ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
ISSN: 0970-020 X
CODEN: OJCHEG
2025, Vol. 41, No.(4):
Pg. 1349-1355

Exploring the Phytochemicals and Anti-diabetic Composition of *Capparis grandis* L.f leaf extracts: An *in-vitro* study

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http://dx.doi.org/10.13005/ojc/410433

(Received: April 16, 2025; Accepted: July 17, 2025)

ABSTRACT

Plants have been used as medicine for centuries, with over eighty percent of the global community, especially in less-developed countries, relying plant-based remedies. *C. grandis* L.f belongs to the family Capparaceae (Caper family). It is used in herbal medicines like Siddha, Ayurveda and Unani in curing number of ailments. This work is undertaken to find the phytochemical compounds existence and anti-diabetic properties in the ethyl acetate, ethanol, aqueous leaf extracts of *C. grandis* using conventional phytochemical tests and anti-diabetic enzyme inhibition assays accordingly. Test results confirm the existence of carbohydrates, phenolic compounds, proteins, cardiac glycosides, glycosides, flavonoids, amino acid, tannins, alkaloids, saponins and phytosterols for qualitative phytochemical examination and anti-diabetic activity evaluation results proves that the ethanolic extract has the most significant enzyme inhibition. This underscores the promising potential of this species for the discovery and development of novel natural drugs.

Keywords: *Capparis grandis*, Qualitative Phytochemical Compounds, Anti-diabetic Activity, Enzyme inhibition.

INTRODUCTION

Medicinally valuable plants are the hub for medicines that are utilized for treatment of numerous illnesses, particularly in the folk system of therapy. Plant parts like fruits, leaves, flowers, stem, gums, resins, etc. are key tools of usage for a disease. These medicinally important plants own specific biochemically active compounds in its various part, and this in turn provide unique physiological action in the body of the human during therapy[1]. Plant derived biologically active chemical compounds from plants are referred to as phytochemicals. They are protective in nature, it is obtained from different resources like nuts, fruits, herbs, vegetables, and whole grains where, above 1000 phytochemical constituents were identified up to date[2].

Medicinal plants contain natural products with biologically active matter, including alkaloids, tannins, terpenoids, flavonoids, carbohydrates, and steroids. The above-mentioned compounds exhibit distinct physiological effects on the human body. They are synthesized through primary or secondary plant metabolism. Secondary metabolites are remarkably diverse, with unclear functions, yet

they play a vital role in various other fields which includes treatments, research works, agriculture, animal medicine[3] etc.

For quality assurance of indigenous medicine, the folk methods are put together and investigated. Ancient records and historical knowledge about the specification and quality analysis of the medicine are later inquired and re-examined by utilizing current scientific analysis techniques[4]. Conducting preliminary phytochemical screenings of plants is essential for discovering and developing new therapeutic agents with enhanced effectiveness. Many research groups globally have reported similar studies[5].

The initiation of novel healing agents comes from plant sources. Herbs are the suppliers of life-restoring medicines utilized in the collection of biomedicines. Among various plant resources, only a few species are being analysed for their medicinal efficacy. So, planned and well-equipped methodologies are needed to discover herbal potential properties[6].

The synonym 'diabetes' is attained out of the Greek synonym 'Diab'- To pass through, which denotes recurrent urination and thirst. 'Mellitus' is acquired out of Latin synonym which means 'sweetened by honey', as it indicates existence of sugars in urine. Herbal plants possess naturally available antioxidants like vitamins C and E, Tannins, Flavonoids, etc, which can protect pancreatic beta cell function and can stop diabetes instigated ROS (Reactive Oxygen Species) emergence[7].

Type-1 DM (Diabetes mellitus) is a severe auto-immune condition accompanied by a rise in blood glucose intensity known as hyperglycaemia that happens because of a deficiency in insulin due to the deficit in pancreatic islet beta cells. This T-1 DM commonly falls during childhood as it is a result of metabolism and endocrine-related conditions[8]. In 2nd. Type DM metabolism of carbohydrates is notable by various malfunctions in metabolism, like insulin aversion in the bony muscles, defective function of pancreatic-beta cells liver and adipose tissue. DM is a chief root of death worldwide, as it creates considerable health issues for mankind. Herbs mark history in utilization as an anti-hyperglycemic agent. They are loaded with phytoconstituents that behave like an antioxidant. An active ingredient discovered from herbs behaves as an impactful anti-diabetic representative, supposing that the particular plant is owned with anti-hyper glycemic and agents of antioxidants[9].

