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Effects on antidiabetic and anti-inflammatory activities of chloroform extract of *Capparis Zeylanica*

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Abstract
To ascertain various phytochemical ingredients in *Capparis zeylanica* leaves extracts by TLC evaluating their antidiabetic property by using *in vitro* assays. The concentrated and dried extracts were subjected to TLC analysis and also the antidiabetic activity was assessed by employing standard *in vitro* techniques. Chloroform extract of *Capparis zeylanica* exhibited significantly lower IC_{50} values for the percentage inhibition of α-amylase (EC_{50} = 64.12 µg/ml) and α-glucosidase (IC_{50} = 62.32 µg/ml) compared to acarbose (IC_{50} = 68.23 µg/ml; IC_{50} = 71.23 µg/ml, respectively) ($p \leq 0.05$). Similarly, chloroform extract of *Capparis zeylanica* had the highest total phenolic content (54.12 mmol GAE/L), albumin denaturation value (55.23 µg/ml) and lipoxygenase inhibition activity (IC_{50} = 59.67 µg/ml). This study provides scientific evidence that chloroform extract of *Capparis zeylanica* have anti-diabetic efficacy. Thus, considering its relative hypoglycemic potency, they may serve as useful therapeutic agents for treating diabetes.

Keywords: antidiabetic; anti-inflammatory; *capparis zeylanica;*

Introduction
Modern natural sciences have comprehensively elucidated biological processes at the biochemical and molecular level that take place in the course of the inflammatory process. In the 1940s, American pathologist Menkin 1960 defined the sixth cardinal and at the same time the only essential biochemical sign of inflammation, i.e. proteolysis. However, the significance of his discovery has been largely neglected, leaving considerable confusion in explanations of the cause effect relationships in pathogenesis and in the approaches to treatment of inflammatory diseases (Maryam Rahimi, 2015) [4]. In particular, it is very often noted that, although inflammation is a defensive, therefore a useful process, its exaggeration or prolonged action may harm the body. However, the reasons for such outcomes are never explicitly stated. Diabetes mellitus is a non-communicable disease often genetic in nature but can be developed due to the life style. In current medicine there is no satisfactory effective therapy or medication to treat diabetes (Ali *et al*., 2006) [1]. Herbal plants having anti-diabetic properties can provide a useful source for the detection of safer economic anti-diabetic drug. Alpha-amylase is type of the intestinal enzyme which play significant role in carbohydrate digestion and glucose absorption. Conquest of the activity of digestive enzymes such as α-amylase, would delay the digestion of starch and oligosaccharides, which in turn decreases the absorption of glucose and therefore reduce the blood glucose (Puls *et al*., 1997) [6]. Inflammation is a severe response by living tissue to any kind of injury. There can be four primary indicators of inflammation: pain, redness, heat or warmness and swelling. When there is injury to any part of the human body, the arterioles in the encircling tissue dilate. This gives a raised blood circulation towards the area (redness) (Pitchai and Manikkam, 2012) [5]. Vasoactive chemicals also increase the permeability (increase pore size) of these arterioles which allows blood cells, chemical substance, blood protein s and fluid to accumulate in that region. This fluid accumulation causes swelling and may compress nerves in the area resulting in pain. In addition, prostaglandins, that might also result in 'irritation' of the nerves and further contribute to pain. Central obesity is associated with low-grade inflammation that promotes type 2 diabetes and cardiovascular disease in obese individuals. The 12- and 5-lipoxygenase (12-LO and 5-LO) enzymes have been linked to inflammatory changes, leading to the development of atherosclerosis. 12-LO has also been linked recently to inflammation and insulin resistance in adipocytes. *Capparis zeylanica* is large climbing shrubs with hooked spines, stems woody, rough, young parts green, rusty tomentose with pungent smell, leaves ovate or elliptic, rusty -tomentose when young, glabrous at maturity, base cuneate, entire, tip mucronate, flowers yellowish-white or white in supra-axillary, solitary, 2-3 pedunculate, berries globose, scarlet red, seeds many. Leaf extract of *C. zeylanica* with black pepper powder is taken twice daily for treatment of dysentery. Leaf juice of *C. zeylanica* is taken orally with cup of fresh gout milk for curing cough cold and also for the treatment of diabetes ripe fruits and leaves are consumed twice for fortnight and during ingestion.

