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In-vitro Antidiabetic & Antioxidant Activity of Different Extracts of Dried Fruits of *Terminalia chebula*

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ABSTRACT

The aim of the present investigation is to study the antidiabetic and antioxidant activity of dried fruits of *Terminalia chebula* by In-vitro models. Dried fruits of *Terminalia chebula* were purchased from the local market. The selected samples were washed properly through water and dried in shade for the further process. Dried samples of all medicinal plants were authenticated by Prof. Gyanendra Tiwari, Senior Botanist and Scientist, Government College of Horticulture, Mandsaur (M.P.). Around 500 gms dried fruits of *Terminalia chebula* were coarsely powdered weighed and filled in Soxhlet apparatus for extraction. First the powdered drug was defatted with petroleum ether (60°C-80°C); Defatted drug was then dried and again filled in soxhlet apparatus for successively extraction with dichloromethane, ethyl acetate, methanol and water as solvent. The extraction was carried out for a period of 72 hrs. The α-amylase inhibition assay was performed using the 3.5-dinitrosalicylic acid (DNSA) method. The different extracts of *Terminalia chebula* were dissolved in buffer (Na2HPO4/NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 25 to 500 µg/mL. The potential antioxidant activity of different extracts of *Terminalia chebula* were determined based on the scavenging activity of the stable DPPH free radical. Among the extracts, the ethyl acetate extract showed the highest alpha–amylase enzyme inhibition activity. Similarly, the IC_{50} values of water and other extracts had less value as compared to ethyl acetate extract

respectively. The standard positive control Acarbose showed higher IC_{50} value. There was a dose dependent increase in the percentage of antioxidant activity for all concentrations tested. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The results give scientific support for the use of the plant in folk medicine for the management of diabetes and its associated complications.

KEYWORDS-

In-vitro Antidiabetic, Antioxidant Activity, Different Extracts, Dried Fruits, *Terminalia chebula.*

INTRODUCTION

Diabetes mellitus is a serious complex multifactorial disorder characterized by hyperglycemia (very high blood glucose level) and glucose intolerance, either due to the relative deficiency in insulin secretion or impaired the effectiveness of insulin's action to enhance glucose uptake. If left untreated, it can lead to severe complications. These complications include hyperlipidemia (abnormal high level of lipid in the blood), oxidative stress, and enzymatic glycation of protein [1]. Considering the fact that diabetes is regarded as a chronic metabolic disease, numerous antidiabetic therapies with conventional drugs are often not a single-dose program as most drugs require frequent injections, sometimes for the entire life of the diabetic patient. However, many of these conventional drugs have been reported for their inefficiency with prominent adverse side effects [2]. These limitations have largely prompted the exploration of management strategies involving the use of medicinal plants reported to be cost-effective antidiabetic agents with fewer reported side effects [3]. However, the majority of these traditional plants have not been scientifically validated for their efficacy in the treatment of diabetes. *Terminalia chebula* is among such plants traditionally used among local healers in the Eastern Cape province of India. [4,5]. As we know that everything in this world change time by time since thousands of year the ear was of ayurveda or herbal origin drug. But last few decades it was replaced by allopathic system of medicine, which was rapidly accepted work wide but latter due to its lots of adverse

effect and safety profile and the people are more believing in natural origin drug [5]. Yet no scientific research has confirmed the efficacy of this plant with regard to diabetes mellitus. Therefore, determination of its efficacy is very important as this plant may play a significant role in the management of diabetes mellitus by various In-vitro models.

MATERIAL & METHODS

Collection of plants

Dried fruits of *Terminalia chebula* were purchased from the local market. The selected samples were washed properly through water and dried in shade for the further process.

Authentification of plants

Dried samples of all medicinal plants were authenticated by Prof. Gyanendra Tiwari, Senior Botanist and Scientist, Government College of Horticulture, Mandsaur (M.P.) and voucher specimen for dried fruits of *Terminalia chebula* were deposited in the herbarium of Department of Pharmacognosy at Pharmacy College for the future reference.

Extraction method by Soxhlet apparatus

Around 500 gms dried fruits of *Terminalia chebula* were coarsely powdered weighed and filled in Soxhlet apparatus for extraction. First the powdered drug was defatted with petroleum ether (60°C-80°C); Defatted drug was then dried and again filled in soxhlet apparatus for successively extraction with dichloromethane, ethyl acetate, methanol and water as solvent. The extraction was carried out for a period of 72 hrs. The extract obtained was dried in vacuum to remove excess solvent and were weighed for the determination of % yields [6].

Preliminary phytochemical tests

Qualitative chemical tests of all extracts were subjected to various chemical tests to detect various phytoconstituents [7].