Cleome droserifolia (Forssk.) Del. belonging to the Capparidaceae family, has a noticeable power over increasing peripheral glycemia, which is observed in rats (before and after glucose intake). The plant extracts have shown significant liver glucose output levels[10]. *C. grandis*-small tree, is commonly utilized in folk medicine systems such as Siddha, Unani, and Ayurveda. It relives ailments since the olden days. Leaves, flowers, and Roots of *C. grandis* are frequently used to remedy several health conditions, e.g. leaf infusion is consumed to address eruptions and swellings. Ethnic people have also used *C. grandis* to treat burns, asthma, and wounds, also used to improve blood health. Additionally, the crushed fresh leaves are applied as a pulp to cure insect bites[11]. Its stem bark is used to cure ailments namely, sterility and paralysis[12].

The objective of this work was an exploration of qualitative phytochemical and *in vitro* anti-diabetic activity studied using the leaf extracts of *Capparis grandis* L.F plant, by identifying the phytochemicals present in the solvent extracts and evaluating their potential antidiabetic effects. This underscores the promising potential of this species for the discovery and development of novel natural drugs.

MATERIALS AND METHODS

Plant material acquisition and taxonomic spotting

Fresh plant matter was gathered from Sanamavu Reserve Forest, Krishnagiri (DT), Tamilnadu, India. Descriptive identification of sample plant is verified by BSI, Agricultural University Campus, Coimbatore, Tamilnadu, India.

Fig. 1. *Capparis grandis* L. f

Sample plant matter was cleansed below running faucet water in order to detach it from External contaminants from the leaves of the plants were separated. Then separated part (Leaves) was air parched in the shaded environment to avoid chemical degradation because of sunlight. The parched leaf matter is finely powdered, stored in airtight container and utilized in the further analysis[13].

Plant material extraction

Finely powdered leaf matter was taken (70 g) and packed in smaller thimbles, and extracted in Soxhlet apparatus using 500 mL of polarity vice solvents including ethanol, ethyl acetate, and aqueous (water).

Before extracting along the subsequent solvents, thimble is allowed to parch each time. At last, the water extract was filtered after the sample was macerated for 24 h in hot water while being stirred constantly. After being concentrated utilizing rotary vacuum vaporization, various solvent extracts are parched. Weighing was done on each solvent's dry extract.

Each solvent's air-dried weight of extract was used to calculate the yield percentage. The extracted extract's stock (1 mg/mL of the relevant solvents) was made for additional testing[14].

After successive extractions, amount of crude extract recovered are weighed and yield percent is measured using the formula below,

$$\text{Extract revovery percent} = \frac{\text{Amount of extract recovered (g) x 100}}{\text{Amout of plant sample (g)}}$$

Phytoconstituent profiling tests for crude extracts

The plant leaf extracts are tested for the existence of significant phytochemical constituents like amino acids, proteins, carbohydrates, tannins, alkaloids, phenolic compounds, saponins, glycosides, flavonoids, phytosterols, and cardiac glycosides according to standard methods[15].

Detection of Carbohydrates[16]

Molish's test

Plant crude extract is mixed with 5 mL of H_2O and purified. 2 drops-alcoholic solution of alpha naphthol is added to 2 mL of the purified sample and 1 mL of Conc. sulphuric acid is slowly mixed down the sides of test tube and let to stand. A form of violet ring appearance detects carbohydrates existence.

Detection of Proteins16

Biuret test

Plant crude extract is liquefied within 10 mL of distilled H_2O and then purified using Whatman No. 1 (filter paper). To 2 mL of purified sample, 1 drop - 2% copper sulphate solution was added. To it, 1 mL of 95% ethanol is added, after that excess of potassium hydroxide pellets. Formation of Purple colour within the ethanol layer detects proteins existence.

Detection of Amino acids[16]

Ninhydrin test

2 drop–Ninhydrin solution that is 10 mg of ninhydrin in 200 mL of acetone is mixed to 2 mL of aqueous filtrate. Purple colour detects amino acids existence.

