PHYTOCHEMICAL EVALUATION AND AMYLASE A GLUCOSIDASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF URTICA DIOICA SEEDS

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Abstract- **Using different in vitro models such as α-amylase and α-glucosidase inhibitory effects and antioxidant activity, the primary goal of this study is to assess the hypoglycemic activity of Urtica dioica. seeds. The antidiabetic and antioxidant activities were explained by the presence of flavonoids and phenolics, which were discovered through phytochemical screening of fruit and seed extracts. Among the many biological benefits shown by flavonoids—a class of polyphenolic compounds—are anti-inflammatory, antihepatotoxic, antiulcer, antidiabetic, antithrombotic, and more. Additionally, they impede the activity of enzymes like xanthine oxidase and aldose reductase. By comparing UDSP with the standard medicine acarbose, which is a particular inhibitor of α glucosidase, and assessing its α amylase and α glucosidase inhibitory activities, the in vitro antidiabetic efficacy of UDSP was assessed. Additionally, acarbose has an inhibiting effect on α amylase. The quantity of glucose that is generated by the activity of α glucosidase can be approximated by utilizing the enzymes glucosidase and peroxidase in an experimental setting. In contrast to acarbose's inhibitory activity, the extract exhibited significantly less α glucosidase inhibition.**

Keywords: **Glucosidase Inhibitory, Antioxidant Activities, Urtica dioica, Seed Extracts.**

INTRODUCTION:

Diabetes or diabetes mellitus has become a burden for the global economy in recent decades. According to the World Health Organization's report, this disease and its complications cause substantial economic loss through direct medical costs and loss of work and wages [1]. Among diabetes cases, type 2 diabetes is much more common and chiefly occurs in adults; however, it is being increasingly noted in adolescents [2]. The pathogenesis of type 2 diabetes is currently attributed to endogenous factors such as genetics and metabolic abnormalities and exogenous factors such as behavior and environment [3]. The type 2 diabetes increases blood sugar level which is considered as a typical symptom in diabetic patients. Monitoring and control of hyperglycemia are the most prevalent methods in the treatment of type 2 diabetes nowadays.

As an endogenous toxin, oxidative stress is considered to be an important determinant of type 2 diabetes complications [4]. The causal relation between oxidative stress and type 2 diabetes has been elucidated through molecular mechanisms [5], whereby the overproduction of reactive oxygen species related to hyperglycemia likely leads to an imbalance of the quantity of antioxidants inside the body and eventually, to oxidative stress. On the other hand, the blood sugar level is crucially determined by the act of digestive enzymes such as α-amylase and α-glucosidase. While α-amylase is responsible for breaking down long-chain carbohydrates, α-glucosidase directly converts carbohydrate to glucose in the small intestine. The inhibition of α-glucosidase has been acknowledged as a therapeutic target for the control of postprandial hyperglycemia, as well as type 2 diabetes [6,7]. Therefore, simultaneously providing antioxidants and α-amylase and α-glucosidase inhibitors through nutriments is a potential and feasible method for the management of type 2 diabetes. However, the origin and dose of ingredients should be scrupulously studied before application and production. Additionally, natural products are recommended owing to their long history of medicinal and beneficial effects on human health [8].

Among natural sources, plants have been the most thoroughly scrutinized thanks to their vast diversity and wide distribution across the Earth. It is easy to derive antioxidant and nutrient components from every part of plants as fruits, leaves, stems, and roots which exhibit a wide range of biological effects such as anti-inflammatory, antibacterial,

antiviral, anti-aging, and anticancer [9].
. The main objective of the present study is to evaluate the hypoglycaemic activity of *Urtica dioica*. seeds using various *in vitro* models like α-amylase and α-glucosidase inhibitory effects and antioxidant activity.
The scope of the present study is attributed in exploring thepotential of the bioactive compounds from the medicinal plants and in revealing their safety & efficacy, there by realizing the promising ethno botanical herbs, towards the development of phytomedicine.

