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Research Article

IN VITRO HYPOGLYCEMIC EVALUATION OF FRACTIONS OF HYDROALCOHOLIC EXTRACT OF HEARTWOOD OF *TECOMELLA UNDULATA* LINN

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ABSTRACT

Objective: The objective of this study was to screen alpha-amylase and alpha-glucosidase inhibitors from the different fractions of crude hydroalcoholic extract of heartwood of *Tecomella undulata* Linn.

Methods: Four fractions of crude hydroalcoholic extract of heartwood of plant were used for *in-vitro* inhibitory assays against digestive enzymes: Alpha-amylase and alpha-glucosidase. For assay, different concentrations (20, 40, 60, 80, and 100 µg/ml) were used for all fractions. A standard protocol was used for preliminary phytochemical screening of different bioactive components present in all fractions.

Results: The fractions have shown moderate to highest inhibitory activity against both enzymes. However, the strong inhibition was revealed by acetone fraction against alpha-amylase with very minimal inhibitory concentrations at inhibitory concentration 50% values when compared with a standard drug acarbose. Several medicinally active phytocomponents such as flavonoids, saponin, anthraquinones, tannins, triterpenoids, and phenols were observed in all studied fractions.

Conclusion: The different fractions prepared from crude hydroalcoholic extract of heartwood of plant are capable of inhibiting alpha-amylase and alpha-glucosidase, and it can be concluded that heartwood of *T. undulata* Linn. is partially active against postprandial hyperglycemia, thus diabetes mellitus.

Keywords: *Tecomella undulata* Linn., Diabetes mellitus, Alpha-amylase, Alpha-glucosidase.

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INTRODUCTION

Diabetes mellitus is a chronic disorder that occurs due to carbohydrate dysmetabolism syndrome and characterized as high plasma glucose level [1,2]. Owing to urbanization, the increase in obesity, bad food habits, older age, and lesser physical activities have become a most prevalent disorder worldwide [3]. The total estimated global health-care expenditures on diabetes mellitus care and treatment have raised from 376 billion U.S. Dollars in 2010 to 490 billion U.S. Dollars as expected by 2030 [4]. According to the timely published reports, the International Diabetes Foundation has clearly indicated that "As the report of 2015, by the end of 2040 the number of diabetic patients will be increased from 415 to 642 million." India has become the second highest country with diabetic patients, 69 million as of 2015 report [5]. India and other countries, including Pakistan, Bangladesh, Sri Lanka, Afghanistan, Nepal, Bhutan, and the Maldives all together are known as Asian Indian people constitute more than 17% of the total world's population chart and they have more chances of coronary heart disease and diabetes mellitus due to their specific phenotype as it can be understand by their high levels of intra-abdominal fat in spite of low body mass index and insulin resistance, which predisposes them to diabetes mellitus and associated coronary heart disease [6]. The current reports suggest that in comparison to fasting blood glucose, the high postprandial plasma glucose does not only be main serious concern due to its harmful effects along with many complications but also increase in mortality rate; hence, it becomes very important to control postprandial hyperglycemia [2].

Therefore, alpha-amylase and alpha-glucosidase inhibition are the most studied therapies to control postprandial hyperglycemia. These are digestive enzymes which are found on the brush border of intestinal cells and are responsible for the preliminary breakdown of starch into glucose [7]. Alpha-amylase is calcium metalloenzyme acts by catalytic action on α-D-(1-4) glycosidic linkages of starch and other carbohydrates into smaller oligosaccharides [8]. The glucosidase by the same hydrolysis method breakdown much complex carbohydrates into simpler monosaccharide units [9]. Inhibition of these enzymes results in a delayed glucose absorption rate which in turn maintains blood glucose in patients with hyperglycemic index [10].

