International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 06-04-2021, Accepted: 04-05-2021, Published: 20-05-2021
Volume 6, Issue 3, 2021, Page No. 238-245

In vitro evaluation of antidiabetic and anti-inflammatory activities of alkaloid rich fraction from the stem of *Coscinium fenestratum*

P Bama[1*], S Sankaranarayanan[2], K Prakash[3]

[1] Professor, Department of Biochemistry, Sri Sairam Siddha Medical College and Research Centre, Poonthadalam, West Tambaram, Chennai, Tamil Nadu, India

[2] Assistant Professor, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India

[3] Assistant Professor, Department of Botany, Arignar Anna Government Arts College, Villupuram, Tamil Nadu, India

Abstract
In this study, alkaloid rich fraction from the stem powder *Coscinium fenestratum*was studied for antidiabetic and anti-inflammatory activities as well as for other phytochemical contents. The antidiabetic activity of alkaloid rich fraction was evaluated with the use of three assays (inhibition of α-amylase, α-glucosidase and glucose uptake in yeast cell). Gas Chromatography and Mass Spectrophotometry analysis was used to determine the phytochemicals. Generally, the examined plants can be devised in two groups; the first one regrouped plants with high antioxidant activity (stem of *Coscinium fenestratum*). The antioxidant activity of extracts showed strong positive correlation with total phenolics and total flavonoids. The crude extracts had a lower level of sugars induced by the inhibitory effect of α-amylase, α-glucosidase and glucose uptake activities. Thus, their enzymatic inhibitory effect might have resulted from a synergism among the alkaloid compounds concerned.

Keywords: evaluation of antidiabetic, anti-inflammatory activities, alkaloid rich fraction, *Coscinium fenestratum*

Introduction

Therapeutic florae are the "mainstay" of old-stylemedication, that resourcesadditional than 3.3 billion peoples are usedmedicinal plants on a regular basis and also control some chronic diseases (Castello *et al*., 2002) [5]. These therapeuticfloraereflect as a rich resources of constituents,these phytocompounds are used to drug development and biochemical synthesis. In addition that these plants performance a serious role in the expansion of human cultures everywhere the whole world. The Indian sub-continent has anidenticalamusing diversity of plant species in anextensive range of ecosystems. There are about 18000 species of angiosperm plants, of which roughly 8.000 species, are measuredremedial and used by traditional communities, mainly tribal communities, or in traditional medicinal systems, such as the Siddha and Ayurveda. Plant-metabolites are organic compounds which can be divided into primary metabolites and secondary metabolites. Primary metabolites are include glucose, starch, polysaccharide, protein, lipids and nucleic acid are helpful for growth and reproductive activity of plant. Plants secondary metabolites are alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides mainly utilize therapeutic efficacy for curing many diseases (Edeoga *et al.*, 2005) [7].

Diabetes mellitus is the most prevalent metabolic syndrome world-wide with an incidence varying between 1 to 8%. The disease arises when insufficient insulin is produced, or when the available insulin does not function properly. Thus diabetes is characterized by hyperglycaemia resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes (Kesari *et al.*, 2007) [10]. The long-term manifestation of diabetes can result in the development of some complications, broadly classified as microvascular or macrovascular disease. Microvascular complications include neuropathy, nephropathy and vision disorders (retinopathy, glaucoma, cataract and corneal diseases), while macrovascular complications include heart disease, stroke and peripheral vascular disease, which can lead to ulcers, gangrene and amputation. Current estimates from different countries in Europe and the United States have shown that diabetes and its complications account for 8-16% of the total health costs for society and this will increase dramatically unless major efforts are made to prevent the ongoing epidemic. There are two major categories of diabetes - insulin dependent diabetes mellitus (Type-1 diabetes mellitus) and non-insulin dependent diabetes mellitus (Type-2 diabetes mellitus). Type-2 diabetes mellitus is associated with hypertension and dyslipidemia (Wadkar *et al*., 2013) [20].

Inflammation is a pathologic disorder that comprisesanextensivecollection of diseases such as rheumatic and immune-mediated conditions with diabetes. There are severalmodern medicines available for directing and controlling inflammatory emergency; steroids and nonsteroid anti-inflammatory drugs, these medicines which are related with opposing effects although in repetition our goal is to apply lowest effective dose by the maximum efficacy with the least adverse effects (Bagad *et al*., 2013) [2]. Thus, we need to apply natural anti-inflammatory factors within medication therapy to achieve increased pharmacological response and the lowest degree of unwanted side effects (Ghasemian *et al*., 2015) [8]. Herbal medicines are promoting subjects in medicine field.

Coscinium fenestratum is commonly known as Tree Turmeric, which islarge dioecioustimbered climber andadditional primitive group, native to the Indo-Malayan region. In India, *C. fenestratum* limited to the few habitats of Western Ghats, generally in the high rainfall gettingrainy evergreen forests and semi-deciduous forests. It has extensively used in Indian medicine Siddha and Ayurveda for the treatment of abdominal problem, chronic fevers, wounds and ulcers. In South India and Sri Lanka stem has long been used as a yellow dye and bitter tonic. The roots and stem are reported to contain alkaloids berlambine, dihydroberlambine, noroxyhydrastine, protoberberine etc. Berberine is bioactive compound of *C. fenestratum*with several bioactivities include antimicrobial activities and inflammatory disorders (Birdsall and Kelly, 1997) [4].