MATERIALS AND METHODS

DRUGS AND CHEMICALS

Acarbose (Biocon Ltd), α-amylase & α-glucosidase (Sisco ResearchLaborotaries Ltd Mumbai), Glucose assay kits (Agappe diagnostics, Kerala), 2,2-diphenyl-1-picryl hydrazyl were purchased from HiMediaLaboratories, Mumbai, ascorbic acid, 2-deoxy-2-ribose, xanthine oxidase, quercetin, kaempferol, hesperidine, rutin, xanthine oxidase, hypoxanthine, pyrocatechol were purchased from Sisco Research Lab, Mumbai andbutylated hydroxy toluene from Loba Cheme. Thiobarbituric acid, trichloroacetic acid, and potato starch were purchased from SD Fine Chemicals Ltd. All other chemicals used in the study were of analytical grade purchased from respective suppliers.

PLANT MATERIAL

Collection and authentication

The fruits of *Urtica dioica* were collected from the local market during the month of June and July 2023.

Preparation of the *Urtica dioica* Seed Extract

The seeds were dried in shade and finely powdered. The powdered seeds were macerated with water and stirred continuously in a mechanical shaker for 4 hours. The preparation was kept aside for 24 h. Itwas again stirred in the mechanical shaker for 4 h and kept aside for 12 h.These contents were taken and filtered through a muslin cloth and the filtrate was distilled to get a dark gummy material. This is then dried, to obtain the *Urtica dioica* seed powder (UDSP) andstored in an airtight container.

UDSP were subjected to qualitative phytochemical tests to determine the presence of various phytoconstituents (Trease and Evans, 2002; Sanni *et al*., 2008) like tannins, phenolics, saponins, flavonoids, terpenoids, alkaloids, proteins and glycosides.

a. Test for tannins and phenolics

To the solution of the extract, a few drops of 0.1% ferric chloride, 1% gelatin solution, 10% lead acetate was added and observed for brownish green or a blue-black color.

b. Test for saponins

About 10 ml of the extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent-froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and thenobserved for the formation of emulsion. When mixed with dilutesulphuric acid and boiled with 90% ethanol, if the initial frothing disappears it confirms the presence of higher concentration of saponins.

c. Test for flavonoids

1. To a portion of the extract concentrated H_2SO_4 was added. A yellow colouration indicates the presence of flavonoids. The yellowcolour disappears on standing.
2. Few drops of 1% $AlCl_3$ solution was added to a portion of extract. Ayellow colouration indicates the presence of flavonoids.
3. A portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates a positive test for flavonoids.

d. Test for terpenoids

About 5 ml of the extract was treated with 2 ml of chloroform and about 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

e. Test for alkaloids

A small portion of the extract was stirred with few drops of dil. HCl and filtered.

1. To the filtrate, Dragendorff's reagent (potassium bismuth iodide solution) was added and an orange brown precipitate indicates the presence of alkaloids.
2. To the filtrate, Mayer's reagent was added and a cream precipitate indicates the presence of alkaloids.

f. Test for proteins

A portion of the extract was mixed with few drops of water and added Millon's test and Biuret reagents. A yellowish-brown precipitate indicates the presence of proteins.

g. Test for glycosides

A portion of the extract was mixed with few drops of Fehling's solution A & B and heated gently. A brick red precipitate indicates the presence of glycosides.

***IN VITRO* HYPOGLYCAEMIC STUDIES**

Glucose can be readily absorbed from the G.I.T. by the presence of enzyme α-amylase and α-glucosidases. Inhibition of these enzymes reduces the postprandial blood glucose levels. Hence *in vitro* α-amylase & α-glucosidase inhibition models were carried out to screen UDSP and evaluate its potential hypoglycaemic activity.

Inhibition of α amylase *in vitro*

PROCEDURE

A 1% starch solution was prepared in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 minutes. The α amylase enzyme was obtained from porcine pancreas andits solution was prepared by mixing 1 mg of α amylase in 250 ml of cold deionised water. The calorimetric reagent was prepared by mixing sodium potassium tartarate (12 g in 8 ml of 2 mM sodium hydroxide and 96 mM of3, 5-dinitrosalicylic acid solution. UDSP extracts were dissolved in5% DMSO (Dimethylsulphoxide) to give a final concentration of 1 mg/ml.

One ml of starch solution was mixed with 1 ml of increasing concentration of the UDSP (100-1000 µg/ml) and mixed by swirling and equilibrated to 20°C. Then added one ml of α amylase solution and incubated at 20°C for 5 minutes to undergo the reaction withthe starch. To the above solution add 1 ml of the colorimetric reagent solution and heated in a water bath for 15 minutes. The reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid corresponds to the maltose generation with the colour change from yellowish orange to winered. Then it is cooled and added 9 ml of deionised water to make a final volume of 13 ml and then the absorbance was recorded at 540 nm for both test and blank using a suitable spectrophotometer (Sigma Aldrich, 1997; Thalapaneni *et al*., 2008)

Assay condition

T = 37°C, pH = 6.9, A540nm, Light path =1 cm, Calorimetric method

Unit definition

One unit will liberate 1mg of maltose from starch in 5 minutes at Ph 6.9 at 20°C and pH 6.9 under specified conditions. The α amylase inhibition was expressed as percentage of inhibitionand the IC50 values determined by linear regression of plots with varying concentration UDSP against the percentage inhibition fromthree separate tests.

4.1.1.* Inhibition of α glucosidase *in vitro

α glucosidase enzyme obtained from yeast as lyophilised powderwas used as the target protein source for the study of the enzyme inhibition using maltose as the substrate. Acarbose is used as positive control and the plant extract is prepared at the concentration of 1 mg/ml with 5% v/v DMSO (Dimethyl sulphoxide). The enzyme and the substrate were dissolved in 0.2 M Tris buffer at pH 8.

PROCEDURE

The enzymatic assay mixture consists of 1 ml of glucosidase enzyme(1U/ml), 1 ml of 37 mM of maltose substrate, 1 ml each of UDSP &acarbose at varying concentration (10µg - 100µg) in 5% v/v DMSO (Dimethyl sulphoxide) which is incubated at 37ºC for 30 min. Afterincubating for 30 min, 0.2 ml of the assay mixture is mixed with 1 ml of thekit reagent. Glucose released in the assay mixture is quantified with commercial glucose oxidase assay kit (GOD-POD Kit, Agappe Diagnostics, Kerala). The enzymatic activity was measured by the amountof glucose released, which was detected spectrophotometrically at 505 nm. The rate of carbohydrate breakdown was determined by calculating the amount of glucose obtained when carbohydrate was completely digested. The enzyme inhibitory activity was determined as the percentage inhibition and the assays were carried out in triplicate & the rate of prevention was calculated. The IC50 of the UDSP required to inhibit the activity of the enzyme by 50% was determined by linear regression of the plots with varying concentration of UDSP Vs percentage inhibition from the three separate tests (Subramanian *et al*., 2008; Thalapaneni *et al*., 2008)

Assay condition

T = 37°C, pH = 6.9, A540nm, Light path =1 cm, Calorimetric method

Unit definition

One unit will liberate 1mg of maltose from starch in 5 minutes at pH 6.9 at 20°C and pH 6.9 under specified conditions.

The α amylase inhibition was expressed as percentage of inhibition and the IC50 values determined by linear regression of plots with varying concentration of UDSP against the percentage inhibition from three separate tests.

STATISTICAL ANALYSIS:
P values are expressed as mean±standard error mean (SEM)and analyzed using GraphPad InStat software.

***IN VITRO* ANTIOXIDANT STUDIES**
DPPH radical scavenging assay (hydrogen donating ability)
The hydrogen donating ability of UDSP was examined inthe presence of DPPH stable radical. One millilitre of 0.3 mM DPPH ethanolsolution was added to 2.5 ml of UDSP solutions of different concentrations in ethanol and allowed to react at room temperature.After 30 min the absorbance values were measured at 518 nm. 1.0 ml ethanol plus 2.5 ml of UDSP solutions were used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus ethanol (2.5 ml) was used as negative control. The positive controls were those using the standard (Ascorbic acid) solutions. Percentage inhibition of DPPH scavenging effect wascalculated and the IC50 values were determined by linear regression of plots with varying concentration of UDSP against the percentage inhibition from three separate tests (Mensor *et al.*, 2001).

Deoxyribose degradation assay (Hydroxyl radical scavengingactivity)
The decomposing effect of UDSP on hydroxyl radicals wasdetermined by the assay of malondialdehyde chromogen formation dueto 2-deoxy 2-ribose degradation. The assay mixture contained in a final volume of 1ml: 100 µl of 28 mM 2-deoxy 2-ribose dissolved in phosphatebuffer, pH 7.4, 500 µl of the UDSP of various concentrations in buffer, 200 µl of 200 mM ferric chloride (1:1 v/v) and 1.04 mM EDTA and100 µl of 1.0 mM hydrogen peroxide and 100 □l of 1.0 µM ascorbic acid. After incubation of the test sample at 37^{o}C for 60 min (Fentons system- generation of hydroxyl radicals). The extent of deoxy ribose degradation by the formed hydroxyl radicals was measured directly using thiobarbituricacid (TBA) test; 1.0 ml TBA (1%) in 0.05M NaoH) and 1.0 ml 2.8% (w/v)trichloroacetic acid were added to the test tubes and heated at 100 ^{o}C for 15 min, cooled and the absorbance was measured at 532 nm against the blank containing deoxyribose and buffer solution. The positive controlswere those using the standard (Quercetin) solutions. Percentage inhibitionof deoxyribose degradation was calculated and the IC50 values were determined by linear regression of plots with varying concentration of UDSP against the percentage inhibition from three separate tests(Gomes *et al.*, 2001)

NBT reduction assay (Superoxide radical scavenging activity)
The capacity of the plant extracts to scavenge the superoxide anion was assayed by using NBT reduction assay. The superoxide anion radical was generated *in vitro* with hypoxanthine and xanthine oxidase. A reaction mixture with a final volume of 3 ml per tube was prepared with 1.4 ml of 50 mM KH_2PO_4-KOH pH 7.4 containing 1 mM EDTA, 0.5 ml of 100 m hypoxanthine, 0.5 ml of 100 µM nitro blue tetrazolium (NBT). The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 ml of phosphate buffer and 0.5 ml of UDSP in aline. The xanthine oxidase was added last. The subsequent rate of NBT reduction (measure of superoxide scavenging activity) was determined on the basis of spectrophotometric determinations of absorbance at 560 nm. Ascorbic acid was used as standard. The results are expressed as thepercentage inhibition of NBT reduction rate with respect to the reaction mixture without test compound (Guzman *et al.*, 2001; Gulcin., 2003).

Reducing power ability
Reducing power ability was measured by mixing 1.0 ml UDSP of varying concentrations (50, 100, 200, 400, 800 µg/ml) in 1 ml ofdistilled water to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of1% potassium ferricyanide and incubated at 50°C for 30 min. Later 2.5 mlof trichloroacetic acid (10%) were added to the mixture and centrifugedat 3000 rpm for 10 min. Finally 2.5 ml from the supernatant were mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbancewas measured spectrophotometrically at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. Allexperiments were done in triplicate using butylated hydroxyltoluene (BHT)as positive control (Yildrim *et al.*, 2001).

Determination of % Inhibition
Percentage antioxidant activity (%AA) was calculated using the formula,

$$\%\ \text{Antioxidant Activity (\%AA)} = 100 - \left[\frac{A_0 - A_1}{A_0} \times 100\right]$$

Where, A0 is the absorbance of the control and A1 is the absorbance ofthe sample.

Determination of 50% Inhibitory Concentration (IC50)
The concentration (mg/ml) of the plant extracts required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extract. The IC50 values were calculated using GraphPad Instat statistical software.

Statistical analysis
All determinations were carried out in triplicate and the values are expressed as the mean ± SEM.

RESULTS

Percentage yield
The percentage yield of the powder of the seeds of *Urtica dioica* seed powder was 17%.

Phytochemical screening
The preliminary phytochemical screening of UDSP showed the presence of flavonoids, alkaloids, saponins, proteins, tannins and phenolics.

Inhibition of α amylase *in vitro*
Urtica dioica seed powder showed α amylase inhibitory activity at the varying concentrations tested (50, 100, 200, 400 and 800µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested (Table 3).

Urtica dioica seed powder (UDSP) showed significant α amylase inhibitory activity at the varying concentrations tested (50, 100, 200, 400 and 800µg/ml). There was a dose dependent increase in the percentage inhibition forall the concentrations tested. UDSP at a concentration of 50 µg/ml showed a percentage inhibition of 16.68, for 100 µg/ml it was 30.70, for 400
µg/ml it was increased to 54.30, 800 µg/ml it was 71.23. The IC_{50} value was found to be 338.66 µg/ml.
Acarbose was used as the standard drug for the determination of α amylaseinhibitory activity. The concentration of acarbose is varied (50, 100, 200, 400 and800µg/ml). Acarbose at a concentration of 100µg/ml exhibited a percentage inhibition of 37.21% and for 800 it was found to be 94.20%. A graded increase inthe percentage inhibition was observed for the increasing concentrations of the drug. The IC_{50} values of acarbose were found to be 170.80. An increase in the IC_{50} value was observed for the plant extract when compared with the standard drug acarbose.

Inhibition of α glucosidase *in vitro*
The study revealed that UDSP had significant α glucosidase inhibitory activity at the varying concentrations tested (50, 100, 200, 400, 800 µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested (Table 4).
UDSP at a concentration of 50µg/ml showed a percentage inhibition of 15.59%, at 200µg/ml it was found to be 36.48% and at 800 µg/ml it increased to
49.29. The IC_{50} value was found to be 429 µg/ml.

Acarbose was used as the standard drug for the determination of α glucosidase inhibitory activity. The concentration of acarbose is varied from 50, 100, 200, 400, 800 µg/ml. Acarbose at a concentration of 100µg/ml exhibited a percentage inhibition of 40.73% and for 800 µg/ml it was found to be 72.56%. Agraded increase in the percentage inhibition was observed for the increasing concentrations of the drug. The IC_{50} values of acarbose were found to be 202.15µg/ml. An increase in the IC_{50} values was observed for the plant extract when compared with the standard drug acarbose (Table 4).

DPPH radical scavenging assay (hydrogen donating ability)
The extracts UDSP at various concentrations demonstrated H-donor activity. The radical scavenging activity of UDSP was determined from the reduction in the absorbance at 518 nm due to scavenging ofthe stable DPPH free radical. The DPPH scavenging potential forUDSP varied at varying concentrations (10, 20, 40, 80, 160 µg/ml) and the resultsare shown in Table 5. UDSP showed the graded increase in percentageof inhibition for all the doses tested and the percentage inhibition ranged from 9.57% to 57.91%. The IC_{50} values of UDSP were found to be

130.06 µg/ml.
Ascorbic acid was used as the reference standard and similar increase in the percentage of inhibition was observed for all the concentrations (10, 20, 40, 80, 160 µg/ml) tested. The DPPH scavenging effect for various extracts was lessthan that of standard compound, ascorbic acid. The IC_{50} value of standard was found to be 53.04µg/ml. DPPH was reduced with the addition of UDSPin a concentration dependent manner**.**

Deoxyribose degradation assay (hydroxyl radical scavenging activity)
Hydroxyl radical scavenging activity was quantified by reaction with thiobarbituric acid and the results are shown in the. The UDSP showed 30.12% of activity at 20µg/ml and it was increased to 54.79 % at 80µg/ml. The IC_{50} value of UDSP were found to be 27 µg/mlrespectively. Quercetin was used as the reference standard and similar increase inthe percentage of inhibition was observed for all the concentrations (5, 10, 20, 40,80 µg/ml) tested. The IC_{50} value of quercetin was found to be 18.5 µg/ml. The degradation of deoxyribose by Fe^{3+}-ascorbate-EDTA-H_2O_2 system was markedly decreased by UDSP tested at various concentrations indicating hydroxyl radical scavenging activity.

5.5. **NBT reduction assay (superoxide radical scavenging activity**)
The UDSP at various concentrations were found to be a scavenger of superoxide anion generated in xanthine oxidase-NBT systems *in vitro* and their activity were comparable to that of ascorbic acid and the results were given in (Table 7). All the concentrations of UDSP offered greater percentage of inhibition with increase in the concentrations. Ascorbic acidwas used as the positive control. The IC_{50} value for UDSP were 285 and 122 µg/ml respectively and that of standard was 39.26 µg/ml.

5.6. **Reducing power ability**
The reductive capabilities of UDSP when compared to the standard BHT were given in Table 8. The reductive ability of the UDSP serves as a significant indicator of its antioxidant activity. The reducing power of UDSP was dose dependent and found to increase with increasing concentrations. UDSP increased the absorbance up to 0.563and 0.394 at 800µg/ml respectively, when the absorbance of the standard at 800µg/ml was 1.397. All the concentrations of UDSP offered higher absorbance values than the control.

Table 1: Preliminary phytochemical screening of UDSP

S.No	Phytochemical compounds	Presence or Absence
1	*Triterpenoids*	**Absence**
2	*Flavonoids*	**Presence**
3	*Glycosides*	**Absence**
4	*Alkaloids*	**Presence**
5	*Saponins*	**Presence**
6	*Carbohydrates*	**Absence**
7	*Proteins*	**Presence**
8	*Tannins and phenolics*	**Presence**

Table 2: Alpha amylase inhibitory activity of UDSP

Sample	Concentration (µg/ml)	% Inhibition	IC $_{50}$ µg/ml
UDSP	50	16.68 ± 1.40	338.66 ± 0.881
	100	30.70 ± 0.98	
	200	41.12 ± 0.46	
	400	54.30 ± 0.52	
	800	71.23 ± 0.97	
Acarbose(standard)	50	22.77 ± 0.21	170.80 ± 0.144
	100	37.21 ± 0.16	
	200	57.49 ± 0.17	
	400	77.98 ± 0.16	
	800	87.70 ± 0.16	

All determinations were carried out in triplicate manner and values areexpressed as the mean ± SEM.

Table 3: Alpha glucosidase inhibitory activity of UDSP

Sample	Concentration (µg/ml)	% Inhibition	IC $_{50}$ µg/ml
UDSP	50	15.59 ± 0.13	429 ± 1.155
	100	28.83 ± 0.14	
	200	36.48 ± 0.34	
	400	49.24 ± 0.35	
	800	61.07 ± 0.34	
Acarbose(standard)	50	22.77 ± 0.21	202.15 ± 1.021
	100	40.73 ± 1.39	
	200	49.34 ± 1.04	
	400	63.48 ± 0.91	
	800	72.56 ± 1.22	

All determinations were carried out in triplicate manner and values areexpressed as the mean ± SEM.

Table 4: Hydrogen donating ability of UDSP using DPPH method

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
UDSP	10	15.54 ± 0.41	130.06 ± 0.096
	20	22.24 ± 0.19	
	40	30.63 ± 0.22	
	80	41.72 ± 0.30	
	160	57.91 ± 0.17	
Ascorbic acid (standard)	10	17.48 ± 0.38	53.04 ± 0.57
	20	32.87 ± 1.38	
	40	47.94 ± 0.30	
	80	68.99 ± 0.07	
	160	84.23 ± 0.21	

All determinations were carried out in triplicate manner andvalues are expressed as the mean ± SEM.

Table 5: Scavenging of hydroxyl radical activity byUDSP using deoxyribose degradation method

Sample	Concentration (µg/ml)	% Inhibition	IC $_{50}$ µg/ml
UDSP	5	16.26 ± 0.09	27 ± 1.60
	10	29.13 ± 0.93	
	20	45.10 ± 0.07	
	40	55.61 ± 0.06	
	80	67.87 ± 0.10	
Quercetin (standard)	5	19.79 ± 0.39	18.5 ± 0.29
	10	35.16 ± 0.21	
	20	57.81 ± 0.28	
	40	73.75 ± 0.23	
	80	92.26 ± 0.21	

All determinations were carried out in triplicate manner and values areexpressed as the mean ± SEM.

Table 6: Superoxide anion scavenging activity of UDSP usingNBT reduction assay

Sample	Concentration (µg/ml)	% Inhibition	IC $_{50}$ µg/ml
UDSP	25	20.22 ± 0.12	122 ± 0.176
	50	33.66 ± 0.15	
	100	47.74 ± 0.09	
	200	61.67 ± 0.18	
	400	71.07 ± 0.10	
Ascorbic acid (standard)	25	33.33 ± 0.03	39.26 ± 0.54
	50	63.87 ± 0.45	
	100	76.78 ± 0.21	
	200	85.79 ± 0.17	
	400	91.82 ± 0.14	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 7: Reductive ability of UDSP

Sample	Concentration (µg/ml)	Absorbance at 700 nm IC $_{50}$ µg/ml
UDSP	50	0.081 ± 0.0008
	100	0.125 ± 0.0023
	200	0.201 ± 0.0017
	400	0.279 ± 0.0029
	800	0.394 ± 0.0011
BHT (standard)	50	0.1647 ± 0.001
	100	0.3252 ± 0.001
	200	0.5862 ± 0.002
	400	0.7635 ± 0.003
	800	1.3972 ± 0.033

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

DISCUSSION

The metabolic condition of carbohydrates, lipids, and proteins known as diabetes mellitus is one of the fastest-growing diseases in the world. Diabetes is characterized by decreased glucose consumption by tissue due to a relative or total shortage of insulin production.

There is an immediate need for alternatives to the current pharmacotherapy of diabetes mellitus due to the fact that these treatments cannot control all the pathological components of the disease, are prohibitively expensive, and are not accessible to many people living in rural areas of developing nations.

Traditional medicine practitioners employ Urtica dioica because, hopefully, it has no negative side effects. People have relied on plants and herbs for medicinal purposes, nutritional supplements, and symptom management in conjunction with pharmaceuticals since ancient times.

Traditional Indian medicine includes the use of the plant Urtica dioica for a variety of conditions, including but not limited to: asthma, cough, diabetes, hemoptysis, internal organ hemorrhages, fever, epilepsy, and pharmacological and phytochemical screening. Those suffering from diabetes can benefit from the seeds and fruits (Indian Medicinal Plants, 1985).

The antidiabetic and antioxidant activities were explained by the presence of flavonoids and phenolics, which were discovered through phytochemical screening of fruit and seed extracts. Among the many biological benefits shown by flavonoids—a class of polyphenolic compounds—are anti-inflammatory, antihepatotoxic, antiulcer, antidiabetic, antithrombotic, and more. Additionally, they impede the activity of enzymes like xanthine oxidase and aldose reductase.

The current investigation aimed to assess the antioxidant and antidiabetic effects of Urtica dioica seeds in a controlled laboratory setting.

By comparing UDSP with the standard medicine acarbose, which is a particular inhibitor of α glucosidase, and assessing its α amylase and α glucosidase inhibitory activities, the in vitro antidiabetic efficacy of UDSP was assessed. Additionally, acarbose has an inhibiting effect on α amylase. The quantity of glucose that is generated by the activity of α glucosidase can be approximated by utilizing the enzymes glucosidase and peroxidase in an experimental setting. In contrast to acarbose's inhibitory activity, the extract exhibited significantly less α glucosidase inhibition. The process by which α amylase works could be because it blocks the starch binding site, and the same could be true for α glucosidase, which inhibits the enzyme, by blocking the oligosaccharide binding site. In order to reduce postprandial hyperglycemia, alpha amylase has been identified as a target for hydrolysis of the internal α 1,4 glucosidic linkages in starch and related polysaccharides. The results of this study suggest that UDSP may have an effect on blood sugar levels.

The pathogenic presentation of diabetes is known to be influenced by free radicals. By scavenging reactive oxygen radicals or preserving the antioxidant defense mechanism, antioxidants battle free radicals and shield us from a host of ailments. Bacterial macromolecules like proteins, polysaccharides, and DNA are susceptible to damage from reactive oxygen species (ROS). ROS encompasses a wide range of substances, including oxygen radicals ($O2^{\cdot}$ and $OH^{\cdot}$) and non-radical oxygen derivatives (H2O2, HOCl, and O3). Furthermore, antioxidant activity could be seen as a crucial attribute for life.

To assess its antioxidant properties in vitro, UDSP has been subjected to a battery of tests, including the DPPH radical scavenging assay, the deoxyribose degradation assay, the NBT reduction assay (for superoxide scavenging activity), and the reducing power ability method (for total antioxidant capacity).
DPPH is a free radical that, when left to its own devices at room temperature, turns ethanol into a violet solution. DPPH is a popular method for determining how effective natural antioxidants are at scavenging free radicals. The deep violet color of DPPH is accompanied by a prominent absorption band at 517 nm in the visible spectrum. A stochiometric discoloration that corresponds to the quantity of electrons taken up occurs when the absorption disappears when the electron pairs up in the presence of free radical scavenging (Mensor et al., 2001). The ability of the test medicines to scavenge free radicals independently is demonstrated by the whitening of DPPH uptake. The ability of UDSP to scavenge DPPH radicals was shown to be concentration-dependent. The presence of hydroxyl groups on the antioxidant molecule is associated with the reduction process of the DPPH molecule, according to the literature. Since UDSP had such high activity, it is safe to assume that it was because of substances that contained accessible hydroxyl groups. Among the most reactive radicals generated by Fenton's reaction in biological systems are hydroxyl radicals. In order to measure the hydroxyl radical scavenging activity, the suppression of free radical degradation of deoxyribose was measured (Guzman et al., 2001). Thiobarbituric acid was used to evaluate the amounts of deoxyribose. Although it was not as effective as the gold standard, quercetin, UDSP did a decent job of scavenging hydroxyl radicals.

The hypoxanthine and xanthine oxidase system was identified utilizing the NBT reduction assay, which allowed for the enzymatic generation of superoxide anion radicals in vitro. Spectrophotometric measurement of the blue complex formazone, which is formed when superoxide degrades NBT, is possible. As UDSP reduces absorbance at 560 nm, it means that the reaction mixture is consuming superoxide anion (Gulcin et al., 2003). The test fraction's ability to block NBT reduction by the superoxide anion radical and, by extension, its superoxide scavenging activity, can be measured by determining the mean rate of rise in absorbance during a one-minute period.

It was found that UDSP's reducing capabilities were comparable to those of BHT. We studied the Fe3+-Fe2+ transformation in the presence of UDSP for the purpose of measuring the reductive ability. A compound's reducing capacity is a good measure of its antioxidant capability. Gulcin et al. (2004) and Amarowiz et al. (2004) found that antioxidants have a number of mechanisms that contribute to their antioxidant activity. These include preventing chain initiation, binding transition metal ion catalysts, decomposing peroxides, preventing continued hydrogen abstraction, having a reductive capacity, and scavenging radicals. As the sample size grew, so did UDSP's lowering power. Here, the hue shifts from yellow to a greenish blue, all dependent on the UDSP's lowering power.

According to the results of this study, Urtica dioica has a strong antioxidant effect in a number of different in vitro test systems.

CONCLUSION

To sum up, the use of alternative medicine and the medicinal benefits of plant-based products has been on the rise in recent years. Urtica dioica seed extract has been found to have stronger inhibitory effects on α amylase and α glucosidase enzymes, which means it slows down glucose absorption and lowers postprandial hyperglycemia, according to the evaluations conducted using different in vitro assay models. A battery of in vitro assays demonstrated the plant's antioxidant capacity. Preliminary chemical analyses revealed that the extract contained polyphenolic chemicals. It follows that these flavonoids are responsible for the aforementioned effects. To validate these actions and investigate the precise mechanism by which the plant components exert their effects, more research employing in vivo models is required.

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