Some clinically proved enzyme inhibitors such as acarbose, voglibose, and miglitol, due to their serious side effects on the liver and gastrointestinal are being avoided to use [11]. Medicinal plants from the ancient time to present day are used widely because they have a vast variety of phytoconstituents which are responsible to treat a number of diseases such as diabetes. Many plants such as *Cichorium intybus* Linn. [12], *Phaseolus vulgaris* Linn. [13], *Phoenix dactylifera* Linn. [14], *Camellia sinensis* [15], and many others have exhibited the inhibitory potential against alpha-amylase and alpha-glucosidase when studied by *in vitro* model. The research, based on antidiabetic plants, especially potent in attenuating the rise in blood glucose level by reducing the postprandial hyperglycemia, is in more demand due to belief of natural, fewer or no side effects, low cost, and easy availability.

In traditional Indian system of medicine, *Tecomella undulata* Linn. (Family, *Bignoniaceae*), has been considered as a valuable agroforestry tree in most arid and semiarid areas for the very high quality of timber with its use as fuel wood and fodder. This is commonly known as Rohira, Rohitaka, and Rohida and has long been used extensively to cure and treat many diseases in both folk and classical stream since long [16]. The plant had been revealed to possess a wide range of biological properties because of the presence of several pharmacologically important phytocomponents such as radermachol [17], clutyl ferulate [18], lapachol [19], β-lapachone [20], α-lapachone [21], and many more which are responsible for the curative action on different diseases such as central analgesic [22],

abortifacient [23], hepatoprotective [24], antimicrobial [25], and many more including diabetes [26,27] as well as other potential health profits.

The heartwood of *T. undulata* Linn. is being soaked in water overnight and consumed as such by the people to cure diabetes by the people of Haryana state of India in the present time. Hence, the intend of this study was to achieve preliminary insight into the mechanism through which it can support in controlling diabetes mellitus and its related complications through *in vitro* assays. We evaluated the potential effect of various fractions prepared from crude hydroalcoholic extract of heartwood of plant to counterbalance the postprandial hyperglycemia which plays a crucial role in the development and progression of diabetic mellitus. Further, bioactive constituents which may be responsible for its antidiabetic property were predicted from preliminary phytochemical screening. Therefore, this could be a novel approach for treating diabetes mellitus patients through carbohydrate metabolizing enzyme α-amylase and α-glucosidase inhibition studies.

METHODS

Plant collection and authentication

The heartwood part was collected from Haryana, India. The plant part was authenticated by Dr. H.B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum NISCAIR, New Delhi. The voucher specimen (Ref. No. NISCAIR/RHMD/Consult/2011-2012/1975/275) of the collected plant part was deposited in the Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak.

Extraction

The heartwood was cleaned with brush to remove unwanted particles, then it was shade dried and coarsely powdered, sieved, and then macerated by a cold maceration process with 70% ethanol for 1 week at room temperature. The method was repeated for 3 times. The mixture was filtered and concentrated using a rotary evaporator (40°C). After drying of this concentrated mixture at 45°C in the oven, the crude extract was obtained. This hydroalcoholic extract was further fractionated into petroleum ether, chloroform, acetone, and remaining hydroalcoholic fraction. For experimental use, all the collected fractions were stored into tightly packed containers to protect them from light, humidity, and other unwanted determinant.

Preliminary phytochemical screening

All the fractions were qualitatively tested for the presence of phytochemicals such as flavanoids, saponins, phenols, cardiac glycosides, alkaloids, proteins, and carbohydrates using standard protocols [28,29].