Detection of Alkaloids[16]

Hager's test

Solvent free plant crude extract is mixed in 5 mL of dil. HCl and then purified. In the filtrate, two ml of Hager's reagent which is saturated aqueous solution of picric acid is mixed. Yellow prominent precipitate detects alkaloids presence.

Detection of Tannins17

Gelatin test

Plant extract is mixed with five mL dist. H_2O and 1% gelatine solution and 10% NaCl and wobbled. white precipitate detects tannin existence.

Detection of Saponins[16]

Frothing test

Plant crude extract is diluted with distilled H_2O and made up to 20 mL. The suspension is agitated within a graduated cylinder for fifteen minutes. 2 cm layer of foam formation detects saponins presence.

Detection of Phenolic compounds17

Ferric chloride test

Plant crude extract is mixed in 5 mL of distilled H_2O. Few drops–solution 5% neutral ferric chloride is then mixed in it. Green colour detects phenolic compounds presence.

Detection of Glycosides[16]

Borntrager's test

The crude plant extract is hydrolysed along Conc. hydrochloric acid about 2 h in water bath and then purified. 2 mL - filtered hydrolysed compound

is mixed with 3 mL of chloroform and agitated. The layer chloroform is separated and 10% ammonia solution is added in it. Pink colour formation detects glycosides presence.

Detection of Flavonoids17

Alkaline reagent test

Aqueous solution of plant crude extract is treated along 10% ammonium hydroxide solution. White bulky precipitate formation detects flavonoids existence.

Detection of Cardiac glycosides17

Keller Killiani test

The plant crude extract is dissolved with one mL glacial acetic acid holding a drop-5% of solution ferric chloride. One mL-Conc. Sulphuric acid is then supported. Brown ring at the boundary detects cardiac glycosides presence.

Detection of Phytosterols[16]

Libermann and Burchard's test

The plant crude extract, two mL of acetic anhydride is dissolved. Along the sides of the test tube 1 or 2 drops - Conc. Sulphuric acid is slowly left. Changes in colour, detects phytosterols presence.

***In vitro* evaluation of anti-diabetic properties**

Assessment of α-Glucosidase Inhibitory Assay

To assess the inhibitory efficacy of alpha-glucosidase, an *in vitro* inhibitory test was executed by utilizing the pNPG (p-nitrophenyl glucopyranoidase) method. 0.075 units of α-glucosidase are diluted with the leaf extracts of *C. grandis* varying in 50-200 µg/mL concentrations. Substrate- pNPG is mixed with the reaction mixture to begin the reaction process. Later, the reaction mixture is placed at 37°C incubation for about 30 minutes. This process is paused by mixing two ml of Sodium carbonate. Hence, the alpha-glucosidase efficacy is calculated by determining the set free amount of pNPG substrate at the absorbance of 400 nm by using the Jenway Genova Nano Spectrophotometer. Acarbose is substituted as standard. The level of alpha-glucosidase inhibitor to restrict fifty percent of the function undergoing the test state defines the IC_{50} (Inhibitory concentration) value[18].

Assessment of α-Amylase Inhibitory Assay

To assess the inhibitory efficacy of alpha-amylase, an *in vitro* inhibitory test was executed by utilizing the DNS (dinitrosalicylic acid) method. Alpha-amylase is diluted in the leaf extracts of *C. grandis* varying in 50-200 µg/mL concentrations. A 0.5 percent starch solution as substrate is mixed to begin the reaction process. Later, the reaction mixture is placed at 37°C incubation for about ten mins. This process is paused by mixing two mL of 3,5-dinitrosalicylic acid- DNS reagent. The mixture of reaction is made warm for about fifteen minutes at 100°C, then dissolved with ten ml of distilled H_2O and kept in an ice bath. Hence, the alpha-amylase efficacy is calculated by determining the absorbance in the Jenway Genova Nano Spectrophotometer at 540 nm. Acarbose is substituted as standard. The level of alpha-amylase inhibitor to restrict fifty percent of the function undergoing the test state defines the IC_{50} (Inhibitory concentration) value[19].

RESULTS

Qualitative phytochemical screening

Table 1: Percentage yield of *Capparis grandis* L.f leaf extracts

Sr. No	Solvent	Yield Percentage Leaf
1	Ethyl acetate	0.869
2	Ethanol	1.95
3	Aqueous	1.24

Table 2: Qualitative phytochemical screening of *Capparis grandis* L. f leaf extracts

Phytochemical compounds	Phytochemical tests	Solvent Extracts Ethyl acetate	Ethanol	Aqueous
Carbohydrates	Molish'stest	✓✓✓	✓✓✓	✓
Proteins	Biuret test	✓	✓✓	✓
Amino acids	Ninhydrin test	✓✓✓	✓✓	x
Alkaloids	Hager's test	✓✓	✓	x
Tannins	Potassium hydroxide test	✓✓	✓✓	x
Saponins	Frothing test	✓✓	✓✓	x
Phenolic compounds	Ferric chloride test	✓✓	✓✓✓	✓
Glycosides	Borntrager's test	✓	✓	✓
Flavonoids	Alkaline reagent test	✓✓✓	✓✓	x
Cardiac glycosides	Keller Killiani test	✓✓	✓✓	✓
Phytosterols	Libermann-Burchard's test	✓	✓✓	x

(✓)-Existence of Phytochemical, (x) - No Phytochemical, (✓)<(✓✓)<(✓✓✓)-Depending on the intensity of the colour

The effects of solvents on the extraction yield of *C. grandis* L.f leaves were tested using organic solvents like (ethanol, and ethyl acetate), and aqueous (water) based on the polarity. Amid the solvents analysed, ethanol extraction yield was high (1.95%), then aqueous extraction (1.24%), and finally ethyl acetate (0.869%) as listed in Table 1. This results that the extraction percentage supports mid-polar solvents[20].

Ethyl acetate and ethanol extract shows the existence of primary metabolite and secondary metabolite phytochemical constituents including carbohydrates, alkaloids, proteins, amino acids, tannins, saponins, phenolic compounds, flavonoids, phytosterols, cardiac glycosides, and glycosides ranging from strong to moderate. But the aqueous (water) extract indicated the presence of proteins, phenolic compounds, carbohydrates, cardiac glycosides, and glycosides respectively[21], as listed in Table 2.

In vitro evaluation of anti-diabetic properties

Table 3: Results of α-Glucosidase Inhibition Assay

Samples	Extracts	α-glucosidase IC_{50} (µg/mL)
Capparis grandis leaf	Ethyl acetate	75.54
	Ethanol	59.53
	Aqueous	78.57
Standard	Acarbose	23.22

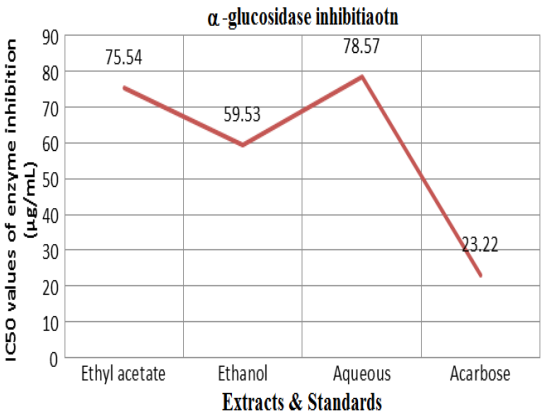

Fig. 2. Inhibition test for α-glucosidase activity

Table 4: Results of α-amylase Inhibition Assay

Samples	Extracts	α-amylase IC_{50} (µg/mL)
Capparis grandis leaf	Ethyl acetate	109.13
	Ethanol	79.64
	Aqueous	88.54
Standard	Acarbose	25.27