Materials and Methods

Plant Material

*Coscinium fenestratum*stem was obtained from Herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamilnadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-443/2021. The stems were sufficiently air-dried in 5 days at the ambient room temperature, while the stem was cut into smaller pieces and air-dried in 7 days.

Phytochemical Screening

The aqueous extract of *Coscinium fenestratum* 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 (Aida *et al*., 2001; Hess *et al*., 1995) [1, 9].

Total Phenolic Content

The total phenolic content (TPC) of aqueous extract of *Coscinium fenestratum*was determined using the method by Singleton, (1965). The aqueous extract (1 mL, 1 mg/mL) was mixed thoroughly with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na_2CO_3, and centrifuged at 13400X *g* for 5 min. The absorbance of upper phase was measured using a spectrophotometer (ELICO (SL150) UV–Vis Spectrophotometer) at 750 nm after 30 min incubation at room temperature. Total phenolic content was expressed as a catechol equivalent.

Estimation of flavanoid

A 1ml aliquot of each aqueous extract of *Coscinium fenestratum*was mixed thoroughly with 1ml of 2% aluminium chloride and 0.5 ml 0f 33% acetic acid followed by the addition of 90% methanol and the content is thoroughly stirred and allowed to stand for 30 minutes (Elfalleh *et al*., 2019). The absorbance was measured at 414 nm using a UV-Visible Spectrophotometer. Quercetin was used as a standard.

Extraction of total alkaloids

Approximately 100 g of stempowdered was soaked in 200 mL of methanol for 48 hours with constant stirring. The methanol extract was filtered and the process was repeated again in order to ensure that no extractable remained in the residues. The methanol was recovered by rotary evaporator at 40 °C. The residue was acidified with 0.1 M H_2SO_4 and finally extracted with chloroform. The organic phase-I confined neutral and acidic materials which was stored while the aqueous phase-I was tested for alkaloids with Dragendorff's reagent. This aqueous phase-I was basified with 20% Na_2CO_3 to pH10 and again extracted with chloroform. The aqueous phase-II obtained contained the water soluble material whereas organic phase-II was washed with H_2O and dried with Na_2SO_4. The chloroform was evaporated in a rotary at 40 °C. The final solvent was totally dried up and the remainder left was weighed to calculate the amount of crude alkaloids (Saddiqe *et al*., 2018) [16].

GC-MS analysis of extract

GC-MS analysis of alkaloid rich fraction of *Coscinium fenestratum*employs that fused silica column and the components were separated using helium as a carrier gas at a constant flow of 1 ml/min. The 1 µl sample extract was injected into the instrument. The initial temperature was set at 100 °C, whereas the injector temperature was set at 250 °C and throughout the process temperature flow was set at the speed of increasing 10 °C/min. The actual separation was observed at 24th minute, for which final temperature was adjusted to 280 °C and run for 5 min.

Antidiabetic Activity

Glucose uptake in yeast cells

Glucose uptake assay by yeast cells was performed according to Cirillo *et al*. (1963). The yeast cellsuspended in distilled water was subjected to repeated centrifugation (3000 × *g*, 5 min) until clear supernatant fluids were obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of ofalkaloid rich fraction of *Coscinium fenestratum*(25 to 100 µg/ml) were added to 1 ml of glucose solution (20 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2500 × *g*, 5 min) and amount of glucose was estimated in the supernatant. Glycomet was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

Increase in glucose uptake%=Abssample-Abscontrol/Abssample×100

Where, Abs sample is the absorbance of test sample and Abs control is the absorbance of control reaction (containing all reagents except the test sample). All the experiments were carried out in triplicates.

Inhibition of alpha-amylase method followed by Narkhede *et al.,* (2011) [14]. In this assay, added 390 µl of 0.02 M phosphate buffer (pH 7), positive control (acarbose), different concentrations of alkaloid rich fraction of *Coscinium fenestratum* and 10 µl of α-amylase enzyme were mixed and incubated at 37°C for 10 min. Added 10 µlof starch to this mixture and again incubated 37°C for 1 h (Megha *et al*., 2013) [12]. After incubation, added 0.1 ml 1% iodine solution and 5 ml of distilled water and optical density was measured at 565 nm. Inhibition of enzyme activity was calculated as follows:

Percentage inhibition=(A−C)×100/ (B−C)

Where, A=Absorbance of the sample, B=Absorbance of blank (without α-amylase), and C=Absorbance of control (without starch).

The inhibitory activity of α-glucosidasemethod 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.

Inhibition of albumin denaturation activity

The anti-inflammatory activity of alkaloid rich fraction of *Coscinium fenestratum* was deliberate by inhibition of albumin denaturation was studied. The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adapted using few drops of 1 N HCl. The differenrt concentration of alkaloid rich fraction were incubated at 37°C for 20 min and then heated to 51°C for 20 min, successively chilled the test sample was measured at 660 nm. The experiment was repeated in triplicate (Montefusco *et al.,* 2013) [13]. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition= (Abs Control−Abs Sample) 100/Abs control.