Alpha-amylase inhibition assay

To perform alpha-amylase (Porcine pancreas extra pure, 9000-90-2, Sisco Research Laboratories Pvt. Ltd.) inhibition activity, the method chosen was slightly modified from Apostolidis *et al*. [30]. 1 ml of 20 mM sodium phosphate buffer at pH 6.9 containing α-amylase solution (1 mg/ml) and 1 ml of sample of different concentrations was incubated at 37°C for 10 minutes. After this, 1 ml of 0.5% starch solution in 20 mM sodium phosphate buffer was added to each test tube. The reaction mixture was again incubated at 25°C for next 10 minutes. In the reaction mixture, 1 ml of color reagent (dinitrosalicylic acid) was added. All the test tubes were kept in a water bath for boiling for 15 minutes and cooled at room temperature. The absorbance for all concentrations was read at 540 nm using ultraviolet (UV)-visible spectrophotometer (Loba life [UV 22]). For blank incubation (to allow for absorbance produced by the extract), in place of enzyme solution, buffer solution was used and absorbance was recorded. Control was conducted in an identical manner replacing the plant extracts with 1 ml dimethyl sulfoxide. Acarbose solution was used as positive control.

The % inhibition was calculated using below-mentioned formula:

100 × {Absorbance of control − Absorbance of sample} ÷ {Absorbance of control}

Alpha-glucosidase inhibition assay

Alpha-glucosidase (maltase, yeast extra pure, 9001-42-7, Sisco Research Laboratories Pvt. Ltd.) inhibition assay was performed by taking 500 µl of alpha-glucosidase (1 U/ml) with previously contained 500 µl of different concentrations of prepared fractions and then all are incubated for 10 minutes at room temperature. From 37 mM of maltose solution, 500 µl was added to each test tube and incubated for next 30 minutes. After this, from glucose kit reagent, 1 ml was added to the reaction mixture and kept aside for 15-20 minutes. Then, 1 ml of a buffer was added to each tube having a reaction mixture. At 505 nm, absorbance was noted down against the reagent blank. Assays were carried out in triplicate.

The inhibition percentage of alpha-glucosidase was calculated using following formula:

100 × {Absorbance of control − Absorbance of sample} ÷ {Absorbance of control}

Both blank and positive controls were carried out simultaneously.

For, alpha-amylase and alpha-glucosidase inhibitory activity, all the fractions concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) were prepared in dimethyl sulfoxide and assays were performed in a dose-dependent manner [31].

Statistical analysis

Mean inhibitory values were calculated and plots of percentage inhibition versus concentrations for each sample was plotted by nonlinear regression analysis to know the inhibitory concentration 50% (IC_{50}) values for both enzymes. The assay was performed in triplicates. Microsoft EXCEL program and graph pad prism 6.0 software were used for data analysis.

RESULTS

The antidiabetic potential of various fractions of heartwood of *T. undulata* Linn. was determined by *in vitro* studies and the results are depicted as that the crude 70% ethanol extract of heartwood of plant the percentage yield obtained was 5.2%. From this crude hydroalcoholic extract, the percentage yield obtained for the different fractions were petroleum-ether (15%), chloroform (24%), acetone (37%), and remaining hydroalcoholic fraction (13%).

The preliminary phytochemical screening showed the presence of different phytoconstituents as flavonoids, saponin, anthraquinones, tannins, and phenol present in all the fractions of hydroalcoholic extract of plant while carbohydrates and proteins are present only in petroleum ether fraction as shown in Table 1.

In our study, we checked the alpha-amylase and alpha-glucosidase inhibitory activities of all the fractions of hydroalcoholic extract of heartwood of plant and the IC_{50} values obtained were compared with the standard drug acarbose.

The absorbance for alpha-amylase was observed at 540 and 505 nm for alpha-glucosidase, and the results are based on graphs plotted between % inhibition of both enzymes and concentrations are shown in Fig. 1 for alpha-amylase and Fig. 2 for alpha-glucosidase inhibition. The % inhibition was calculated in mean±standard error of the mean values.

