Research Article

In vitro Alpha Glucosidase Inhibitory Activity and GC-MS Analysis of *Capparis Zeylanica* Linn

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ABSTRACT

The present work intended to analyze the inhibition of α-Glucosidase using hexane, ethyl acetate, methanol and aqueous extracts of *Capparis zeylanica*. *In vitro* study of α-glucosidase inhibitory activity was carried out using *Capparis zeylanica* leaf extracts. Methanol extract inhibited α-glucosidase (32.92±0.52) at 1000µg/ml, followed by aqueous extract (30.67±0.69). On performing the various analytical techniques, the results concluded that leaves have more or less similar phytoconstituents. In total, 4 compounds were identified in GC-MS analysis of methanol extract of *Capparis zeylanica.* The results of this study present the evidence that *Capparis zeylanica* given either systemically or topically has alpha glucosidase inhibition properties.

Keywords: Plant extracts, *Capparis zeylanica*, Alpha glucosidase inhibition, GC-MS.

INTRODUCTION

Nature has been a potential source of many therapeutic agents for thousands of years and an impressive number of modern drugs have been derived from natural resources, e.g. plants[1]. The combination of α-glucosidase inhibitors and antioxidants will become more effective for the prophylaxis of Type2 diabetes[2].

α-Glucosidase inhibitors (AGIs) offer an alternative; they are designed to specifically delay the digestion of complex carbohydrates, thus significantly reducing postprandial glycemic and insulinemic excursions[3]. Diabetes mellitus type II is a serious disease with rising prevalence. α-Glucosidase enzymes in the intestinal lumen and in the brush border membrane play important role in carbohydrate digestion to degrade starch and oligosaccharides convert them to monosaccharides before they can be absorbed. Natural inhibitors from dietary plants have lower inhibitory activity against α-amylase and a stronger inhibitory activity against α-glucosidase and can be used as effective therapy for postprandial hyperglycemia with minimal side effects[4]. An effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering great potential for discovery of new antidiabetic drugs[6-8].

Capparis zeylanica, Linn belongs to the family Capparidaceae. It is commonly known as the Indian caper, is a climbing shrub found throughout India and has been used as a 'Rasayana' drug in the traditional medicine. Modern phytochemical screening of the plant has shown the presence of fatty acids[8] and flavonoids in the leaves[9]. Flavonoids have been known to possess antioxidant, antineoplastic, antiulcer, anti-inflammatory and antimicrobial activities[10, 11]. The present study is to investigate its α-Glucosidase properties in detail using various *In vitro* studies.

MATERIALS AND METHODS

Plant material

Leaves of healthy *Capparis zeylanica* plants were collected from the botanical garden of MC College campus, Chennai, South India. The species was identified and authenticated by Dr. Narasiman. A, Head, Department of Plant Biotechnology and Biotechnology, MC College, Chennai. The freshly collected healthy plant leaves were washed thoroughly, shade dried in open air and grounded into powder.

Crude extract preparation

The leaf powder (200g) was soaked serially in hexane, ethyl acetate, methanol and water in the ratio 1:3 for 72 hrs respectively with intermittent shaking. After 72 hrs, the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The filtrate was air dried to yield 9g of hexane extract, 12g of ethyl acetate extract, 18g of methanol extract and 8g of aqueous extract and stored at 4ºC in air tight containers until the assay.

Chemicals and reagents

Maleate buffer (100mM, pH 6.0), Ice-cold PBS, Maltose 40mM (Substrate), Crude α-glucosidase enzyme, maleate buffer (100mM, pH 6.0), Phosphate buffer saline (PBS); pH 6.8.

Isolation of α-glucosidase crude enzyme

After fasting for 24hrs, anesthetize the rat with chloroform. The small intestine below duodenum and above cecum was cut, rinsed with ice cold saline, and homogenized with 12 ml of maleate buffer (100mM, pH

6.0). After centrifugation at 5000 rpm for 10min at 4ºC, the homogenate was used as the α-glucosidase solution.

In vitro α-glucosidase inhibitory assay

In order to investigate the inhibitory effect of the leaf extracts of *Capparis zeylanica*, an *in vitro* α-glucosidase inhibition test was performed. α-Glucosidase from yeast is used extensively as a screening material for α-glucosidase inhibitors, but the results do not always agree with those obtained in mammals. Therefore, we used the rat small intestine homogenate as α-glucosidase. The inhibitory effect was measured using the method slightly modified[12].