Heat-Induced Hemolysis

The reaction mixture (2 ml) consisted of 1 ml alkaloid rich fraction of *Coscinium fenestratum*of different concentrations (25-100 µg/ml) and 1 ml of 10% red blood cells (RBCs) suspension, in its place of the test sample, the only saline was added to the control test tube. Diclofenac sodium was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min (Sadique *et al.,* 1989) [17]. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was executed in triplicates for all the test samples. The percentage inhibition of hemolysis was calculated as follows:

Percentage inhibition = (Abs Control−Abs sample)100/Abs control.

Inhibition of lipoxygenase activity

5-LOX inhibition assay was performed using the principle of 1-4 diene (linoleic acid) oxidations to 1-3-diene. Briefly, an aliquot of the stock solution (50 µL, in dimethyl sulfoxide (DMSO) and tween 20 mixture; 29:1, w/w) of different concentration of alkaloid rich fraction of *Coscinium fenestratum*was placed in a 3 mL cuvette, followed by addition of pre-warmed 0.1 M potassium phosphate buffer (2.95 mL, pH 6.3) and linoleic acid solution (48 µL). Thereafter, ice-cold buffer (potassium phosphate; 12 µL) was added with 5-LOX (100 U) and absorbance recorded at 234 nm (Baylac and Racine, 2003). The control was prepared with DMSO: tween 20 mixture (no enzyme inhibition).

Statistical Analyses

Statistical evaluation was carried out by the SPSS software (SPSS Inc, Chicago, USA, ver. 13.0). Descriptive statistics were ascertained for all the contemplated attributes. Analyses were carried out in triplicate and the means of all parameters were examined for significance ($p < 0.05$) by analysis of variance (ANOVA).

Results and Discussion

Phytochemicals properties of *Coscinium fenestratum*

In this study, phytochemical screening of the *Coscinium fenestratum* was done to assess the availability of bioactive secondary metabolities. The presence of phytochemicals such as, flavonoids, alkaloids, tannins, steroids, phenols, saponins and terpenoids were detected.In generally therapeutic plants contained some secondary metabolites significantly contribute in the direction of the biological activities correspondingantidiabetic, hypoglycemic, antimicrobial, antiinflammatory, antioxidant, antimalarial activities (Negi *et al*., 2011) [15].

Table 1: Phytochemical screening of *Coscinium fenestratum*

Sl. No.	Phytochemical Constituents	Observation	Aqueous stem extract of *Coscinium fenestratum*
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	+

+ indicate positive result; -- Indicate negative result

The examinationpresented that total phenolics (45.23 ±0.78 µg GAE/g plant extracts). Total flavonoid contents in the *Coscinium fenestratum* were calculated as rutin equivalent (mg/g) using the equation based on the calibration curve: y = 0.0182x - 0.0222, R2 = 0.9962, where y wasthe absorbance and x was the rutin equivalent (mg/g).The experimental result showed the presence of appreciable amount of flavonoid 23.15±0.51 mg/gm) in the Aqueous stem extract of *Coscinium fenestratum.*

Analysis of GC-MS

The GC–MS analysis of the extract of the *Coscinium fenestratum* exhibited the occurrence of numerous phytochemicals which importantly subsidize to the biological activity of this plant. In this study, GC–MS analysis exhibited the existence of 8 compounds from the alkaloid rich fraction extract (Fig-1). The imperativephytochemical determined in GC–MS analysis were, 4-Piperidine, phytol, 9,12-Octadecandionoic acidand Trifluoroaceteoxy tetradecene.