The *in vitro* alpha-amylase activity (Figure 2) had displayed that the acetone fraction has shown maximum % inhibition range from 18.33±0.248 to 50.75±0.274 with the IC_{50} value at a concentration of 0.76 mg/ml. This is most effective concentration to inhibit 50% of enzymes as compared to acarbose with IC_{50} value 0.86 mg/ml, % inhibition range from 17.91±0.495 to 57.95±1.17. The chloroform fraction has demonstrated enzyme inhibition range from 27.27±0.616 to 59.84±0.383 at IC_{50} value of 0.82 mg/ml while hydroalcoholic

Table 1: Preliminary phytochemical screening for phytoconstituents present in fractions of hydroalcoholic extract of heartwood of *T. undulata* Linn.

Phytocomponents	Fractions			
	Petroleum ether	Chloroform	Acetone	Remaining hydroalcoholic
Carbohydrates	+ve	−ve	−ve	−ve
Proteins	+ve	−ve	−ve	−ve
Flavonoids	+ve	+ve	+ve	+ve
Triterpenoids	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	+ve	+ve
Phenols	+ve	+ve	+ve	+ve
Anthraquinones	+ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve
Steroids	−ve	−ve	−ve	−ve

−ve: Absent, +ve: Present. *T. undulata*: *Tecomella undulata*

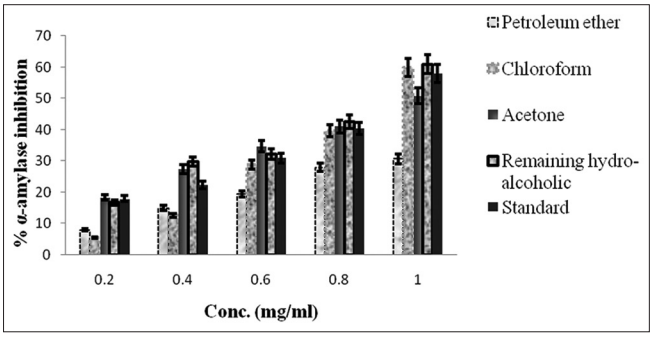

Fig. 1: % Alpha-amylase enzyme inhibition by the different fractions of hydroalcoholic extract of heartwood of *Tecomella undulata* Linn. Standard: Acarbose. Data are expressed as mean±standard error of mean (n=3)

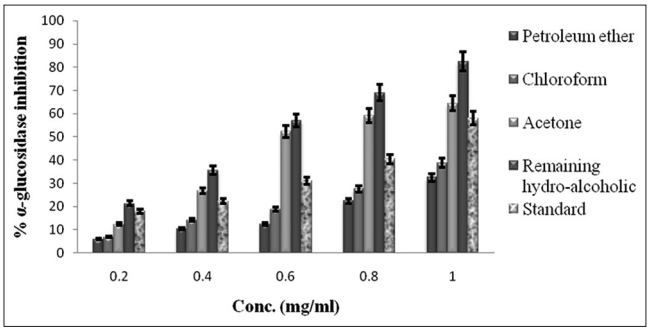

Fig. 2: % Alpha-glucosidase enzyme inhibition by the different fractions of hydroalcoholic extract of heartwood of *Tecomella undulata* Linn. Standard: Acarbose. Data are expressed as mean±standard error of mean (n=3)

fraction has shown inhibition from 16.68±0.230 to 60.86±0.289 with IC_{50} was 0.91 mg/ml as compared to acarbose exhibiting moderate to high inhibition potential. Alpha-amylase inhibitory activity induced by fractions of 70% ethanol extract of heartwood of plant is shown in Fig. 1.

The alpha-glucosidase inhibition studies of fractions of heartwood of plant at different concentrations were performed, and the results are given in Fig. 2.

The profound alpha-glucosidase inhibition (Figure 2) activity was exhibited by the acetone fraction with % inhibition range from 21.36±0.239 to 82.34±0.325 and 50% inhibition at 0.63 mg/ml as compared to standard drug acarbose. The hydroalcoholic fraction has also shown substantial inhibition concentration at 1.0 mg/ml and it was found from 12.38±0.265 to 64.34±0.287 as compared to acarbose, respectively. Acarbose has shown % inhibition range from 17.91±0.495 to 57.95±1.17. However, the chloroform and the petroleum ether fraction were not able to perform any type of enzyme inhibition potential at 50% inhibition concentration.