Materials and Methods
Plant Materials
The leaves of *Capparis zeylanica* were collected from Government siddha medical college, herbal garden, Arumbakkam, Chennai, Tamilnadu, during June 2021 and it was taxonomically identified and authenticated as leaves of *Capparis zeylanica* by Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106, Tamil Nadu. A voucher specimen was deposited in the herbarium for future reference (Ref.No. 312mGSMCmMB/2021.

Preparation of Extracts

The leaves of *Capparis zeylanica* was thoroughly cleaned, dried under the shade and coarsely powdered. The polyphenol extract was prepared according to a previously reported method Kumarappan *et al*. (2012). The powdered plant material was mixed with 70% aqueous-methanol and stored at room temperature for 5 days. After 5 days, it was filtered and the solvent was evaporated. The residue was dis-solved in water, and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with chloroform. Extraction of the polyphenols was performed for 36 h at room temperature, and the combined chloroform layer was then concentrated. The residue was lyophilised. The extract obtained was dried and stored in an airtight container at 4◦C. The yield of the dry poly-phenolic extract was 30.5% (w/w). The dried extract was dissolved in Milli-Q water and used for further study.

Phytochemical Screening

The aqueous leaves extract of *Capparis zeylanica* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973; Trease and Evans 1983).

Thin Layer Chromatography

Thin layer chromatography of chloroform extract of *Capparis zeylanica* was performed using standard procedures (Harborne 1973). The polyphenol extract was placed carefully in precoated aluminum silica gel 60 F, Merck F 254 using a microcapillary tube. The spots were allowed to dry for few minutes and the TLC plate was placed in the solvent mixture, Toluene, acetone and Formic acid (6:6:1). After drying, the TLC plates were observed under UV at 240nm and 360 nm in UV TLC viewer. The Rf value of the spots was calculated by using the standard formula,

$$\text{Retention factor Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Alpha-Amylase Inhibitory Assay

The Alpha-amylase inhibitory assay of methanol and aqueous extracts of *X. americana* was evaluated according to a previously described method by Lena *et al*., 2008). In brief, 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 ml of dinitrosalicylic acid color reagent. At this time, the test tubes were placed in a water bath (100 °C for 5 min) and cooled until room temperature was attained. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as standard drug.

Inhibitory Activity of A-Glucosidase

The inhibitory activity of α-glucosidase method was followed by. The first step carried out substrate of starch solution (2% w/v maltose or sucrose, 1 mL) with Tris buffer (0.2 M, pH 8) and various concentrations of alkaloid rich fraction of *Coscinium fenestratum* for 5 min at 37°C. The reaction was initiated by adding α-glucosidase enzyme (1 mL of 1 U/mL yeast α-glucosidase) to the reaction mixture, followed by incubation for 10 min at 37°C. The reaction was terminated by heating the contents in a boiling water bath. 3, 5-dinitrosalicylic acid (1 mL) was added with the product before being incubated for 5 min and added with distilled water (9 mL). The amount of liberated glucose was measured by glucose oxidase peroxidase method.

Lipoxygenase Inhibition Assay

A spectrophotometric assay for determination of LOX activity was used as reported (Kemal *et al*., 1987) with slight modification. Soybean 15-lipoxygenase (15-LOX) was used for the assay. Inhibition experiments were run by measuring the loss of soybean 15-LOX activity (5µg) with 0.2µM linoleic acid (Sigma) as the substrate prepared in solubilized state (Kemal *et al.,* 1987) in 0.2M borate buffer (pH 9.0). Inhibition studies in presence of various concentrations of extracts (5, 10, 15, 20 µg/mL) and reference compound *viz*., quercetin was recorded at 234 nm using UV-Vis spectrophotometer. The inhibitory effect of the extracts was also expressed as percentage of enzyme activity inhibition. IC_{50} indicating the concentration required to inhibit 50 % LOX activity was also calculated. Values of hydroperoxide content and lipoxygenase activity were calculated from equation,

Specific activity (LOX) =ΔA. V/ε.l.c

Where, ΔA is the value of absorbance increase per min, V is the volume of incubation mixture, ε is the extinction coefficient for linoleic acid (25 x 10-3 mol/l/cm), l is the length of the cuvette (1 cm) and c is the concentration of enzyme in mg (0.005). The values are mean of three independent experiments.

***In-Vitro* Protein Denaturation Inhibition Activity**

In vitro anti-inflammatory activity of chloroform extract of *Capparis zeylanica*, the method used was "inhibition of protein denaturation" (Mishra *et al.,* 2011) using diclofenac sodium a standard. The test solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution (Chloroform extract of *Capparis zeylanica*). The test control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution. Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Various concentrations (25, 50, 75, 100µg/ml) of extract and diclofenac sodium (standard) were taken, respectively. All the solutions were adjusted to pH 6.3 using 1 N HCl. The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm.

Statistical Analysis

All experiments were performed in triplicates (n = 3) and the data are presented as the mean ± standard error. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software Version 20 (IBM).

Result and Discussion

Phytochemical Screenings of *Capparis Zeylanica*

The phytochemical screening of the aqueous leaves extract of *Capparis zeylanica* extract were studied presently showed the presence of alkaloids, flavonoids, polyphenols, and saponins (Table -1).

Table 1: Phytochemical screenings of aqueous leaves extract of *Capparis zeylanica*

Sl. No.	Phytochemical Constituents	Observation	Aqueous leaves extract of *Capparis zeylanica*
1	Alkaloids-Dragendorff's Test-Mayers test	Orange / red precipitate Yellow or white precipitate	+ +
2.	Flavonoids-Alkalai Reagent-Lead acetate test	Intense yellow colour Precipitate formed	+ +
3.	Glycosides Keller-Killiani test	Reddish brown colour ring formed	-
4.	Tannin-$FeCl_3$ test	Blue black coloration	-
5.	Saponins-Frothing test	Foam	+
6.	Terpenoids-*Salkowski test*	Dark reddish brown color in interface	-
7.	Polyphenols-Ferrozine test	Raddish blue	+
8.	Anthocyanin test Ammonia	Ammonia layer yellow in color	-

+ Positive result;--Negative result

The partial characterization of chloroform extract of *capparis zeylanica* by TLC

The chloroform extract of *Capparis zeylanica* loaded onPre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of Toluene, tetrahydrofuran and acetic acidin the ratio of 1:0.5:0.1 were efficient to extract the antioxidant compound it is used for further studies. The developed plate was viewed under UV 240nm and 360nm (Table-3

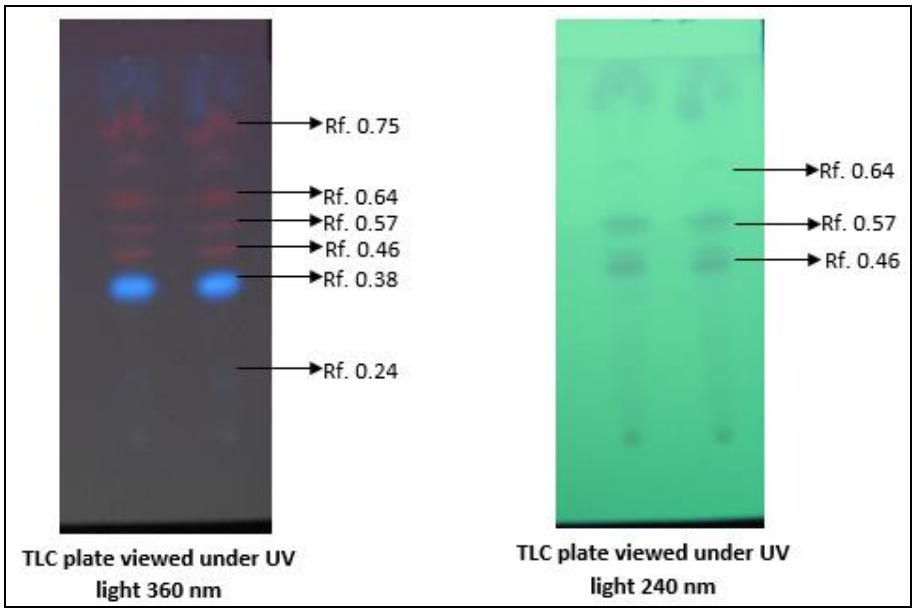

Fig 1: The Partial characterization of chloroform extract of *Capparis zeylanica* by TLC

Alpha-amylase inhibition

Inhibitory effects of α-amylase confirmed that chloroform extract of *Capparis zeylanica* at concentrations of 25-100 µg/ml (Fig-2). The maximum inhibition was observed at highest concentration of 100 µg/ml exhibited of 77.23% as compared to standard acarbose which showed significantly lower inhibition of 73.26% at the same concentration. Although different classes of drugs are available to control type 2 diabetes, still it is a challenging task to bring a better molecule which is devoid of undesirable adverse effects than existing drugs. In Indian traditional medicine systems, the number of medicinal plants has been used since ancient time to effectively treat diabetes (Tiwari and Rao, 2002) [8].

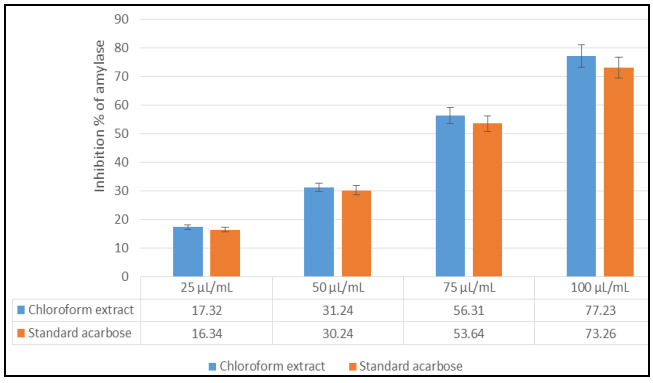

Fig 2: Effects of Inhibitory activity of a-amylase by Capparis zeylanica

Alpha-Glucosidase Inhibition

Another results of antidiabetic activity using α- glucosidase inhibitory assay of the chloroform extract of *Capparis zeylanica* are shown in Fig-3. The chloroform extract of *Capparis zeylanica* revealed a significant inhibitory action of α-glucosidase enzyme. The percentage inhibition ranges from 19.64% to 80.23% for lowest concentration to highest concentration. Thus the inhibition of the activity of α-glucosidase by chloroform the key enzymes for carbohydrate metabolism in the small intestine are pancreatic *α*-glucosidase which convert consumed polysaccharides to monosaccharides (Brenner and Stevens, 2006) [2].

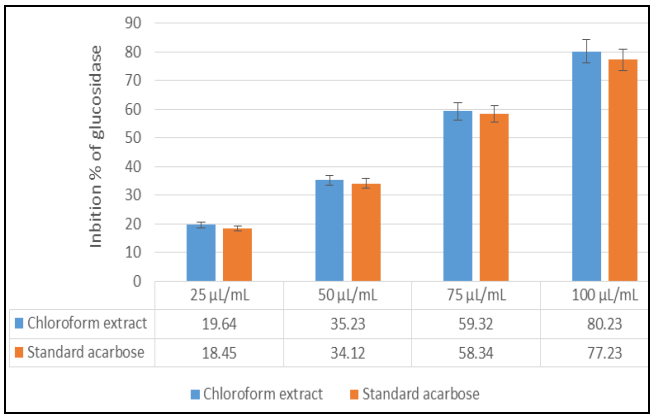

Fig 3: Effects of Inhibitory activity of a- glucosidase by Capparis zeylanica

Albumin Denaturation Inhibition

As fragment of the analysis on the mechanism of the anti-inflammatory activity, the capability to protein denaturation of chloroform extract of *Capparis zeylanica* was recorded. It was effective in inhibiting albumin denaturation in Fig-4. Maximum inhibition was recorded in chloroform extract of *Capparis zeylanica* 76.31% at 100 µg/ml was. Diclofenac sodium was used standard drug, which showed the maximum inhibition of 74.32% at the concentration of 100 µg/ml. The EC_{50} value of extract found to be 55.23 µg/ml which is higher than standard (Diclofenac Sodium) value 59.67 µg/ml. Denaturation protein is recorded as reason of inflammation, some of inflammatory drugs salicylic acid, phenylbutazone and diclofenac sodium have showing dose-dependent ability to thermally induced protein denaturation (Sakat *et al*., 2009) [7].

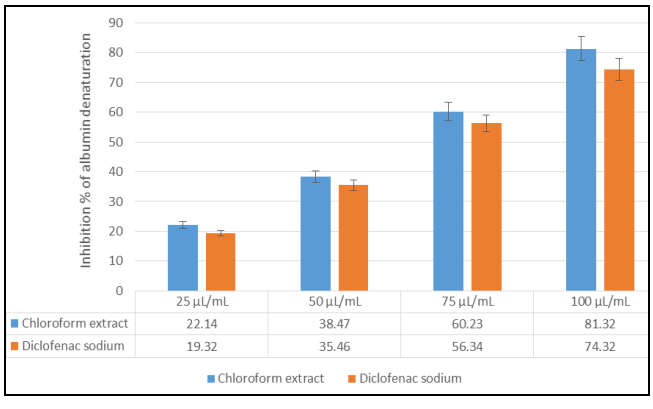

Fig 4: Effect of albumin denaturation inhibition by chloroform extract of *Copparis zeylanica*

Inhibition of 5-Lipoxygenase

Chloroform extract of *Capparis zeylanica* exhibited inhibitory activity of 5-Lipoxygenase compared to the standard diclofenac sodium. Chloroform extract of *Capparis zeylanica* recorded comparatively higher anti-inflammatory activity with regard to Lipoxygenase (EC_{50} 51.23 mg/mL) than the standard (Fig-5). Most of these anti-inflammatory agents have validated and proved to be potential anti-inflammatory agents. In plants, compounds such as alkaloids, terpenoids and saponins have been found to be responsible to cure inflammatory disorders (Dawei *et al.*, 2004).

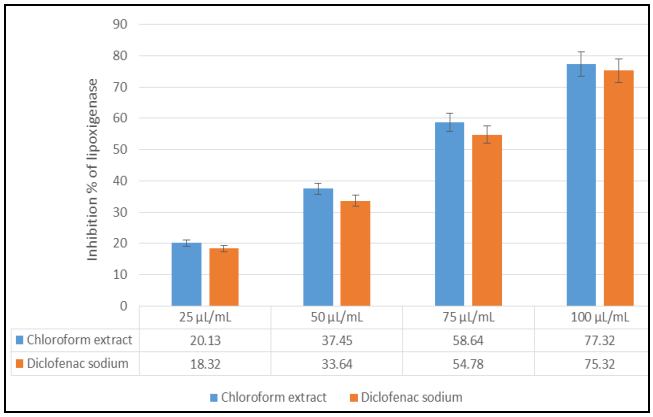

Fig 5: Effect of inhibitory activity of 5-Lipoxygenase by Chloroform extract of Capparis zeylanica

Conclusion

The investigation confirms that Chloroform extract of *Capparis zeylanica* exhibited highest antidiabetic and anti-inflammatory activity; Additional studies on needed for purification, characterization and structural elucidation of bioactive compounds from chloroform and also confirm its antidiabetic and anti-inflammatory property by *in vivo* studies. This study provides scientific evidence that leaves of *Capparis zeylanica* have anti-diabetic efficacy.

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