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PRELIMINARY PHYTOCHEMICAL STUDY, *IN VITRO* ANTI-INFLAMMATORY AND ANTI-DIABETIC ACTIVITY OF DIFFERENT EXTRACTS OF *CISSUS QUADRANGULARIS* LINN

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ABSTRACT

The present study was aimed to evaluate the phytochemistry, anti-inflammatory and anti-diabetic activity of different extracts of aerial parts of *Cissus quadrangularis* plant. Initially, the plant material was collected authenticated and dried for powdering. The dried material was powdered in mechanical grinder and the coarse powder thus obtained was extracted in soxhlet apparatus with the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol. The dried extracts thus obtained were subjected to preliminary phytochemical evaluation. Anti-inflammatory activity of all the extracts was evaluated in vitro by different approaches viz., inhibition of protein denaturation, proteinase inhibitory activity. Anti-diabetic activity was evaluated in vitro by inhibition of α-amylase and α-glucosidase activity. In the preliminary phytochemical evaluation, presence of alkaloids, phenolic compounds, flavanones and flavonoids, carbohydrates, proteins, terpenoids, sterols, saponins and gum and mucilage were found in the tested extracts. The methanol and ethyl acetate extracts showed a significant presence of majority of phytochemicals comparing with other two, the petroleum ether, chloroform extracts. The methanol extract showed significant activity in the in vitro evaluation of anti-inflammatory and anti-diabetic activity. These results are useful for further investigation in the future.

KEYWORDS: *Cissus quadrangularis*, Preliminary phytochemical evaluation, *In vitro* anti-inflammatory activity, *In vitro* anti-diabetic activity.

INTRODUCTION

Throughout history, medicinal plants have been extensively used to treat various diseases and infections worldwide. The plant-based treatment methods found in numerous traditional healing systems have consistently inspired researchers to seek new drug molecules.[1,2] Indeed, several medications derived from natural sources, including antibiotics like penicillin, anti-malarials such as quinine and artemisinin, hypolipidemics like lovastatin, immunosuppressants such as cyclosporine, and anti-cancer agents like paclitaxel, have transformed therapeutic practices.[3] Recent studies suggest that nearly two-thirds of the medicines approved globally originate from plants.[4]

The organic compounds found in plants are responsible for their specific physiological effects. These secondary metabolites are chemically and taxonomically unique substances, including alkaloids, glycosides, flavonoids, essential oils, saponins, resins, and phenols.[5] These phytochemicals are located in various parts of the plants, such as the root, stem, flower, fruit, seed, and exudates, and can be utilized to treat a range of chronic and infectious diseases. A systematic evaluation of plants used in traditional healing systems is necessary to provide more promising data regarding their medicinal value, which may help address the increasing demand for novel agents to combat infections and diseases.[6,7] In this context, *Cissus quadrangularis*, a plant of ethno medicinal significance, was chosen for the current study.

C. quadrangularis is a succulent, creeper plant, native to India, Bangladesh and Sri Lanka. It is also found in Africa, Southeast Asia.[8] It can be cultivated in plains coastal areas, jungles and wastelands up to 500m elevation. Plant is propagated using cuttings. Plant flowers in the month of June-December. Plant material occurs as pieces of varying lengths; stem quadrangular, 4-winged, internodes 4-15cm long and 1-2cm thick. The surface is smooth, glabrous, buff coloured with greenish tinge, angular portion reddish-brown; no taste and odour. Leaves are simple 2.5-5cm long, broadly ovate or reniform, sometimes 3-7 lobed, denticulate, glabrous, cordate, rounded, truncate or cuneate at the base; petioles 6-12mm long; stipules small broadly ovate, obtuse. Flowers are in shortly peduncle cymes with spreading

umbellate branches. Calyx is cup shaped, truncate or very obscurely lobed. Petals are 4, ovate-oblong, short, stout. Berry is obovoid or globose, scarcely 6mm, long apiculate, red when ripe, 1- (very rarely 2) seeded.[9] Now, the present study was focused on the preliminary phytochemical evaluation, *in vitro* anti-inflammatory and anti-diabetic evaluation of *C. quadrangularis* extracts, an attempt to provide a direction for further studies.

MATERIALS AND METHODS

Plant collection and identification

The aerial parts of *Cissus quadrangularis* was collected from Melpalai, a small village located in the Devicode Panchayath of *Melpuram Block in Kaniyakumari District* of Tamil Nadu State. Identification and authentication of the collected plant material was done by Dr. S. Sukumaran, Associate Professor, Dept. of Botany & Research Centre and Dr. G. Johnsi Christobel, Head., Dept. of Botany & Research Centre, Nesamony Memorial Christian College, Marthandam, Kanyakumari District-629 165, Tamil Nadu.

