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Antioxidant, anti-arthritic and hypoglycemic activity of *Oxalis corniculata* Linn. leaf extracts

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Abstract: *Oxalis corniculata* (Linn,) is a subtropical plant belonging to Oxalidaceae family. The dried leaves of *Oxalis corniculata* Linn. (Family:Acanthaceae) were extracted using pet ether (non-polar), ethyl acetate (mid-polar) and ethanol (highly polar). All three extracts were subjected to qualitative phytochemical screening to identify the phytoconstituents present. The in-vitro anti-arthritic activity of the extracts was carried out by egg albumin denaturation method, where diclofenac was taken as standard. Ethanol extract exhibited potent anti-arthritic activity by significantly (p<0.05) inhibiting the denaturation of egg albumin at 50, 100 and 200 µg/mL concentrations. Anti-diabetic effect was examined by α-amylase and α-glucosidase inhibitory activity, taking Acarbose as standard. Ethanol extract inhibited both the enzymessubstantially (p<0.05) at 50, 100 and 200 µg/mL concentrations, unveiling potential anti-diabetic activity.
Key words: *Oxalis corniculata*Linn., phytochemical screening, anti-arthritic, anti-diabetic.

Introduction

Rheumatoid arthritis is an autoimmune disorder characterized by synovial membrane inflammation, pain, degeneration of cartilage, hyperplasia and restricted joint movement.[1, 2] Though many drugs are marketed for the treatment of rheumatoid arthritis, the development of better drugs is encouraged.[3] The drugs frequently used for the treatment of rheumatoid arthritis are nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and biological agents like interleukin-1beta(IL-1ß) antagonists and tumour necrosis factor alpha (TNF-α). But the success rate of these drugs is narrow and they are characterized by side effects such as cardiovascular risks, gastrointestinal disturbances, nephropathy and infections. Moreover the use of drugs like interleukin-1beta(IL-1ß) antagonists and tumour necrosis factor alpha (TNF- α) might cause side effects that are life threatning.[4, 5]

Diabetes or hyperglycemia is a metabolic disorder which can be categorized as Type 1 and Type 2.[6] The major input of glucose in blood comes from the hydrolysis of dietary starch. The two key enzymes involved in breakdown of starch and its absorption in the intestines are α-amylase and α-glucosidase respectively. The inhibition of these two enzymes triggers the decrease in blood glucose level.[7] The drugs presently used for regulating hyperglycemia are metformin, acarbose, tolbutamide, miglitol, glimepride etc. Though these drugs can efficiently control the increased glucose levels but their inevitable side effects like nausea, gastrointestinal infections, stomach upsets, liver toxicity, weight gain and many others could not be neglected.[8, 9]

Oxalis corniculata (Linn,) is a subtropical plant belonging to Oxalidaceae family. It is commonly known as the creeping wood sorrel. It is a delicate, procumbent herb with palmately three foliolate leaves. It is a

plant native of India which grows in damp shady places, plantations, roadside, lawns etc.[10, 11] Traditionally this plant is used as antiseptic, anti-inflammatory, anti-diabetic, digestive, antibacterial, diuretic, hepatopathic and cardiopathic agent. It also cures diarrhea, dysentery, skin diseases etc.[12, 13]

In the present study, leaves of *O. corniculata* were extracted by using a non-polar (pet ether), mid-polar (ethyl acetate) and highly polar (ethanol) solvent. The three extracts were subjected to qualitative phytochemical screening to detect the important phytoconstituents present. All three extracts were evaluated for antioxidant, anti-arthritic and hypoglycemic activity.

Materials

The solvents and commercially available reagents were of analytical grade and used without further purification. The chemicals and the solvents used for extraction and *in vitro* activities were acquired from SD Fine Chem. Ltd, Mumbai. The UV readings were noted in Jasco V-670 spectrophotometer.

Experimental

Extract Preparation

Oxalis corniculata Linn. plants were collected from Hoskote, Bengaluru and authenticated in Plant Anatomy Research centre, Chennai. The leaves were plucked and washed thoroughly. The leaves were shade dried at 25-30 ºC. The dried leaves were ground into powder using a grinder. The powdered leaves were extracted in a sohxlet extractor using a less polar, mid polar and high polar solvent i.e, pet ether, ethyl acetate and ethanol respectively. Extraction was completed in 36hrs and the solvents were evaporated in an electric water bath. The qualitative phytochemical screening was performed for all three extracts to identify the phytoconstituents present.

Antioxidant activity

DPPH assay

The antioxidant activity of the extracts were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.[14, 15] 0.1 M DPPH solution and different concentrations of the extracts were prepared in methanol (25, 50 and 100 µg/mL). 3 mL of each concentration of the extracts was mixed with 1 mL DPPH solution. Standard Ascorbic acid samples were prepared in the similar manner. 3 mL methanol with 1 mL DPPH was taken as control. The control and the samples were incubated in dark at room temperature for 30mins. The absorbance was recorded in a UV spectrophotometer at 517 nm. In its radical form, DPPH exhibits a higher absorbance at 517 nm but in presence of a radical species, it shows a decrease in absorbance, thus, changing its colour from light violet to yellow. The IC_{50} value of the samples was calculated by plotting a linear regression graph.

The DPPH activity was measured by the following formula:

DPPH scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$

Where, A_0 is the absorbance of the control and A1 is the absorbance of the plant extracts.

H_2O_2 assay

The hydrogen peroxide scavenging activity of the samples was assessed by a reported procedure.[16]20 mM hydrogen peroxide solution was prepared in phosphate buffer saline (PBS, pH 7.4). 50 µg/mL, 100 µg/mL and 200 µg/mL concentrations of the extracts and the standard (ascorbic acid) were prepared. 2 mL of hydrogen peroxide solution was added to 1 mL of each concentration of extracts and the standard. A blank was prepared using phosphate buffer. The samples were incubated at room temperature (25-30 °C) for 10 mins. After incubation the absorbance was measured at 230 nm. This assay shares a similar principle as the DPPH assay. Upon oxidation of H_2O_2, the absorbance of H_2O_2 decreases. The IC_{50} value was measured from a regression graph. The antioxidant activity was measured by the following formula:

H_2O_2 scavenging activity (%) = $[(A_0 - A_1) / A_0] X 100$

Where, A_0 is the absorbance of the control and A1 is the absorbance of the plant extracts.

Anti-arthritic activity

Protein denaturation using egg albumin

The pet ether, ethyl acetate and ethanol extracts were assessed for anti-arthritic activity.[17, 18] Anti-arthritic activity was examined egg albumin denaturation technique. Phosphate buffer of pH 6.4 was prepared. 50 µg/mL, 100 µg/mL and 200 µg/mL concentrations of all three extracts were prepared in distilled water. Albumin was isolated from a hen egg. 2 mL extract of each concentration, 0.2 mL of egg albumin and 2.8 mL of phosphate buffer were mixed to form the samples. A standard sample was prepared by taking 2 mL of Diclofenac sodium of the same concentration as the extract. A control without any drug or extract was also taken. All samples along with the control were incubated for 20 mins at 37 °C and then at 70 °C for 5 mins. The absorbance was noted at 660 nm. The principle behind this activity was very simple. The protein rich egg albumin is likely to get denatured on heating. Anti-arthritic activity was measured as the ability of the sample to prevent the denaturation of protein. The extent of prevention would indicate the potency of the samples. The formula used for calculating the anti-arthritic activity was:

$$\% \text{ Inhibition} = \frac{100 \times \text{Absorbance of Test}}{1 - \text{Absorbance of Control}}$$

Hypoglycemic activity

α-amylase inhibition assay

The α-Amylase inhibitory assay was performed by a simple procedure.[19, 20]50 µg/ml, 100 µg/ml and 200 µg/ml concentrations of extracts and standard (Acarbose) were prepared in phosphate buffer (pH 6.9). 1 mL of standard and extracts of each concentration was added to an equal volume of α-amylase enzyme solution. The mixture was incubated for 20 mins at room temperature. The reaction was initiated by adding 2 mL starch solution (0.5% w/v in phosphate buffer) and incubating the sample for 20 mins at 37 °C. At the end of incubation 2ml of DNS solution was added and the reaction was terminated by maintaining the samples at 100 °C for 15mins. A control was taken without addition of standard or extract. The initiation of the reaction triggers the breakdown of starch into simpler molecules by amylase enzyme. The extent of inhibition of amylase enzyme by the extracts is measured by UV spectroscopy. The absorbance was measured for all samples at 540 nm. The formula used for calculating the anti-arthritic activity was:

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

α-glucosidase inhibition assay

α-glucosidase inhibitory activity was evaluated by a reported procedure with little modification.[21,22] 0.5mMpNPG (4-nitrophenyl α-D-glucopyranoside) solution was prepared in 0.1 M phosphate buffer (pH 6.9). 50 µg/ml, 100 µg/ml and 200 µg/ml concentrations of extracts and standard (Acarbose) were prepared in phosphate buffer (pH 6.9). 0.1 U/mL of α-glucosidase solution was prepared in 0.01 M phosphate buffer (pH 6.0). 5 mL phosphate buffer (0.1 M), 2.5 mL pNPG, 1 mL test sample and 2.5 mL α-glucosidase solution were mixed. The reaction mixture was incubated for 20 min at 37 °C. The reaction was terminated by adding 10 mL sodium carbonate solution (0.1 M). Absorbance was measured at 405 nm. As the reaction proceeds α-glucosidase causes enzymatic hydrolysis of pNPG releasing p-nitrophenol. The extract is expected to inhibit the α-glucosidase activity and prevent the hydrolysis. A control was taken where extract was replaced by methanol. The α-glucosidase inhibition was measured in terms of percentage inhibition using the following formula:

Percentage of inhibition = $\frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$

Results and Discussion

Qualitative Phytochemical Screening

The qualitative phytochemical analysis of the extracts indicated the presence of proteins, phytosterols and flavonoids in pet ether extract, carbohydrates and phytosterols in ethyl acetate extract and carbohydrates, phenols, sterols and terpenoids in ethanol extract. The results are tabulated in Table 1:

Table 1: Qualitative Phytochemical Screening Results

Phytoconstituents	Pet ether	Ethyl acetate	Ethanol
Alkaloids	-	-	-
Carbohydrates	-	+	+
Glycosides	-	-	-
Phytosterols	+	+	+
Phenols	-	-	+
Proteins	+	-	-
Terpenoids	-	-	+
Saponins	-	-	-
Flavanoids	+	-	-

"+" = present, "-" = absent

Antioxidant Activity

DPPH assay

The results of DPPH assay are tabulated in Table 2. Pet ether extract exhibited potent antioxidant activity followed by ethanol extract and ethyl acetate extract. At all three concentrations ascorbic acid exhibited better result; however pet ether and ethanol extract showed equally potent results. Ethyl acetate extract exhibited moderate activity at all three concentrations. The potency of pet ether extract could be attributed to the presence of flavonoids and sterols.

Table 2. DPPH and H_2O_2 inhibition activity

Sl. No	Sample		DPPH	H_2O_2
		Conc. (µg/mL)	% Inhibition (%)	% Inhibition (%)
1	Control		0.858	1.0087
2	Ascorbic acid (STD)	50	76,34	77.24
		100	80.65	86.25
		200	85.43	91.02
3	Pet Ether Extract	50	73.31	75.71
		100	78.32	82.75
		200	82.28	89.94
4	Ethanol Extract	50	65.5	66.10
		100	72.61	77.93
		200	77.50	84.28
5	Ethyl Acetate Extract	50	64.10	64.37
		100	71.79	77.08
		200	76.10	80.57

H_2O_2 assay

The results of H_2O_2 assay were similar to DPPH assay. The percentage inhibition values are presented in Table 2. Pet ether and ethanol extract displayed potent antioxidant activity compared to ascorbic acid whereas ethyl acetate extract displayed moderate activity. The activity of pet ether extract was closely comparable to the standard.

Anti-arthritic activity

The anti-arthritic effect of pet ether extract, ethyl acetate extract and ethanol extract of *O. corniculata* was compared to standard Diclofenac sodium. The percentage inhibition by the extracts is shown in Table3. Ethanol extract showed good activity compared to the standard followed by pet ether extract. Ethyl acetate extract exhibited the least activity of all three extracts. At all three concentrations the extent of inhibition presented by ethanol extract was comparable to diclofenac. Thus it can be considered to have potent anti-arthritic activity whereas pet ether and ethyl acetate extract exhibited moderate activity.

Table 3. Anti-arthritic activity

Sl. No	Sample	Conc. (µg/mL)	% Inhibition (%)
1	Control		0.1308
2	Diclofenac sodium (STD)	50	28.30
		100	69.85
		200	91.32
3	Ethanol Extract	50	22.84
		100	65.52
		200	83.21
4	Pet Ether Extract	50	17.54
		100	58.91
		200	78.11
5	Ethyl Acetate Extract	50	13.12
		100	49.13
		200	67.05

Table 4.α–amylase and α-glucosidase inhibition assay

Sl. No	Sample		α amylase	α glucosidase
		Conc. (µg/mL)	% Inhibition (%)	% Inhibition (%)
1	Control		0.9891	0.5270
2	Acarbose (STD)	50	63.72	59.49
		100	75.36	71.19
		200	88.26	83.81
3	Ethanol Extract	50	56.75	51.02
		100	69.62	65.35
		200	81.41	80.75
4	Pet Ether Extract	50	49.29	45.14
		100	60.98	58.63
		200	77.60	74.11
5	Ethyl Acetate Extract	50	43.91	35.63
		100	56.75	49.62
		200	71.38	63.14

α-amylase inhibition

The α-amylase inhibition of the extracts is presented in Table 4. Ethanol extract revealed better α amylase inhibitory activity than pet ether and ethyl acetate extract. As compared to acarboseethanol extract demonstrated potent results. However pet ether extract also inhibited the α-amylase enzyme effectively.Though the activity exhibited by ethyl acetate extract was the least among the three extracts but it can be considered to possess moderate inhibitory activity.

α-glucosidase inhibition assay

The α-glucosidase inhibitory activity of the extracts is presented in Table 4.The results of α-glucosidase inhibitory assay were quite similar to α-amylase inhibitory assay. The inhibitory activity of ethanol extract was comparable to acarbose whereas pet ether extract exhibited slightly lower activity than ethanol extract. Ethyl acetate extract demonstrated fair inhibition of α-glucosidase enzyme. Both ethanol and pet ether extract could be considered to possess potent activity.

Conclusion

The pet ether, ethanol and ethyl acetate extracts of the leaves of *O. corniculata* were qualitatively screened for the presence of various phytoconstituents. The extracts were assessed for *in vitro* antioxidant, anti-arthritic and hypoglycemic activity. Pet ether extract exhibited potent antioxidant activity in both DPPH assay and H_2O_2 inhibition activity. This might be attributed to the presence of flavonoids and sterols in it. In the protein denaturation assay to determine anti-arthritic activity ethanol extract exhibited effective results followed by pet ether extract and ethyl acetate extract. The presence of terpenoids might be responsible for the potent activity of ethanol extract. Ethanol extract also exhibited good hypoglycemic activity in α-amylase as well as α-glucosidase inhibition assay.

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