grover *et al.,* 2002; Bnouham *et al.,* 2006; Mentreddy, 2007; Sales *et al.,* 2012). A number of them are known to exert their antihyperglycemic activity via the inhibition of carbohydrate hydrolyzing enzymes. Therefore, natural inhibitors from plant sources can offer an attractive strategy for the effective control of postprandial hyperglycemia without or less unwanted secondary effects (Ali *et al.,* 2006; Tundis *et al.,* 2010). The potential role of medicinal plants as inhibitors of alpha-amylase and alpha-glucosidase has been reviewed by several authors. A variety of plants have been reported to show an enzymatic inhibitory activity and so many are relevant to the treatment of type 2 diabetes

(Benalla *et al.*, 2010; Sudha *et al.*, 2011; Sales *et al.*, 2012). The search for new pharmacologically active agents obtained by screening natural sources such as medicinal plants or their extracts can lead to potent and specific inhibitors for alpha-amylase (Tarling *et al.*, 2008). With this scenario, present study was conducted with the main objectives of phytochemical screening and determination of *in-vitro* alpha-amylase inhibition activity of rhizome extract of *Curcuma longa*.

Materials and Methods

Collection of Plant Material

The rhizomes *C. longa* were purchased from the local market of Bengaluru, Karnataka, India and washed several times with running tap water to remove adhered dirt and debris. The rhizomes were finally washed once with distilled water, and then shade dried at room temperature. The dried rhizomes were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered rhizomes of *C. longa* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of distilled water. Extract was concentrated by distilling the solvent in a rotary flash evaporator. The extract was preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Phytochemical screening was carried out on the aqueous(aq.) extract of rhizome of *C. longa* by using standard procedure to detect constituents as described by Sofora (1993); Trease and Evans (1989) and Harborne (1973).

Test for Alkaloids

Approximately 0.2g of aq. extract of rhizome of *C. longa* was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins and Phenolic Compounds

The aq. extract of rhizome of *C. longa* in small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green coloration indicates the presence of tannins and phenolic compounds.

Test for Glycosides

About 0.6g of aq. extract of rhizome of *C. longa* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugars

The aq. extract of rhizome of *C. longa* was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

Test for Saponins

About 0.2g of aq. extract of rhizome of *C. longa* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids

0.2g of aq. extract of rhizome of *C. longa* was dissolved in diluted 10% NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for Steroids

2 mL of acetic anhydride was added to 0.5g of aq. extract of rhizome of *C. longa* and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for Terpenoids

0.3g of aq. extract of rhizome of *C. longa* was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

Test for Proteins and Amino Acids

To the 0.3g of aq. extract of rhizome of *C. longa* few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue coloration indicates the presence of proteins and amino acids.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the aq. extract of rhizome of *C. longa* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725 nm increases linearly with the concentration of phenolics in the reaction medium (Singleton *et al.*, 1999). The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in aq. extract of rhizome of *C. longa* (Ordonez *et al.*, 2006). The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Tannins

The tannin concentration was determined for aq. extract of rhizome of *C. longa* following a modified version of the vanillin-HCl method (Chanwitheesuk *et al.*, 2005).

In-vitro Alpha-amylase Inhibition Assay

The alpha-amylase inhibition assay was performed as per the modified method of Adefegha and Oboh (2012). Briefly, 100µl of alpha-amylase (1mg/ml) was preincubated for 10 mins with different concentrations of aq. extract of rhizome of *C. longa* (25, 50, 100 µg/ml) and 100µl of 0.1M phosphate buffer (pH 7.0). After incubation, 100µl of starch (0.1%) solution was added and incubated at room temperature for further 30min.

This was followed by addition of 1ml dinitro salicylic acid (DNS) reagent to stop the reaction and incubation in boiling water bath for 5 minutes. The reaction mixture was removed from water bath, cooled at room temperature and diluted to 10 ml with distilled water. The alpha-amylase activity was determined by measuring maltose released from starch at 540 nm.

The results were expressed as a percentage of inhibition. The same procedure was done with Acarbose (1mg/ml stock) which was used as standard. The inhibition percentage was calculated by using below formula; IC_{50} value was calculated by using regression analysis (Uddin *et al.,* 2014).

% Inhibition = $(A_{standard} - A_{extract}) / A_{standard}) \times 100$

Results and Discussion

The major phytochemicals found in aq. extract of rhizome of *C. longa* were found to be alkaloids, flavonoids, saponins, phenolic compounds, and tannins. While, the phytochemicals proteins & amino acids, reducing sugars, steroids, and terpenoids were found absent in aq. extract of rhizome of *C. longa* (Table 1).