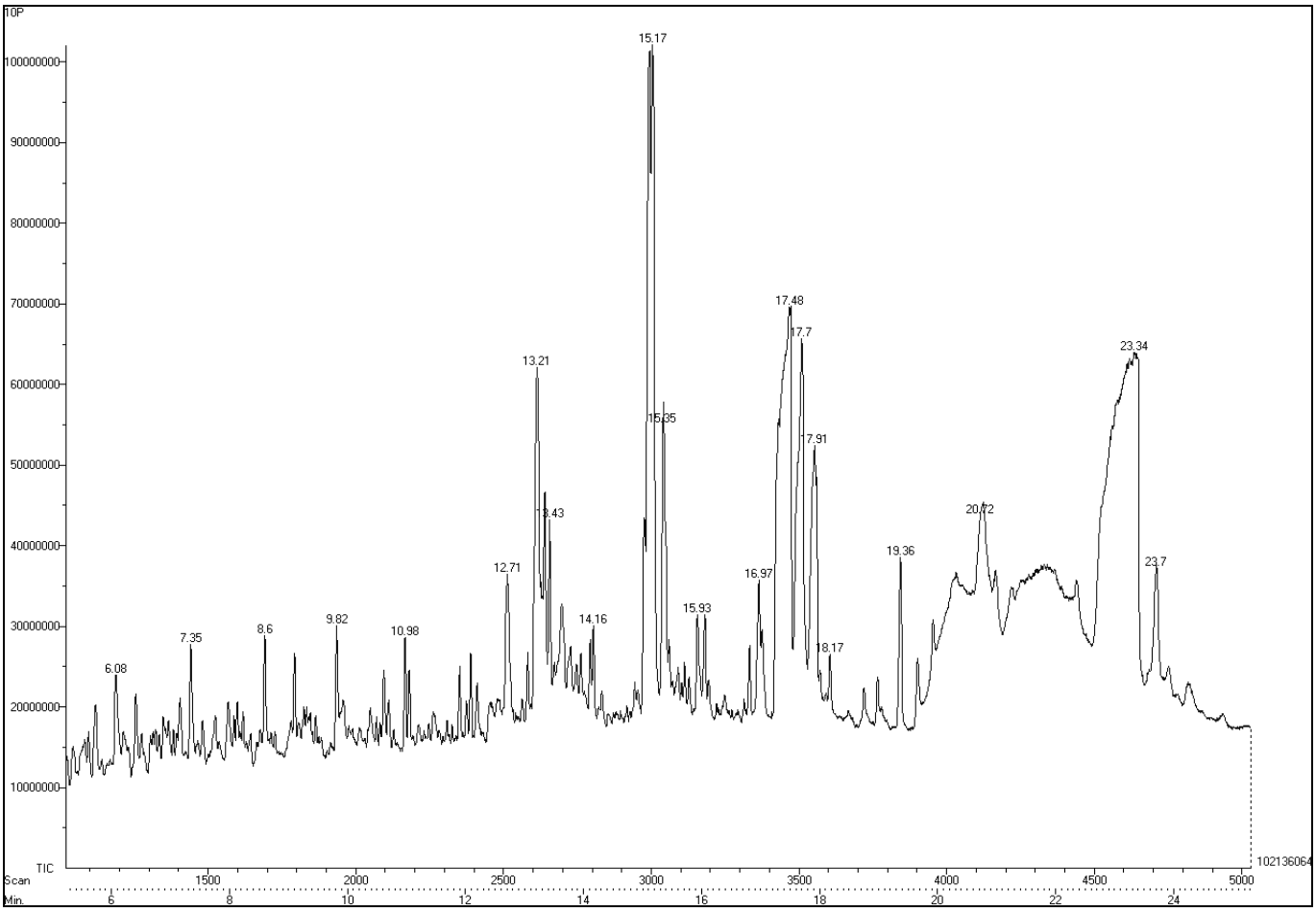

Fig 1: Consolidated compound peak of *Coscinium fenestratum* extract

Table 2: Consolidated compound peak of *Coscinium fenestratum* extract

S. No	Compound Name	Major Peak	Retention Time
1	1-Tetracosanol		7.25
2	1-Hexacosanol		8.6
3	4-Piperidine		12.71
4	7-Tetradecana		13.21
5	9,12-Octadecandionoic acid		15.17
6	Trans-11Tetradecenylacetate		17.48
7	Trifluoroaceteoxy tetradecene		23.23

Glucose uptake in yeast cells

Different concentrations of alkaloid rich fraction of *Coscinium fenestratum* were subjected to *in vitro* glucose uptake assay employing yeast as model. The percentage of glucose uptake in yeast cells by the alkaloid rich fraction was compared with standard drug diclofenac sodium (Fig-2). Alkaloid rich fraction of *Coscinium fenestratum* exhibited highest percentage of glucose uptake 73.64%, which was almost near to the standard 71.23%at 100 µg/ml concentration. Results also indicated that alkaloid rich fraction had nearly same effectiveness in increasing the glucose uptake by yeast cells as compared to standard drug acarbose.

Type II diabetes categorized by lack of insulin triggering augmented close in blood glucose level and it be contingent on the uptake of glucose by the cells (Shori, 2015) [19].

The increased concentration of alkaloid rich fraction similarly increased proportion of glucose uptake in yeast cells.

This result indicated that high concentrations of alkaloid rich fraction displayed high glucose uptake.

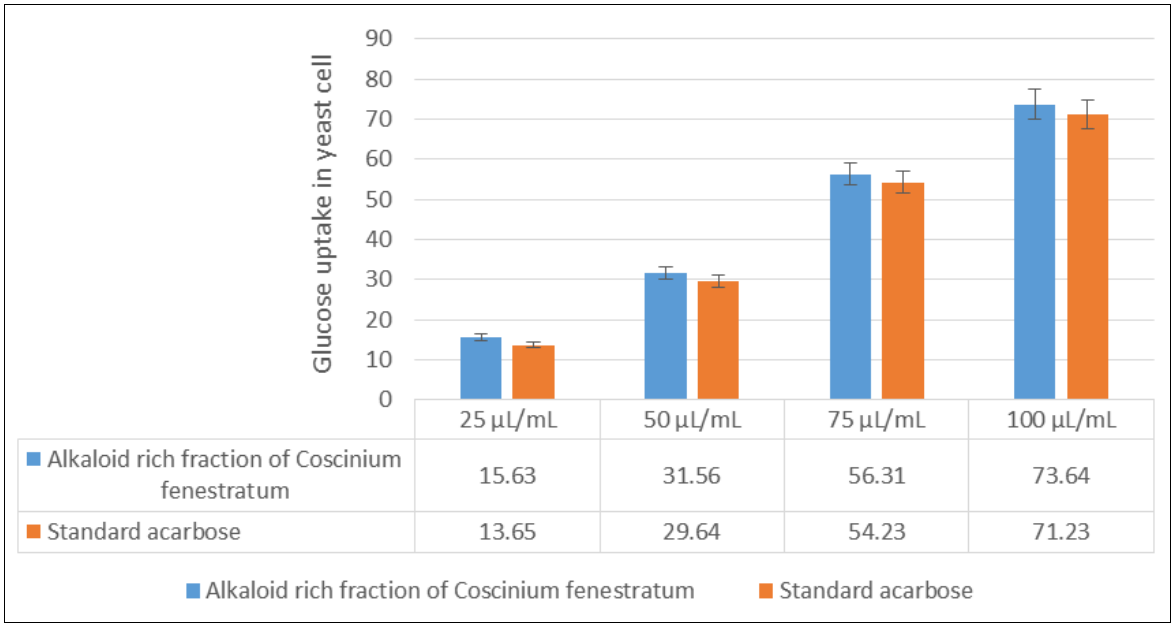

Fig 2: Effects of glucose uptake in yeast cells by alkaloid rich fraction of *Coscinium fenestratum*