Preparation of powdered material and extraction

Powdering and extraction of collected plant material was done in reference with the previous literature.[10-15] The aerial part of the collected plant was dried in shade for about three weeks, powdered the dried material by using mechanical grinder and stored in the airtight container for further studies. Initially, the powdered material was extracted with the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol in the soxhlet apparatus assembly. For that, about 50g of dried coarse powder was weighed, moistened with the selected solvents, packed in the extracting apparatus and extracted with 500ml of each solvent individually. After each extraction, subsequent extraction was done by using the same dried marc. Each extract was filtered, distilled off the solvent to obtain the dried extract. The percentage yield of each dried extracts obtained was calculated.

Preliminary phytochemical screening

The preliminary phytochemical screening (test for alkaloids, glycosides, phenolic compounds and tannins, flavonones and flavonoids, carbohydrates, proteins and aminoacids, terpenoids, saponins, gum and mucilage and volatile oil) of the collected extracts were carried out in reference with the standard procedure.[10-14]

In vitro anti-inflammatory activity

Anti-inflammatory activity of all the extracts was evaluated by different approaches viz., inhibition of protein denaturation, proteinase inhibitory activity. Standard procedures [16-18] were utilized with suitable modifications in all the methods of evaluation. Experiments were performed in triplicate.

Inhibition of protein denaturation

Reaction mixture (0.5ml) was prepared using bovine serum albumin (0.45ml; 5% aq. solution) and plant extracts (0.05ml) in different concentration (50, 100, 150, 200µg/ml). pH of the reaction mixture was adjusted to 6.3 using 1N HCl. Distilled water was used as normal control and ibuprofen was employed as standard control. The reaction mixture was incubated at 37°C for 20min and then heated to 57°C for 20min. After cooling, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube and the absorbance was measured spectrophotometrically (660 nm). The percentage inhibition of protein denaturation was calculated by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

The concentration (µg/ml) of the drug required to denature 50% protein was calculated from the graph.

Proteinase inhibitory activity

The reaction mixture (2ml) composed of 0.06mg trypsin, 1ml of 20 mM Trypsin HCl buffer (pH 7.4) and 1 ml test sample of different concentration (50, 100, 150 and 200µg/ml) was incubated at 37°C for 5min and then 1ml of 0.8%w/v casein was added. The mixture was again incubated for additional 20min. 2ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer blank. Ibuprofen was used as standard control. The percentage inhibition of proteinase inhibitory activity was calculated by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

The concentration (µg/ml) of the drug required to denature 50% proteinase (IC_{50} value) was calculated from the graph.

In vitro anti-diabetic activity

Inhibition of α–amylase activity

Evaluation of α–amylase inhibition activity of *C. quadrangularis* extracts was done in accordance with the previous literature[19,20] with slight modification. Starch solution (0.5% w/v) used as the substrate in this evaluation was prepared by dissolving potato starch in 20mM phosphate-buffered saline (pH 6.9) and kept this mixture in the boiling water bath for 15min. to get a clear solution. The enzyme solution used in the evaluation was prepared by dissolving porcine pancreatic α-amylase in 20mM phosphate-buffered saline (pH 6.9) to obtain the concentration of 1U/ml. The test extracts (10–50µg/ml) and the standard (acarbose) solution (100-1000µg/ml) was prepared in dimethyl sulfoxide (DMSO) solvent. DNS solution was used as the colour reagent ((20ml 96mM 3,5-dinitro salicylic acid, 12gm sodium potassium tartrate in 8ml of 2M NaOH and 12ml de-ionized water).

Three sets, control, positive control, and test were prepared. In case of test sample preparation, a mixture of 1ml each of test extract and enzyme solution was incubated at 25°C for 30min. To this mixture, 1ml starch solution was added and again incubated at 25°C for 20min. The reaction was stopped by adding DNS solution (1ml) and kept in the boiling water bath for

15min. Cooled and the final volume was made with distilled water and the absorbance was measured at 540nm. In case of control, the test extract was replaced with 1ml of DMSO. Acarbose was used as a positive control. Percentage inhibition and half-maximal inhibitory concentration (IC_{50}) scores were calculated. Percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} \times 100$$

Abs C – absorbance of the control; Abs S – absorbance of the sample (test extract/standard).

Inhibition of α-glucosidase activity

α-Glucosidase inhibitory activity of *C. quadrangularis* extracts was evaluated as per the previous literature [20-22] with slight modification. Three sets, control, positive control, and test were prepared in DMSO. Control tube contains DMSO, enzyme and substrate. In positive control, 100µl of Acarbose (0.1-3.2µg/ml) was used instead of test extracts.