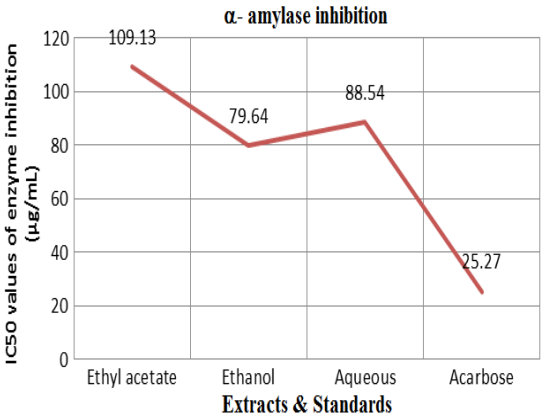

Fig. 3. Inhibition test for α-amylase activity

Starch supplies vital energy that is obtained from the eating process of humans. Starch and sugar are split into Glucose by the enzymes involved like, alpha-amylase and alpha-glucosidase. Anti-diabetic inhibitors like alpha-glucosidase and alpha-amylase should lower the hyperglycemia of postprandial. Commonly used drugs, namely voglibose, Miglitol and Acarbose, are effective and also show side effects, which include gastrointestinal problems like flatulence, diarrhea and bloating. So, the search continues to make new alpha-amylase and alpha-glucosidase inhibitor drugs that balance the T2D from PPHG with minimal risks[23]. In this research work, ethanol, ethyl acetate and aqueous leaf crude extracts of *C. grandis* (CGL) were tested for their efficacy on alpha-glucosidase and alpha-amylase action.

It manifested that CGL ethanol extracts notably act as an inhibitor against anti-diabetic enzymes. (Fig. 1) and (Table 3) shows the IC_{50} values of enzyme inhibition (µg/mL) of alpha-glucosidase where, the inhibitory effect of ethyl acetate extract (75.54), ethanol extract (59.53), aqueous extract (78.57), and the standard acarbose exhibits (23.22) at a concentration of 50-200 (µg/ml) respectively.

Similarly, (Fig. 2) and (Table 4) shows the IC_{50} values of enzyme inhibition (µg/mL) of alpha-amylase where, the inhibitory effect of ethyl acetate extract (109.13), ethanol extract (79.64), aqueous extract (88.54), and the standard acarbose exhibits (25.27) with a level of 50-200 (µg/ml) respectively.

DISCUSSION

Leaf extracts were exposed to different qualitative assays to test for the existence of primary

metabolite and secondary metabolite phytochemical constituents including carbohydrates, alkaloids, proteins, amino acids, tannins, saponins, phenolic compounds, flavonoids, phytosterols, cardiac glycosides, and glycosides. ethyl acetate and ethanol extract show the existence of mentioned phytoconstituents ranging from strong to moderate. But the aqueous (water) extract indicated the presence of proteins, phenolic compounds, and carbohydrates, cardiac glycosides, and glycosides respectively[21], as listed in Table 2. So, the leaf extracts of *C. grandis* which are employed in screening of primary phytochemical constituents show that the plant species is rich in plant phytoconstituents[22].

The test animals-mice dealt with the acarbose drug diminished the breakdown of starch and sucrose[24]. Therefore, the inhibitory efficacy of CGL ethanol extracts on alpha-glucosidase and alpha-amylase activity results may provide in developing drugs that are effective and have minimal risks.

Phytochemical analysis of the *C. sepiaria* leaf extract showed the existence of the bioactive compounds; flavonoids, tannins, alkaloids, saponins, steroids, and glycosides. These compounds are recognized for their function in regulating carbohydrate metabolism, stimulation of insulin secretion and antioxidant effects, which together play an important part in diabetes management[25].

CONCLUSION

The preliminary phytochemical profile testing of *C. grandis* leaf extracts it is summarized that, the solvent extraction yield of *C. grandis* was higher in ethanol when compared with aqueous, and ethyl acetate solvents, and it has phenolic compounds, carbohydrates, cardiac glycosides, glycosides, amino acid, proteins, flavonoids, tannins, alkaloids, phytosterols, and saponins, with strong to moderate presence and completely absent nature too in the plant crude extract used, these phytoconstituents pave way for developing novel drugs to address ailments.

Therapeutic plants contain numerous amounts of biologically active compounds that control as well as treat end number of diseases, and the cure for diabetes is also one among them. This research work provides evidence where the *C. grandis* leaf (CGL) extracts are investigated for inhibiting diabetes causing enzymes, The obtained results convey that CGL ethanolic extracts contain promising and unique anti-diabetic agents that suppress the starch and sugar enzymes indigestion. Even though commercial medicines like voglibose, Miglitol, and Acarbose are effective than CGL ethanol extracts in treating diabetes, the CGL ethanol extracts might be beneficial in a way that can cure or control diabetes mellitus in a risk-free predicament.

ACKNOWLEDGEMENT

The authors are thankful to the Management, Nirmala College for Women, for providing Support.

Conflict of interest

The authors declare that they have no conflict of interest.

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