Alpha-Amylase Inhibition

Inhibitory effects of α-amylase confirmed that alkaloid rich fraction of *Coscinium fenestratum* at concentrations of 25-100 µg/ml(Fig. 3). The maximum inhibition was observed at highest concentration of 100 µg/ml exhibited of 78.23% as compared to standard acarbose which showed significantly lower inhibition of 75.32% at the same concentration. Alpha-amylase is type of the intestinal enzyme which play important role in carbohydrate digestion and glucose absorption (Worthington, 1993). Since alkaloid rich fraction, further studies have to conduct on the isolation, and characterization of the compounds in authority for the activity.

Alpha-Glucosidase Inhibition

Another results of antidiabetic activity using α- glucosidase inhibitory assay of the alkaloid rich fraction of *Coscinium fenestratum* are shown in Fig-4. The alkaloid rich fraction revealed a significant inhibitory action of α-glucosidase enzyme. The percentage inhibition ranges from 14.56% to70.23% for lowestconcentration to highestconcentration. Thus the inhibition of the activity of α- glucosidase by alkaloid rich fraction of *Coscinium fenestratum*desiredinterruption the degradation of carbohydrate, which would in chancereason a reduction in the absorption of glucose, as a result the decrease of postprandial blood glucose level advancement (Mai *et al*., 2007) [11].

Albumin Denaturation Inhibition

As fragment of the analysis on the mechanism of the anti-inflammatory activity, the capability to protein denaturation of alkaloid rich fraction of *Coscinium fenestratum*was recorded. It was effective in inhibiting albumin denaturation in Fig. 5. Maximum inhibition was recorded in alkaloid rich fraction of *Coscinium fenestratum*76.31% at 100 µg/ml was. Diclofenac sodium was used standard drug, which showed the maximum inhibition of 68.32% at the concentration of 100 µg/ml. The EC_{50} value of extract found to be 58.61 µg/ml which is higher than standard (Diclofenac sodium) value 61.23 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) [18]. In this study, protein denaturation inhibition study was performed also same way.

Heat-Induced Hemolysis

The alkaloid rich fraction of *Coscinium fenestratum* inhibited the heat-induced hemolysis of RBCs to varying degrees as per Fig. 6. The maximum inhibition of 68.23% at 100 µg/ml was observed alkaloid rich fraction and standarddiclofenac sodium showed lower inhibition of 65.20% at the same concentration. The EC_{50} value of test extract found to be 71.23 µg/ml and 74.56 µg/ml for standard.These clarifications produce a scientific basis for the use of this therapeutic plant in traditional medicine for the treatment of inflammatory diseases.

Inhibition of 5-Lipoxygenase

Alkaloid rich fraction of *Coscinium fenestratum*exhibited inhibitory activity of 5-Lipoxygenase compared to the standard diclofenac sodium. Alkaloid rich fraction of *Coscinium fenestratum* recorded comparatively higher anti-inflammatory activity with regard to Lipoxygenase (EC_{50} 81.23 mg/mL) than the standard (Fig-7). Most of these anti-inflammatory agents have validated and proved to be potential anti-inflammatory agents. In plants, compounds such as alkaloids, terpenoidsand saponins have been found to be responsible to cure inflammatory disorders (Dawei *et al*., 2004) [6].