The assay mixture consisted of 100mM maleate buffer (pH 6.0), 2% (w/v) sugar substrate solution (100µl), and the test extracts (50-1000mg/ml). Acarbose was used as reference drug. The reaction mixture was preincubated for 5min at 37°C, and the reaction was initiated by adding α-glucosidase solution (50µl), followed by incubation for 10 min at 37°C. The glucose released in the reaction mixture was determined using Accuzyme, GOD-POD kit. OD was read at 505 nm. The rate of carbohydrate decomposition was calculated as percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

All the OD values must by divided by standard value and then multiplied by 100 which gives rise to glucose in (mg/dl)

$$\% \text{ Inhibition: } \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Gas chromatography-mass spectrometry analysis of plant extracts (GC-MS)

Since, methanol extract shows better α-glucosidase activity when compared to the other three extracts, GC/MS analysis of the methanol extract was performed using a Shimadzu GC/MS (GC-17A) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 mm ID, film thickness 0.25 µm). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. Injector and MS transfer line temperature were set at 260 and 320°C respectively. The oven temperature was programmed from 60-320°C at 3°C/min, increase then held isothermal for 11 min and finally raised to 320°C at 10°C/min. Diluted samples (1/100, v/v in methanol) of 1.0 µl were injected manually in the split less mode. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Mass spectrometer: Shimadzu GC/MS (GC-17A) system recording at 70 eV; scan time 1.5s; mass range 40–300 amu. Software adopted to handle mass spectra and chromatograms was a ChemStation.

RESULTS AND DISCUSSIONS

Inhibition of α-glucosidase inhibitory activity

The present study investigated, *in vitro* α-glucosidase inhibitory activity by using the four different leaf extracts of *Capparis zeylanica*. Methanol extract inhibited α-glucosidase (32.92±0.52) at 1000µg/ml, followed by aqueous extract (30.67±0.69). Hexane and ethyl acetate extracts were found to show moderate inhibition (Table 1). Acarbose was used as reference. The resulted values were expressed as Mean±Standard Error.

Our extracts showed promising results in the inhibition of α-glucosidase. Aqueous extract from the gall of *Rhus chinensis* (AEGRC) is an enzyme responsible for the digestion of carbohydrate to monosaccharides in the process of intestinal absorption, which inhibited *Bacillus* α-glucosidase activity with an IC_{50} of 0.9µg/ml[13]. Living organisms use enzyme inhibitors as a major tool to regulate glycolytic activities of alpha amylase. In most of the cases the mechanism of inhibition occurs through the direct blockage of the active center at several sub sites of the enzyme[14].

GC-MS

Four compounds were identified in methanol extract of *Capparis zeylanica*. The active principles with their retention time (RT) and percentage peak of the individual compounds were presented in the table 2.

Table 1: α- Glucosidase inhibitory activity of leaf extracts of *Capparis zeylanica*

Concentration (µg/ml)	α-glucosidase inhibitory activity				
	Hexane	Ethyl acetate	Methanol	Aqueous	Acarbose
50	5.02±0.52	3.63±0.69	2.71±1.04	12.13±0.52	74.56
100	6.58±0.69	9.01±0.52	2.94±0.35	13.51±0.17	81.28
200	7.10±0.62	9.70±0.17	3.46±0.69	14.03±0.34	88.01
400	7.27±0.86	11.26±0.62	4.50±0.17	19.41±0.86	91.81
500	7.97±0.17	11.78±0.35	4.62±0.26	23.05±0.34	94.15
1000	29.98±0.86	25.99±0.69	**32.92±0.52**	30.67±0.69	96.78

Values are expressed as Mean±S.E., *n* = 3

Table 2: Components identified in methanol extract of *Capparis zeylanica*

No	RT	Name of the compound	Peak Area %
1	10.875	O-Acetyl epipachy sandrine	44.86
2	10.942	Mixture of (A,E)-1,10-dihydroxy-(2.2)metacyclophane	45.05
3	25.088	DOP; 1,2-Benzene dicarboxylic acid, bis(2-ethylhexyl)ether	2.51
4	27.173	Spinacene	7.58

CONCLUSION

The results of this investigation present evidence that *Capparis zeylanica* leaf extract has α-glucosidase activity. The identification of active principles, as well as a relative absence of the toxic effects which could support the popular use of this plant in traditional medicine in the treatment of some ailments associated with cough, asthma, inflammation, fevers, cholera and also useful as poultice in gout and rheumatism. Nevertheless the mechanism of action for such activity is remained to be confirmed.

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