DISCUSSION

Diabetes mellitus has increasing global prevalence and incidence and it is endocrinological and metabolic disorder which is characterized by chronic hyperglycemia [32]. In this situation, the insulin-producing β-cell mass gets defected and resulted into defects in insulin secretion, action, or both. Inflammatory cytokinins, circulatory free fatty acids, and hyperglycemia with including other mechanisms have been projected for β-cell destruction [33]. Ethnobotanical survey clearly indicates that in India, more than 800 plants have antidiabetic potential but still unfortunately out of these only few medicinal plants were explored scientifically for their antihyperglycemic activity [34]. From ancient time till modern era, medicinal plants have long been studied to treat and alleviate several diseases such as diabetes as they are the good source of principal medicinally active phytocomponents which are reported to show good antidiabetic activity. Some phytochemical components such as myricetin, quercetin, and luteolin flavonoids have shown potent pancreatic alpha-amylase inhibition with very minimal IC_{50} concentration of 0.5 mg/ml as reported previously [35]. Polyphenol compounds from *Ipomoea batatas* (Family: Convolvulaceae) and *Currants* species possess alpha-glucosidase inhibitory activities [36]. Similar attributes have also been documented for flavonols [37] and have found profound significance as potent hypoglycemic agents [38]. Other phytochemicals such as alkaloids, glycosides, steroids, carbohydrates, terpenoids, saponins, dietary fibers, and amino acids affect various metabolic events, which affect the level of serum glucose directly or indirectly.

Alpha-amylase and alpha-glucosidase convert dietary polysaccharide units into monosaccharide units mainly glucose in the digestive tract. This sugar molecule is further absorbed by epithelial cells of intestine using sodium-dependent, carrier-mediated active transport pump [39]. Inhibition of these enzymes decreases the hydrolysis of complex carbohydrate units into simple sugar units and helps in the management of postprandial hyperglycemia thus diabetes mellitus [40,41]. In our *in vitro* study, the acetone fraction has exhibited remarkable alpha-glucosidase inhibition as compared to alpha-amylase. On the other hand, the petroleum ether fraction demonstrated no inhibition of both the enzymes. The chloroform fraction has proved very good inhibition effect on alpha-glucosidase, but it was not able to inhibit the alpha-amylase. The hydroalcoholic fraction has given very good inhibitory action on alpha-glucosidase, but not on α-amylase as it is noticed in the IC_{50} values results.

From the literature, it is declared that the extraction solvents, nonpolar to polar are a rich source of phytochemicals. Hence, all the fractions

were extracted into nonpolar to polar solvents to ensure complete extraction to get maximum number of phytoconstituents in the preliminary phytochemical screening [42]; However, according to our studies, we observed the presence of some bioactive phytoconstituents such as flavonoids, saponins, anthraquinones, tannins, and phenols in fractions. From the *in vitro* experimental work, it is observed that the positive inhibition effect on both alpha-amylase and alpha-glucosidase by the fractions of crude hydroalcoholic extract of heartwood of plant could be due to singly or in combination of these phytoconstituents found in fractions.

Thus, this study suggests that hydroalcoholic extract of heartwood of *T. undulata* Linn. being as a good source of timber in arid regions of India possess the potentiality to minimize or cure the diabetes mellitus by managing postprandial hyperglycemia inhibiting the carbohydrate metabolizing alpha-amylase and alpha-glucosidase enzymes.

CONCLUSION

From the study, it can be concluded that the different fractions of hydroalcoholic extract of heartwood part of *T. undulata* Linn. contains several bioactive phytochemicals, which shows a beneficial effect to control postprandial hyperglycemia thus, render absorption of glucose in the intestine. These studies will be helpful in further exploring the plant for *in-vivo* antidiabetic activities.

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