100µl of test extracts (25-400µg/ml) in DMSO were pre-incubated at 37°C for 20min with 20µl of α–glucosidase (maltase) enzyme. Then the reaction was started with the addition of 50µl of the substrate solution [p-nitrophenyl glucopyranoside (pNPG) in 20mM phosphate buffer (pH 6.9)] to the mixture and again incubated at 37°C for 20min. and the reaction was stopped by the addition of 50µl of 0.1M Na_2CO_3. The *α*-glucosidase activity was determined by measuring the yellow coloured p-nitrophenol released from pNPG at 405nm. Percentage inhibition was calculated by using the formula previously employed in the evaluation of α–amylase inhibition activity. IC_{50} values were determined graphically from the plots of percentage inhibition *Vs* log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

RESULTS AND DISCUSSION

Processing and extraction of plant material

The aerial parts of *C. quadrangularis* was collected, dried in shade, made into coarse powder, extracted by soxhlation with solvents of increasing polarity viz., Petroleum ether, Chloroform, Ethyl acetate and Methanol. In the analysis of colour, consistency and percentage yield of extracts, it was found that all the extracts were thick solid mass in consistency, in case of colour, all the extracts were brownish green in colour. The percentage yield analysis revealed that the petroleum ether extract gave 5.27%w/w, the chloroform extract gave 7.30%w/w, ethyl acetate and methanol extract gave 10.50 and 12.40%w/w of dried extract respectively (Table 1).

Table 1: Percentage yield of the extracts of *C. quadrangularis.*

Extract	Extraction method	Colour of the dried extract	Physical nature of dried extract	% yield (%w/w)
Petroleum ether	Soxhlation	Brownish green	Thick solid mass	5.27
Chloroform		Brownish green	Thick solid mass	7.30
Ethyl acetate		Brownish green	Thick solid mass	10.50
Methanol		Brownish green	Thick solid mass	12.40

Phytochemical evaluation

In the preliminary phytochemical evaluation, presence of alkaloids, phenolic compounds, flavanones and flavonoids, carbohydrates, proteins, terpenoids, sterols, saponins and gum and mucilage were found in the tested extracts. All the tested extracts showed negative results in the tests for glycosides and volatile oil. Alkaloids was found in all the tested extracts notably, ethyl acetate and methanol extracts showed its significant presence. A similar results was found in the test for phenolic compounds and saponins. In case of flavanones and flavonoids, terpenoids and sterols, the chloroform, ethyl acetate and methanol extracts showed its significant presence. All the tested extracts showed positive results for the test for carbohydrates and proteins (Table 2).

Table 2: Preliminary phytochemical evaluation of *C. quadrangularis* extracts.

S. No.	Chemical Test	I	II	III	IV
1	**Alkaloids**				
a	Mayer's test	+	+	++	++
b	Wagner's test	+	+	++	++
c	Hager's test	+	+	++	++
d	Dragendorff's test	+	+	++	++
2	**Glycosides**				
a	Legal test	-	-	-	-
b	Baljet test	-	-	-	-
c	Borntrager's test	-	-	-	-
d	Modified Borntrager's test	-	-	-	-
3	**Phenolic compounds**				
a	Ferric chloride test	+	+	++	++
b	Lead acetate test	+	+	++	++

c	Gelatin test	+	+	++	++
4	**Flavanones and flavonoids**				
a	Aqueous NaoH test	+	++	++	++
b	Ammonia test	+	++	++	++
c	Shinoda test	+	++	++	++
5	**Carbohydrates**				
a	Molisch's test	+	+	+	+
b	Fehling's test	+	+	+	+
c	Benedict's test	+	+	+	+
6	**Proteins and Amino acids**				
a	Millon's test	+	+	+	+
b	Biuret test	+	+	+	+
c	Ninhydrin test	+	+	+	+
7	**Terpenoids**				
a	Salkowski test	+	++	++	++
8	**Sterols**				
a	Libermann-Burchard test	+	++	++	++
b	Salkowski test	+	++	++	++
9	**Saponins**				
a	Foams test/froth test	+	+	++	++
b	Haemolysis test	+	+	++	++
10	**Gum & mucilage**	+	+	+	+
11	**Volatile oil**	-	-	-	-

I – Petroleum ether extract; **II** – Chloroform extract; **III** – Ethyl acetate extract; **IV** – Methanol extract; (+) – Positive result; (++) – Significant positive result; (-) – Negative result