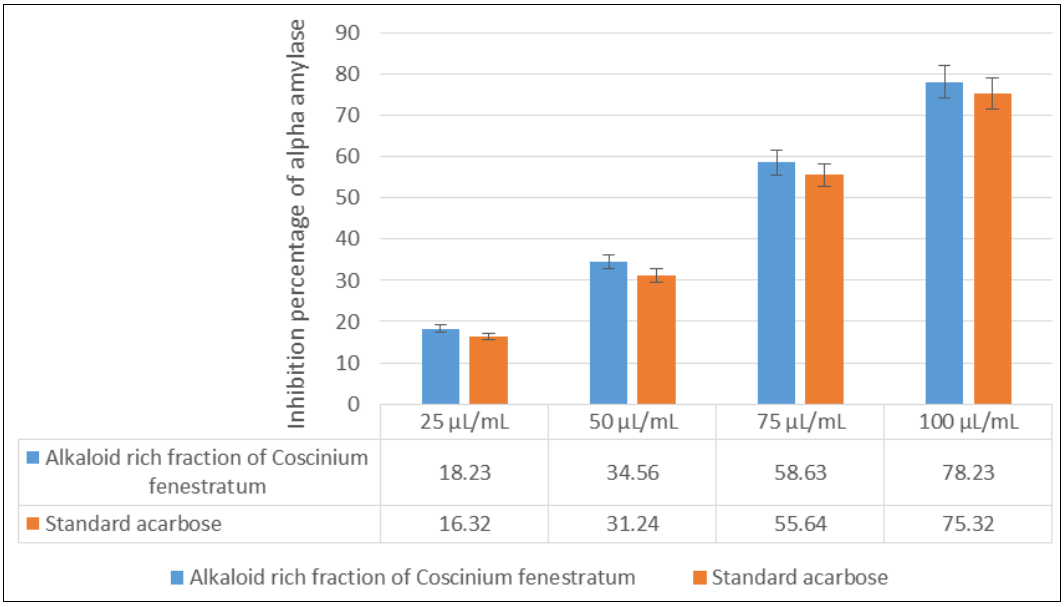

Fig 3: Effects of alpha-amylase inhibition by alkaloid rich fraction of *Coscinium fenestratum*

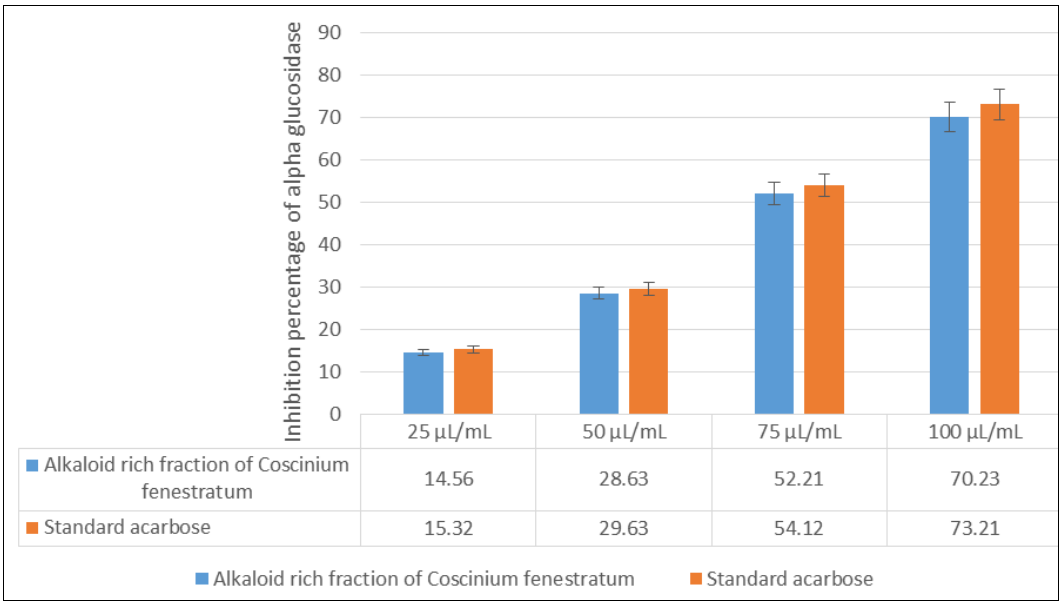

Fig 4: Effects of alpha-glucosidase inhibition by alkaloid rich fraction of *Coscinium fenestratum*

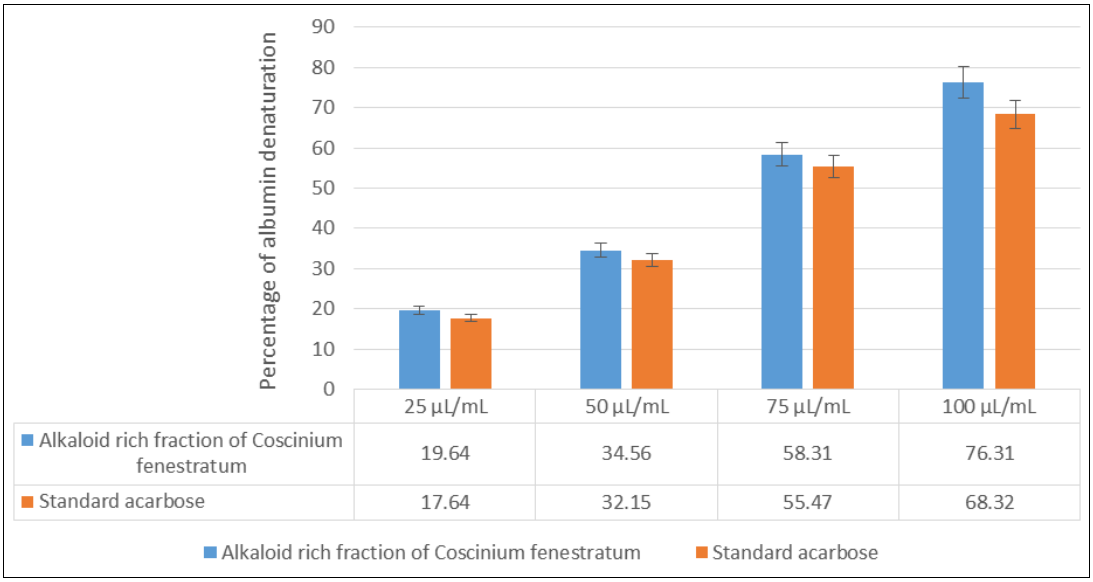

Fig 5: Albumin denaturation inhibition of alkaloid rich fraction of *Coscinium fenestratum*