Determinations of α-Amylase Inhibition Activity

The α-amylase inhibition assay was performed using the 3.5-dinitrosalicylic acid (DNSA) method. The different extracts of *Terminalia chebula* were dissolved in buffer (Na2HPO4/NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 25 to 500 µg/mL. A volume of 200 µL of α- amylase solution (2 units/mL) was mixed with 200 µL of the extract and was incubated for 10 mins at 30°C.

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Thereafter, 200 µL of the starch solution (1% in water (w/v)) was added to each tube and incubated for 3 mins. The reaction was terminated by the addition of 200 µL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3.5-dinitrosalicylic acid solution) and was boiled for 10 mins in a water bath at 85° C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µL of the buffer. A blank reaction was similarly prepared using the plant extracts at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose and the reaction was performed similarly to the reaction with plant extract as mentioned above [7]. The inhibition of α-amylase was expressed as percentage of inhibition and was calculated by the following equation:

Inhibition (%)= [(Ac − Acb) − (As − Asb)/(Ac − Acb)] × 100,

Where Ac- absorbance of control; Acb-absorbance of control blank; As-absorbance of sample; and Asb-absorbance of sample blank.

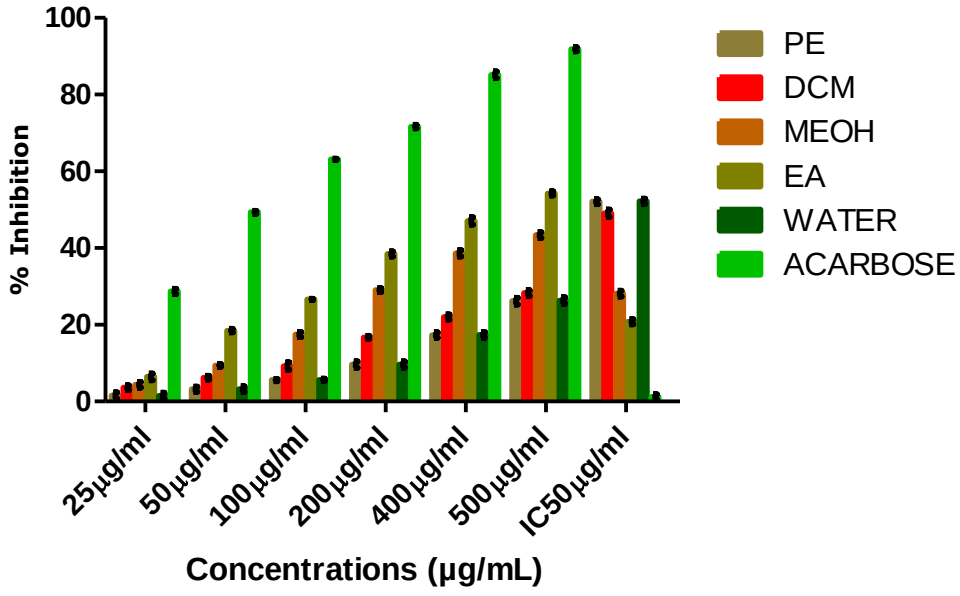

Figure No. 1: Effect of different extracts on α-amylase inhibition assay

In-vitro Antioxidant Activity in DPPH Assay Model

The free radical scavenging activity of the different extracts of *Terminalia chebula* and ascorbic acid were determined in vitro by diphenyl-2-picrylhydrazyl assays according to the method described earlier. The potential antioxidant activity of different extracts of *Terminalia chebula* were determined based on the scavenging activity of the stable DPPH free radical. Aliquots of 100µL of solution containing different concentrations ranging from 25 to 500µg/mL were added to 3.9 mL of a 0.004% solution of DPPH. Absorbance at 517 nm was determined after 30 mins, and the percent inhibition activity was calculated. IC_{50} values denote the concentration of the sample required to scavenge 50% DPPH-free radicals [8]. The percentage (%) of the scavenging of the DPPH-free radical was calculated by the formula: (A0-A1)/A0 X 100, Where-A0 is absorbance of the control and A1 is absorbance of the extract/standard.

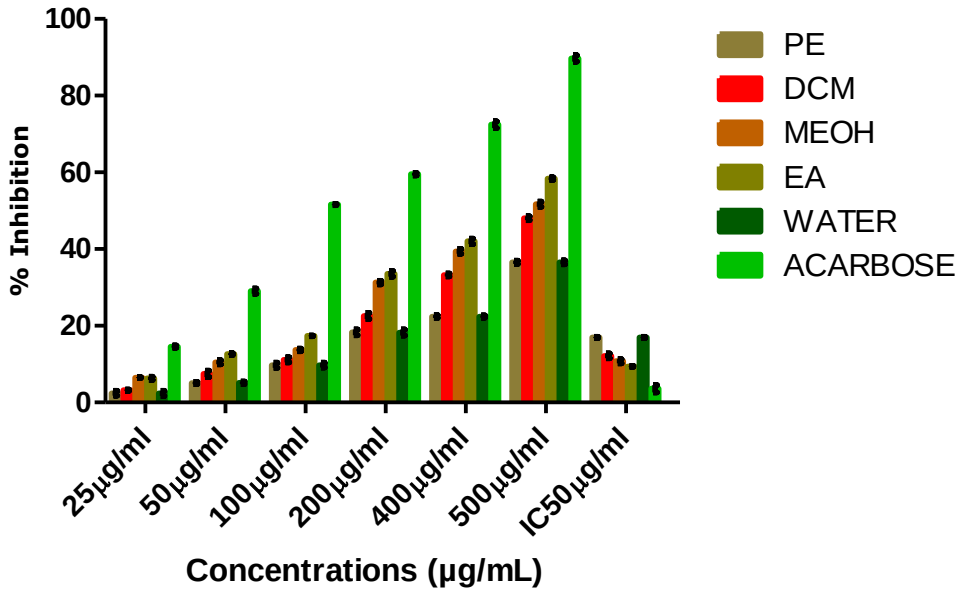

Figure No. 2: Effect of different extracts on In-vitro Antioxidant Activity in DPPH Assay

RESULTS

Preliminary Phytochemical Screening

Preliminary phytochemical screening was done for the different crude extracts and they resulted in the presence of Saponins, Tannins, Terpenoids, Phenols, Flavonoids, Glycosides, Steroids, and Anthraquinones. Alkaloids were also present in the phytochemical

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screening.

In vitro α-Amylase Inhibition Activity

In this study, different crude extracts were evaluated for their possible α-amylase inhibitory activities alongside acarbose as a positive control. The α-amylase inhibitory activities and IC_{50} values of the acarbose and different crude extracts are summarized in Table. Concentration dependent inhibition was observed for various concentrations of the tested extracts and the standard. Among the extracts, the ethyl acetate extract showed the highest alpha–amylase enzyme inhibition activity. Similarly, the IC_{50} values of water and other extracts had less value as compared to ethyl acetate extract respectively. The standard positive control Acarbose showed higher IC_{50} value.

Antioxidant Activity

Antioxidant activities of the different crude extracts were tested for antioxidant activity using DPPH free radical. There was a dose dependent increase in the percentage of antioxidant activity for all concentrations tested. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid and the different extracts varied from 25 to 500µg/mL. Among extracts, the highest inhibitory activities were shown by the crude extract.

DISCUSSION

Numerous new bioactive phytochemicals isolated from the plants having hypoglycemic and anti-hyperglycemic effects prove the same anti-diabetic activity and sometimes even more potent than already recognized oral hypoglycemic agents [9,10]. The present study investigated the in vitro antidiabetic and antioxidant activity of the different extracts. The preliminary phytochemical screening of the crude extract showed the likely existence of Saponins, Tannins, Terpenoids, Phenols, Flavonoids, Glycosides, Steroids, and Anthraquinones. As a result, these secondary metabolites which were found in crude extracts may, therefore, be accountable for the observed glucose suppressive and anti-hyperglycemic activity of the extract and some of the bioactive constituents in this study could act synergistically or independently enhancing the action of glycolytic and glyconeogenic enzymes.

It is well known that reduction of postprandial hyperglycemia can be achieved by inhibiting intestinal α-glucosidase and pancreatic α-amylase activity via delayed carbohydrate digestion [11]. The plant

crude extract showed pancreatic α-amylase inhibitory activity. The search for new group of agents from natural resources, especially from medicinal plants, becomes an attractive approach for the treatment of postprandial hyperglycemia. As shown in Table, all doses of the crude extract demonstrated a dose dependent reduction in α- amylase activity. The most important inhibition appeared in the ethyl acetate solvent fraction while the aqueous fraction showed the weakest effect. The α amylase inhibitory activity in ethyl acetate extract is most likely to be due to semi-polar compounds and is worth investigating further and isolating pure active compounds. Flavonoids, Tannins and Phenolic acids are a major group of polyphenolic compounds that have been reported to possess inhibitory activity against α-amylase [12-15]. In this study, the phytochemical analysis revealed that the extracts are rich in polyphenolic components, this suggests that the bioactive exerting the inhibitory effect against α-amylase may be present in all plant extracts at different concentrations. The greatest anti-oxidant activity was observed in the crude extract while the lowest antioxidant activity was observed in aqueous extract. The attractive fact of our study was that the crude extracts showed outstanding antioxidant potential [16]. The result of the anti-oxidant activity of extracts showed a dose dependent antioxidant activity.

CONCLUSIONS

The results also verified that inhibition of intestinal α-amylase and free radical scavenging activity by the extracts may contribute to the antihyperglycemic and anti-hyperlipidemic activity. The results give scientific support for the use of the plant in folk medicine for the management of diabetes and its associated complications. Further studies to find out the mechanism of this plant for its antidiabetogenic effect and there is a need for bioactivity guided investigation to isolate the lead compound responsible for antidiabetic activity.

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