The results of quantitative estimation of aq. extract of rhizome of *C. longa* was represented in Table 2. Results delineated that total phenolic quantity was found to highest (36.34 GAE) followed by total flavonoids (21.46 GAE), and quantities of tannins (4.82 GAE).

The results of alpha-amylase inhibition percentage and the IC_{50} values of Standard (Acarbose) and aq. extract of rhizome of *C. longa* was represented in Table 3. Results depicted that there was a dose dependent inhibition (%) of alpha-amylase was observed with standard (Acarbose) and aq. extract of rhizome of *C. longa.*

The IC_{50} value exhibited by standard (Acarbose) and aq. extract of rhizome of *C. longa* was found to be31.23 µg/ml and 51.28 µg/ml respectively indicating that alpha-amylase inhibition potential of aq. extract of rhizome of *C. longa* was comparable with that standard (Acarbose).

The knowledge about the role of herbal extracts in management of type 2 diabetes is known since ancient times and currently being used in Ayurveda for the treatment of diabetes. However, due to lack of sustained scientific evidence, these medicinal plants have not gained much importance (Sudha *et al.,* 2011). In the intestine, digestive enzymes like alpha-glucosidase and alpha-amylase break down starch into glucose and maltose (Bhat *et al.,* 2011). As a result, Type 2 diabetes is managed by using enzyme inhibitors of such enzymes *viz.* alpha-glucosidase and alpha-amylase (Winterbourn and Metodiewa, 1994).

The results of alpha-amylase inhibition assay in our study depicted that maximum alpha-amylase inhibition percent (43.58%) observed with aq. extract of rhizome of *C. longa* at the concentration 100µg/ml with IC_{50} value of 51.28 µg/ml. These findings indicated that aq. extract of rhizome of *C. longa* may be used to decrease glucose availability from the intestine from digestible carbohydrates, hence may be used as an oral anti-hyperglycemic agent.

In concurrence with our study findings, literature reports evidenced that Rhizome of *C. longa* is known to possess therapeutic activities and used as an antidiabetic agent (Sastry, 2005; Sharma and Vijnana, 2006; Chunekar, 2010; Pandey and Vijnana, 2002). According to Ponnusamy *et al.,* (2011) Isopropanol and acetone extracts of *C. longa* were found to inhibit starch hydrolytic activity of human pancreatic amylase. Furthermore, Prabhakar *et al.,* (2013) reported amylase inhibition activity of methanolic and aqueous extracts of *C. longa*.

Table.1 Photochemical screening of aq. extract of rhizome of *C. longa*

Phytochemical Components	Aq. Extract of Rhizome of *C. longa*
Alkaloids	+
Flavonoids	+
Glycosides	-
Proteins and Amino acids	-
Reducing sugar	-
Saponins	+
Steroids	-
Phenolic compounds	+
Tannins	+
Terpenoids	-

Table.2 Quantitative estimation of phytochemicals of aq. extract of rhizome of *C. longa*

Phytochemical Components	Aq. Extract of Rhizome of *C. longa*
Total phenolics	36.64 GAE
Total flavonoids	21.46 GAE
Tannins	4.82 GAE

Table.3 Effect of aq. extract of rhizome of *C. longa* on alpha-amylase inhibition activity

Sample	Conc. (µg/ml)	Inhibition (%)	IC_{50}
Standard	25	62.13	31.23
	50	85.46	
	100	86.64	
Aq. extract of rhizome of *C. longa*	25	11.82	51.28
	50	17.65	
	100	43.58	

Values were expressed as Mean; n=3

The major phytochemicals found in aq. extract of rhizome of *C. longa* were found to be alkaloids, flavonoids, saponins, phenolic compounds, and tannins. Furthermore, results of quantitative estimation of aq. extract of rhizome of *C. longa* delineated that total phenolic quantity was found to highest followed by total flavonoids, and quantities of tannins. According to Bhosale *et al.*, (2018) in presence of rhizome of *C. longa* extract, the mode of inhibition was found to be of mixed type in which active constituents in extract is expected to bind with either free or bound form of enzyme. The increase in the value of km in presence of rhizome of *C. longa* extract indicated that the active constituent binds with free form of enzyme.

The results of the present study clearly demonstrated that aq. extract of rhizome of *C.*

longa exhibited alpha-amylase inhibition activity with IC_{50} value 51. 28 µg/ml which was comparable with that of the IC_{50} value of standard acarbose. Hence, findings of our study supported that the aq. extract of rhizome of *C. longa*would prove to be a probable drug molecule for the treatment of postprandial hyperglycemia. However, further studies could be recommended to carried to ascertain the exact phytoconstituent and mechanism of action responsible for alpha-amylase inhibition activity.

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