In the present study, phytochemicals such as alkaloids, phenolic compounds, flavanones and flavonoids, terpenoids, sterols, saponins were found in the tested extracts. Previous literature[23] stated that alkaloids are a huge class of natural occurring organic molecules, which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. Alkaloids are extremely divergent chemical structures including heterocyclic ring systems and encompass more than 20,000 different molecules in organisms. They are present not only in human daily life, in food and drinks but also as stimulant drugs, medicines, narcotics insecticides and in many physiological activities. They showed strong biological effects on animal and human organisms even in very small doses. Alkaloids show several pharmacological activities on human health such as anti-cancer, anti-inflammatory, Anti-malarial, Anti-microbial, Anti-hypertensive, Anti-diabetic, Anti-oxidant. Alkaloids directly act on the central nervous system in the human body and also affect nucleic acid, DNA (Deoxy Ribonucleic acid), RNA (Ribonucleic acid), membrane permeability and proteins.

Phytosterols have been proposed to exert a wide number of pharmacological properties, including the potential to reduce total and low-density lipoprotein (LDL) cholesterol levels and thereby decreasing the risk of cardiovascular diseases. Other health-promoting effects of phytosterols include anti-obesity, anti-diabetic, anti-microbial, anti-inflammatory, and immunomodulatory effects. Also, anticancer effects have been strongly suggested, as phytosterol-rich diets may reduce the risk of cancer by 20%.[24]

Terpenoids, the most abundant compounds in natural products, are a set of important secondary metabolites in plants with diverse structures. Terpenoids play key roles in plant growth and development, response to the environment, and physiological processes. As raw materials, terpenoids were also widely used in pharmaceuticals, food, and cosmetics industries. Terpenoids possess antitumor, anti-inflammatory, antibacterial, antiviral, antimalarial effects, promote transdermal absorption, prevent and treat cardiovascular diseases, and have hypoglycemic activities. In addition, previous studies have also found that terpenoids have many potential applications, such as insect resistance, immunoregulation, antioxidation, antiaging, and neuroprotection. Terpenoids have a complex structure with diverse effects and different mechanisms of action.[25]

Saponin isolated from medicinal plants is a naturally occurring bioorganic molecule with high molecular weight and its aglycone (water non-soluble part) nucleus having 27 to 30 carbon atoms besides one or two sugar moieties (water soluble part) containing at least 6 or 12 carbon atoms respectively. The complexity of saponin chemistry maybe considered as a gap for many scientists and researchers to understand the relationship between the chemical structure and its medical or pharmaceutical behaviour. Recently, the increase in demand of saponin applications was observed due to various biological, medicinal, and pharmaceutical actions. Literature shows that saponins exhibit a biological role and medicinal properties such as hemolytic factor anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal,

anticancer, cytotoxic and molluscicidal action. In addition, saponins are reported to exhibit cholesterol-lowering action in animals and human.[26]

Flavonoids are phytochemical compounds present in many plants, fruits, vegetables, and leaves, with potential applications in medicinal chemistry. Flavonoids possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects. These biological activities depend upon the type of flavonoid, its (possible) mode of action, and its bioavailability. These cost-effective medicinal components have significant biological activities, and their effectiveness has been proved for a variety of diseases.[27] Phenolic phytochemicals play a variety of protective roles against abiotic stresses, such as UV light, or abiotic stresses, namely predator and pathogen attacks. Phenolic phytochemicals are utilized by humans to treat several ailments including bacterial, protozoal, fungal, and viral infections, inflammation, diabetes, and cancer.[28]

***In vitro* anti-inflammatory activity**

In vitro anti-inflammatory evaluation was done by inhibition of protein denaturation and proteinase inhibitory activity method. In the inhibition of protein denaturation assay, the results showed a concentration-dependent rise of activity. Among all the tested extracts, the methanol extract (200µg/ml) showed maximum percentage inhibition of 77.20±1.05, followed by the ethyl acetate extract in the same concentration revealed percentage inhibition of 67.20±1.30 (Table 3). Proteinase inhibitory activity, another one method employed for the evaluation also showed a concentration-dependent rise of activity. In this method also, the methanol extract in 200µg/ml concentration showed the maximum percentage inhibition of 70.20±0.55, followed by the ethyl acetate extract in the same concentration showed 64.05±0.27 as percentage inhibition (Table 4).

Table 3: *In vitro* anti-inflammatory evaluation by protein denaturation inhibition method.