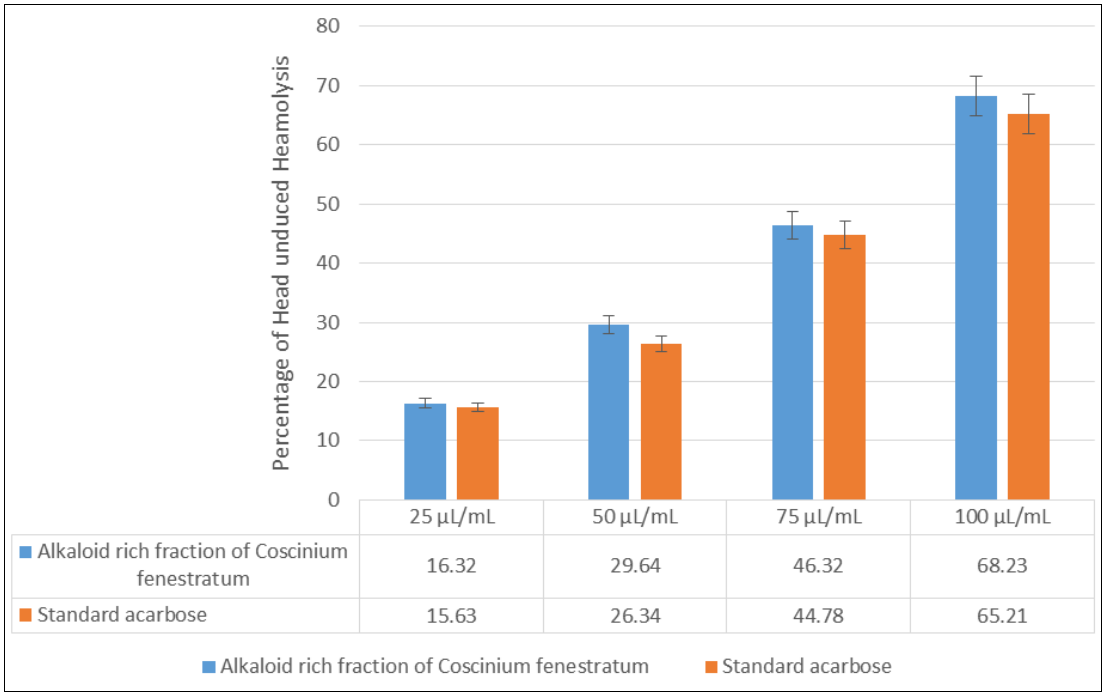

Fig 6: Heat-induced hemolysis of alkaloid rich fraction of *Coscinium fenestratum*

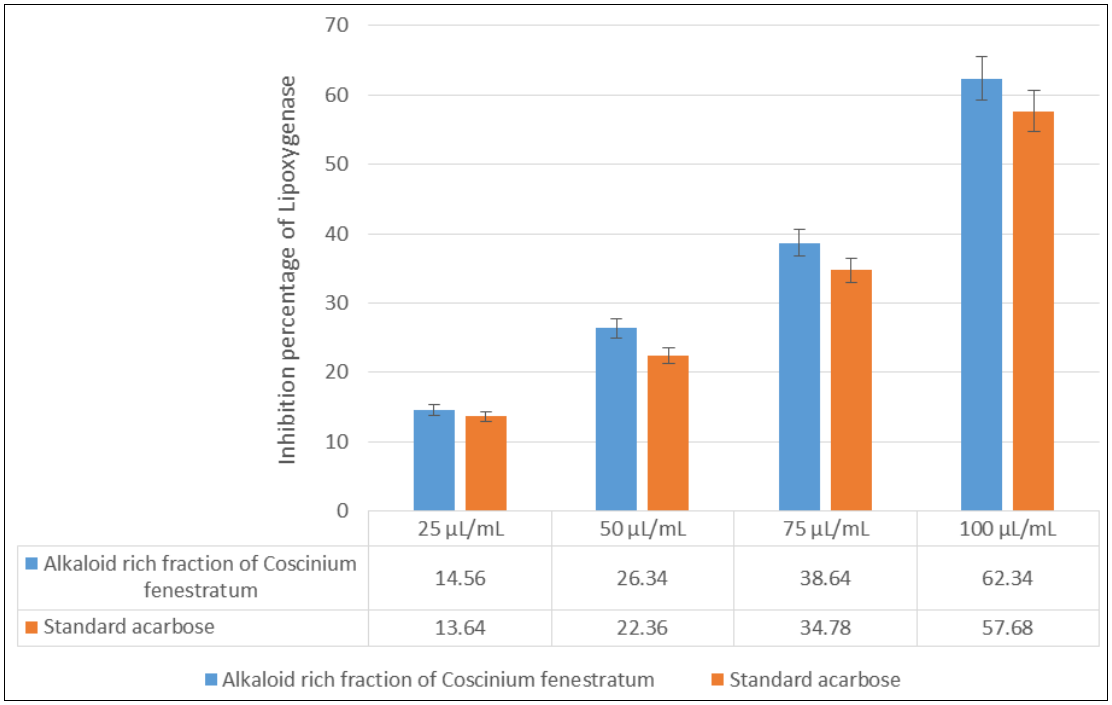

Fig 7: Inhibition of 5-lipoxygenase by alkaloid rich fraction of *Coscinium fenestratum*

Conclusion

Inthis present study concludedthat alkaloid rich fraction of *Coscinium fenestratum* potentially used in the management of diabetes mellitus and inflammatory diseases. In addition, this study exhibits for the first time the inhibitory activities of amylase glucosidase and LOX of alkaloid rich fraction. Therefore, data existing in this section could be supposed as an inventive involvement to the literature.Additional studies might be directed to examine the mode of action of these extracts in interrelating with the antidiabetic, and inflammatory pathways in animal models.These data convey new care for the traditional utilization of *Coscinium fenestratum*and display that alkaloid rich fraction could be used as antidiabetic and anti-inflammatory agents.

Acknowledgement

We thank Mr.SaiPrakashLeomuthu, CEO Sairam Institutions, Mr.SathishKumar CBO Sairam Institutions. Dr.S.Mathukumar M. D. (S), Principal Sri Sairam Siddha Medical College West Tambaram for Support and Encouragement to carry out the study.We also thank Commissioner Indian System of Medicine & Homeopathy, Joint Director Indian System of Medicine& Homeopathy and Principal Goverment Siddha Medical College,

Arumbakkam, Chennai-600 106 for Continues Support throughout the study.

Reference

1. Aida P, Rosa V, Blamea F, Tomas A, Salvador C. *Paraguyan* plants used in traditional medicine. Short communication. J. Ethnopharm, 2001:16:93-98.
2. Bagad AS, Joseph JA, Bhaskaran N, Agarwal A. Comparative evaluation of anti-inflammatory activity of curcuminoids, turmerones, and aqueous extract of *Curcuma longa*," Advances in Pharmacological Sciences,2013:2013:Article ID 805756, p7.
3. Baylac S, Racine P. Inhibition of 5-Lipoxygenase by Essential Oils and Other Natural Fragment Extracts. International Journal of Aromatherapy, 2003:13(2–3):138–142.
4. Birdsall TCND, GSND Kelly. Berberine: Therapeutic potential of an alkaloid found in several medicinal plants. Alt. Med. Rev,1997:207:94-103.
5. Castello MC, Phatak A, Chandra N, Sharon M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixaorellana L Ind. J. Exp. Biol,2002:40(12):1378-1381.
6. Dawei Wen, Yuping Liu, Wei Li, Huwei Liu. Separation methods for antibacterial and antirheumatism agents in plant medicines. Journal of Chromatography B,2004:812(1-2):101-117.
7. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotech,2005:4(7):685-688.
8. Ghasemian M, Owlia MB. A different look at pulsed glucocorticoid protocols; is high dose oral prednisolone really necessary just after initiation of pulse therapy?" Journal of Case Reports in Practice,2015:3(1):p1–3.
9. Hess SC, Brum RL, Honda NK, Cruz AB, Moretto E, Cruz RB, *et al*. Antibacterial activity and phytochemical analysis of *Vochysiadivergens* (*Vochysiaceae*). J. Ethnopharm,1995:47(2):97-100.
10. Kesari AN, Kesari S, Singh SK, Gupta RK, Watal G. Studies on the glycemic and lipidemic effect of Murrayakoenigii in experimental animals. J. Ethnopharmacol,2007:112:305-311.
11. Mai TT, Thu NN, Tien PG, Van Chuyen N: Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. J NutrSciVitaminol (Tokyo),2007:53:267–76.
12. Megha GC, Bhoomi BJ, Kinnari NM. In vitro anti-diabetic and antiinflammatory activity of stem bark of Bauhinia purpurea. Bull Pharm Med Sci,2013:1:139-50.
13. Montefusco-Pereira CV, de Carvalho MJ, de AraújoBoleti AP, Teixeira LS, Matos HR, Lima ES. Antioxidant, anti-inflammatory, and hypoglycemic effects of the leaf extract from PassifloraNitidaKunth. ApplBiochemBiotechnol,2013:170:1367-78.
14. Narkhede MB, Ajimire PV, Wagh AE, Manoj M, Shivashanmugam AT. In vitro antidiabetic activity of Caesalpinadigyna (R.) methanol root extract. Asian J Plant Sci,2011:1:101-6.
15. Negi JS, Singh P, Rawat B. Chemical constituents and biological importance of Swertia: a review. Curr. Res. Chem,2011:3:1-15.
16. Saddiqe Z, Wahab U, Maimoona A, Raheel R, Iram M. Alkaloid rich fraction of *Daturaalba* Rumph. exNees leaves possesses antitumor and antimitotic activity. South African Journal of Botany,2018:117:282-287.
17. Sadique J, Al-Rqobah NA, Bughaith MF, El-Gindy AR. The bioactivity of certain plants on the stabilization of RBC membrane system. Fitoterapia,1989:60:525-35.
18. Sakat SS, Tupe PN, Juvekar AR. In-vitro anti-inflammatory activity of aqueous and methanol extracts aqueous and methanol extracts of Erythrinaindica leaves. Pharamacol Online,2009:3:221-9.
19. Shori AB. Screening of antidiabetic and antioxidant activities of medicinal plants. Journal of Integrative Medicine,2015:13(5):297-305.
20. Wadkar KA, Magdum CS, