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In Vitro Evaluation of Antioxidant and Hypoglycemic Potential of Extracts from Different Parts of *Xanthium Strumarium* L

Research Article

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Abstract

Introduction: Indian medicinal plants used in the Ayurveda traditional system to treat diabetes and to scavenge free radicals are a valuable source of novel anti-diabetic agents. Alpha amylase inhibitors offer an effective strategy to lower the level of postprandial hyperglycemia via control of starch breakdown. Plant extracts also possesses free radical scavengers which can be used to treat various diseases like cancer. Extracts of *Xanthium Strumarium* L. have been considered as hypoglycemic and antioxidant agents.

Materials and Methods: In this study, we evaluated antioxidant potential and hypoglycemic potential of methanolic extracts of root, stem, leaves and fruits of the plant by DPPH method and DNSA assay respectively.

Results: Results showed that all plant parts showed very good antioxidant and hypoglycemic activities with low IC_{50} values.

Discussion: results of present study showed that maximum salivary alpha amylase inhibitory activity was shown by methanolic extract of root with minimum IC_{50} value i.e.46.70 µg/ml and maximum free radical scavenging activity was shown by methanolic extract of fruits with minimum IC_{50} value i.e. 0.016 µg/ml. Root extract also show good antioxidant potential.

Conclusion: The results of this study direct researchers to evaluate the therapeutic potential of secondary metabolites in the management of post prandial hyperglycemia and type II diabetes mellitus either alone or in combinatorial therapy.

Keywords: Alpha amylase inhibitors; Postprandial hyperglycemia; Free radical scavenging activity; DPPH assay; DNSA method etc

Introduction

Nature has been a source of medicinal agents for many years. Majority of the traditional medicines used in healthcare are obtained from plants [1]. In spite of several advancements in the field of synthetic drug chemistry and antibiotics, plants continue to be one of the major raw materials for drug streating various human ailments. Clinical and pharmaceutical investigations have in fact elevated the status of medicinal plants by identifying the role of active principles present in them and elaborating on their mode of action in human and animal systems [2].

One important therapeutic approach for treating diabetes is by decreasing the post prandial increase in blood glucose level. Alpha amylase inhibitors play major role in the management of post prandial hyperglycemia. It inhibits the action of alpha amylase enzyme leading to a reduction in starch hydrolysis to maltose and consequentially lower post prandial hyperglycemia [3]. Various medications are

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available for the treatment of Type II diabetes such as bigunides, sulfonylureas, thiazolidinedione etc. But they have also exhibited a number of undesired side effects associated with their uses and thus suggesting other effective alternatives. Medicinal plants have been an exemplary source in the treatment of various diseases including DM particularly in developing countries like India, because of high cost and poor availability of current therapies [4].

Oxidative stress is an important risk factor in the pathogens of numerous chronic diseases. Free radicles and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. ROS are also said to be responsible for human aging [5].

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxides and thus inhibit the oxidation mechanisms that lead to degenerative diseases. Herbal plants considered as good antioxidant since ancient times [5].

Xanthium Strumarium L. belongs to family Asteraceae, commonly known as cocklebur, is annual herb commonly found in India, North America, Brazil, China and Malaysia. Various plant parts have been traditionally used for treatment of various diseases like eczema, fever, salivation, congestive heart diseases, nephritis, toxemia of pregnancy, hypertension, premenstrual tension etc.

In this study we tried to find out antioxidant and hypoglycemic potential of methanolic extracts of roots, stem, leaves and fruits of *Xanthium Strumarium* L. We also confirmed antioxidant potential of the extracts due to presence of flavonoids in the plant parts by using High Performance Thin Layer Chromatography (HPTLC).

Materials and Methods

Collection of Plant material

Carefully inspected healthy plant parts (root, stem, leaves and fruits) of *Xanthium Strumarium* L. were collected from Jaipur in the month of September-October 2017. The selected plants were botanically identified and authenticated. The plant parts were washed and dried at room temperature (27-30°C) for 15-20 days maintaining hygienic conditions. After complete drying, plant materials were grounded to form powder using a domestic electric grinder and then stored in brown bottles to conduct the experimental protocols.

Preparation of extracts

10 gram of each selected plant parts were dissolved in 100 ml of methanol separately and kept in shaking incubator (40 RPM) at room temperature for 24 hours. Then extracts were filtered by using Whatmann's filter paper No. 1 and then used for further experiments.

Evaluation of hypoglycemic potential

The inhibition assay was performed using the chromogenic DNSA method [7]. The total assay mixture composed of 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1ml of salivary amylase and 400 µl extracts at concentration from 0.3-1.5 $mgml^{-1}$(w/v) were incubated at 37°C for 10 min. After pre-incubation, 580 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature, diluted and the absorbance were measured at 540 nm. The control represented 100% enzyme activity and did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate control with the extract in the reaction mixture except for the enzyme was included.

The % inhibition of alpha amylase was calculated as follows:

% inhibition of salivary alpha amylase = (absorbance of Control-absorbance of test / absorbance of control) * 100

Evaluation of antioxidant potential

The antioxidant activity of plant extracts were determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [8]. Experiments were initiated by preparing a 0.004% w/v solution of DPPH and 1mg/ml solution of different plant extracts in methanol. 2 ml of the methanolic solution of DPPH was added to a sample solution (0.1 ml). An equal amount of alcohol was added to the control. The setup was left at dark in room temperature and the absorption was monitored after 30 minutes at 515 nm.

The percentage of free radical scavenging activity was calculated from the following equation-

% radical scavenging activity = (absorbance of Control-absorbance of test / absorbance of control) * 100

Statistical Data Analysis

All experiments were performed in 3 different sets with each set in triplicates. The data were expressed as mean ± SEM (standard error of the mean). Statistical difference, ANOVA and linear regression analysis were performed using Graph pad prism 5 statistical software. The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values. The IC_{50} values were defined as the concentration of the extract that showed 50% inhibition.

Evaluation of Antioxidant activity by HPTLC

HPTLC was performed of all extracts by using standard mobile phase and visualized plates at 315 nm. Plates were run in mobile phase (ethyl acetate: acetic acid: formic acid: water). Then one plate was derivetized by dipping it into natural product reagent A (NP reagent A) which is specific for flavonoids. Another plate was dipped into DPPH and kept in dark for 1 minute. Then both plates were compared.

Results

The results revealed that methanolic extracts (at concentration of 20 µg/ml -100 µg/ml) of selected parts of plant exhibited different degree of alpha amylase inhibitory activity and free radical scavenging activity. Methanolic extract of roots of the plant showed 45.75±0.12 % to 62.42±0.13 % salivary alpha amylase inhibition with an IC_{50}

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value of 46.70 µg/ml and 24.47±0.09 to 78.16± 0.12 % free radical scavenging activity with an IC_{50} value of 50.16 µg/ml.

Methanolic extract of stem of the plant showed 7.00 ± 0.14 % to 11.86±0.07 % salivary alpha amylase inhibition with an IC_{50} value of 273.27 µg/ml and 19.19±0.13 % to 63.56± 0.10% free radical scavenging activity with an IC_{50} value of 74.70 µg/ml.

Methanolic extract of leaves of the plants showed 4.32±0.21% to 17.29±0.11 % inhibition of salivary alpha amylase with an IC_{50} value of 309.09 µg/ml and 15.43±0.11% to 61.19± 0.12 % free radical scavenging activity with an IC_{50} value of 80.89 µg/ml.

Methanolic extract of fruits of the plant showed 6.67±0.09 % to 53.46±0.17 % inhibition of salivary alpha amylase with an IC_{50} value 85.28 µg/ml and 40.75± 0.09 % to 82.47± 0.12 % free radical scavenging activity with an IC_{50} value of 0.016 µg/ml.

Percent inhibition and IC_{50} values of different extracts are shown in Table 1 and 2 and graphically represented in Figure 1-8.

Results of HPTLC showed that flavonoids were seen as blue-green bands in fluorescence after dipping into NP reagent A and the second plate which was dipped into DPPH, was turned into violet color having yellow bands (at places where antioxidant compounds were present). On comparisons of both plates it was conformed that antioxidant potential of methanolic extracts of the plant was due to presence of flavonoids as in the presence of DPPH, bands showing flavonoids (compared with band in NP reagents) changed its color from purple to yellow as shown in Figure 9-11.

Table 1: Salivary alpha amylase inhibitory activity of extracts of different parts of *Xanthium Strumarium* L.

Name of plant part	% Inhibition (at concentration of 20-100 µg/ml)	IC_{50} value (µg/ml)
Root	45.75±0.12 – 62.42±0.13	46.70
Stem	7.00 ± 0.14– 11.86±0.07	273.27
Leaves	4.32±0.21– 17.29±0.11	309.09
Fruits	6.67±0.09 – 53.46±0.17	85.28

Table 2: Free radical scavenging activity of extracts of different parts of *Xanthium Strumarium* L.

Name of plant part	% inhibition (at concentration of 20 -100 µg/ml)	IC_{50} value (µg/ml)
Root	24.47±0.09 to 78.16± 0.12	50.16
Stem	19.19±0.13 to 63.56± 0.10	74.70
Leaves	15.43±0.11 to 61.19± 0.12	80.89
Fruits	40.75± 0.09 to 82.47± 0.12	0.016

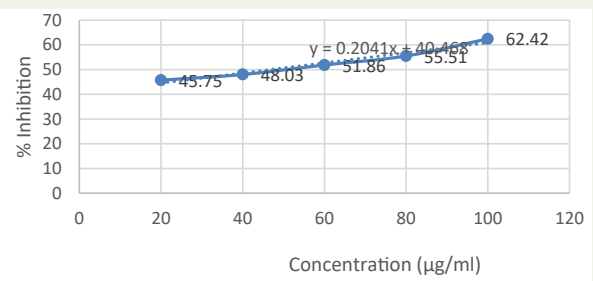

Figure 1: Hypoglycemic activity of methanolic extract of roots of *Xanthium Strumarium* L.

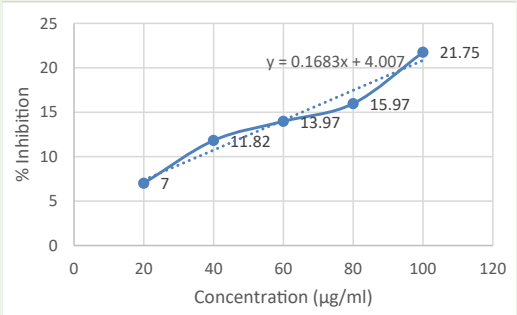

Figure 2: Hypoglycemic activity of methanic extracts of stem of *Xanthium Strumarium* L.

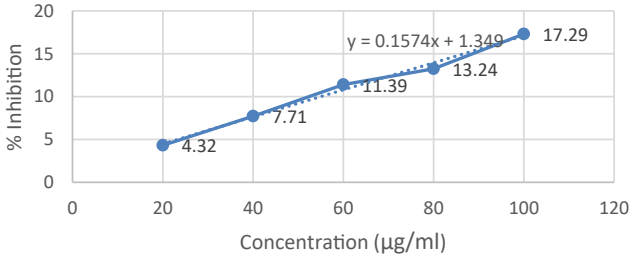

Figure 3: Hypoglycemic activity of methanolic extracts of leaves of *Xanthium Strumarium* L.

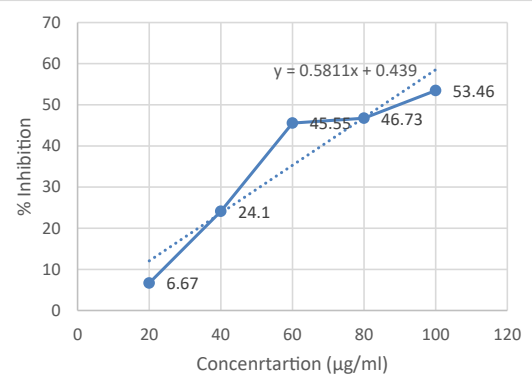

Figure 4: Hypoglycemic activity of methanolic extract of fruits of *Xanthium Strumarium* L.

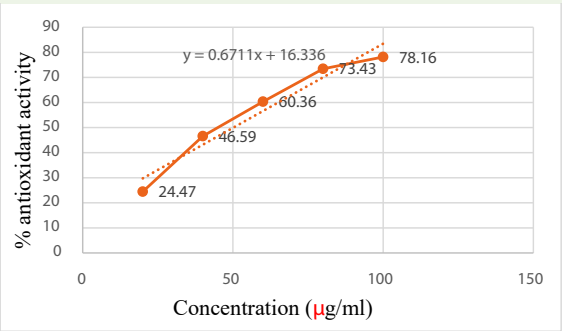

Figure 5: Free radical scavenging activity of methanolic extract of root of *X. strumarium.*

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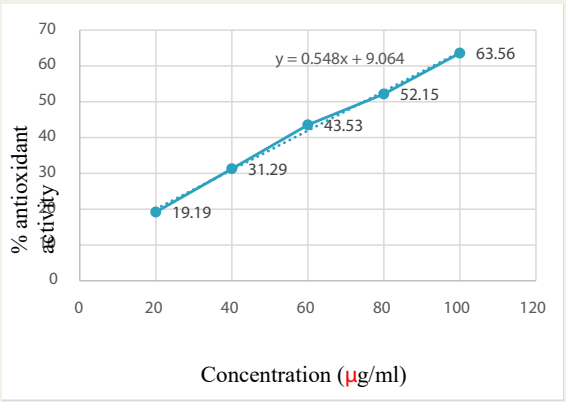

Figure 6: Free radical scavenging activity of methanolic extract of stem of *X. strumarium.*

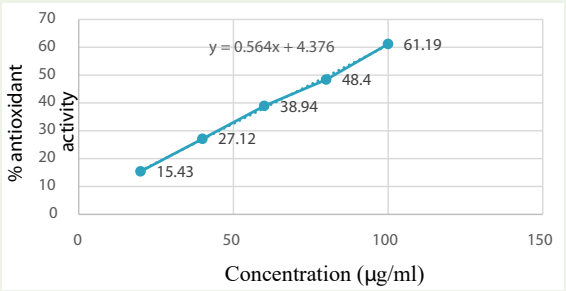

Figure 7: Free radical scavenging activity of methanolic extract of leaves of *X. strumarium.*

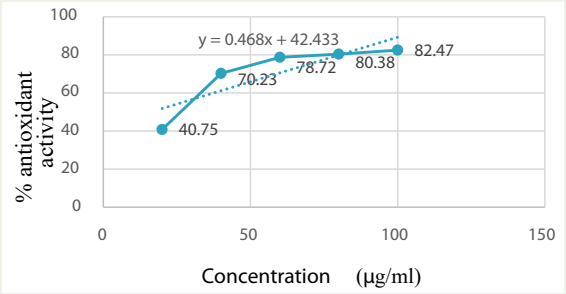

Figure 8: Free radical scavenging activity of methanolic extract of fruits of *X. strumarium.*

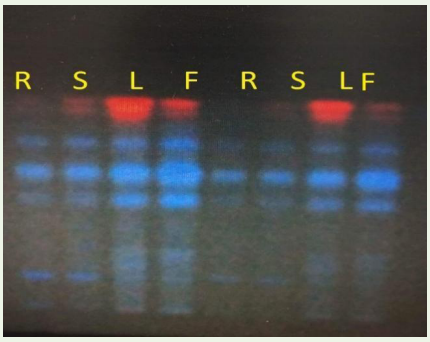

Figure 9: HPTLC performance of plant extracts and visualization of bands at 315 nm before derivetization.

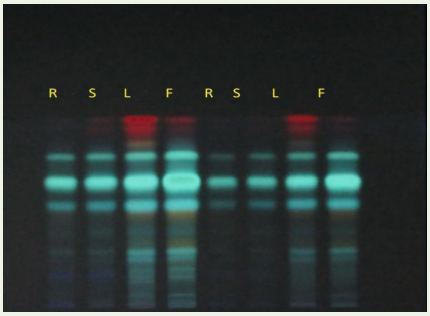

Figure 10: HPTLC performance of plant extracts and visualization of bands after derivetization in Natural Product A reagent.

Figure 11: HPTLC performance of plant extracts and visualization of bands after derivetization in DPPH.

Discussion

Drugs that reduce post prandial hyperglycemia by suppressing hydrolysis of starch such as alpha amylase inhibitors have been found useful in the control of diabetes mellitus [9]. Many herbal extracts have been reported for their anti-diabetic activities and are currently being used in Ayurveda for the treatment of diabetes. However, such medicinal plants have not gained much importance as medicines due to the lack of sustained scientific evidences [10].

In previous study, alcoholic extract of aerial parts of the plant showed alpha amylase inhibitory activity with an IC_{50} value of 587.62 µg/ml [11].

In present study, maximum salivary alpha amylase inhibitory activity was shown by methanolic extract of root with minimum IC_{50} value i.e. 46.70 µg/ml.

1,1-diphenyl-2-picrylhydrazyl assay is a sensitive method widely used to assess the free radical scavenging activity of plant extracts or isolated phytochemicals. DPPH is a stable free radical which accepts an electron or hydrogen radical to turn into a stable diamagnetic molecule. This test possesses many advantages compared with other methods, such as good stability, sensitivity, feasibility, and handiness [12].

Previous studies show that leaf methanol extract of the plant showed free radical scavenging activity by DPPH assay with an IC_{50} value of 0.09 mg/ml [13].

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In present study, maximum free radical scavenging activity was shown by methanolic extract of fruits with minimum IC_{50} value i.e. 0.016 µg/ml. Root extract also showed good antioxidant potential.

Conclusion

From the results, it can be concluded that methanolic extracts of *X. strumarium* L. possess potent antioxidant activity and hypoglycemic activity and can be used as a source of natural hypoglycemic agent and natural antioxidants for medicinal uses against cancer, ageing, autoimmune diseases, and other related to free radicals and thus replacing the synthetic ones. The methanol extracts of plant exhibited very good antioxidant and hypoglycemic potential for different assays. Further investigation of individual compounds with their *in vivo* hypoglycemic and antioxidant activities and different mechanisms is needed.

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