Conc. (µg/ml)	% inhibition				
	Std. (Ibu.)	Test extracts			
		1	2	3	4
50	52.14±0.57	23.10±0.51	27.60±0.45	33.79±1.20	45.03±1.21
100	65.75±0.26	34.50±0.24	39.11±0.56	47.14±1.03	58.18±0.85
150	73.19±0.38	41.25±0.30	47.35±0.20	59.18±1.10	65.40±0.10
200	81.10±2.35	53.41±1.09	55.24±1.55	67.20±1.30	77.20±1.05
IC_{50}	59.27	225.17	165.30	136.40	107.15

Table 4: *In vitro* anti-inflammatory evaluation by proteinase inhibitory activity.

Conc. (µg/ml)	% inhibition				
	Std. (Ibuprofen)	Test extracts			
		1	2	3	4
50	47.20±0.35	15.36±0.80	19.50±0.20	28.17±0.52	37.19±0.29
100	55.27±0.27	23.62±0.25	27.49±0.50	42.33±0.34	46.39±0.40
150	68.77±0.25	31.49±0.31	48.26±0.30	55.60±0.10	59.01±0.39
200	79.13±0.40	43.15±0.09	59.14±0.55	64.05±0.27	70.20±0.55
IC_{50}	75.31	219.70	149.65	120.11	105.20

Conc.-Concentration; Std.-Standard; 1-Petroleum ether; 2-Chloroform; 3-Ethyl acetate; 4-Methanol

***In vitro* anti-diabetic evaluation**

The *in vitro* anti-diabetic activity of petroleum ether, chloroform, ethyl acetate, and methanol extracts of the whole plant *C. quadrangularis* was evaluated by the inhibition of α–amylase and α–glucosidase activity. The results of α–amylase inhibitory evaluation of test extracts and the standard, acarbose are shown in Table 5. The results clearly indicated that a significant α–amylase inhibition was produced by methanol and ethyl acetate extracts comparing with the standard drug acarbose, particularly, these extracts showed a significant dose-dependent rise in percentage inhibitory activity against α–amylase enzyme comparing with other tested extracts with IC_{50} value 121.50µg/ml and 110.81µg/ml respectively.

Table 5: α–amylase inhibitory activity of test extracts and standard drug.

Conc. (µg/ml)		% inhibition of α–amylase activity				
		Std.	Test extracts			
Std	Test	Acarbose	Pet. ether	Chloroform	Et. acetate	Methanol
100	10	20.22±0.47	4.25±0.23	7.27±0.76	9.91±0.18	11.90±0.52
200	20	51.40±1.00	13.70±0.71	23.56±0.63	25.60±0.32	26.28±1.32
400	30	72.13±1.65	29.21±0.44	37.80±0.56	39.07±0.67	42.57±1.40
800	40	81.60±2.30	49.32±0.17	52.70±0.10	57.78±0.29	59.72±2.27

1000	50	95.12±2.20	61.08±0.23	61.58±1.27	69.60±1.53	79.35±2.73
IC_{50}		92.57	142.57	133.12	121.50	110.81

The results of α–glucosidase inhibition assay are shown in Table 6. From the results, it was found that the methanol and ethyl acetate extracts showed a significant activity comparing with the standard drug, acarbose. Similar with α–amylase inhibition assay, these extracts showed a significant dose-dependent rise in percentage inhibitory activity against α–glucosidase enzyme comparing with other tested extracts with IC_{50} value 137.27µg/ml and 239.49µg/ml.

Table 6: Effect of test extracts and standard on α–glucosidase inhibition assay.

Conc. (µg/ml)		Std.	% inhibition of α–glucosidase activity			
			Test extracts			
Std.	**Test**	**Acarbose**	**Pet. ether**	**Chloroform**	**Et. acetate**	**Methanol**
0.1	25	15.32±0.55	3.43±0.74	5.08±0.52	6.88±0.70	9.24±0.14
0.2	50	38.47±1.35	11.51 ±0.15	17.55±0.43	19.48±0.55	21.50±0.55
0.4	100	52.78±2.85	23.63±0.79	29.10±0.22	33.11±0.60	39.53±1.31
0.8	200	75.20±2.52	42.13±0.11	45.71±0.53	51.60±0.38	59.32±1.20
1.6	400	95.18±2.49	59.07±0.27	61.80±0.27	72.51±0.29	85.11±2.31
IC_{50}		119.20	281.41	253.80	239.49	137.27

CONCLUSION

The phytochemicals identified in the preliminary phytochemical assessment of this study may be accountable for the biological activities demonstrated by the extracts. In conclusion, this research indicated that the solvent methanol produced remarkable outcomes, as shown from the beginning, including extraction yields and the diverse pharmacological activities evaluated. In addition to the methanol extract, the ethyl acetate extract also showed significant results across all previously mentioned criteria. Future studies focusing on in vivo research may provide even more impactful findings.

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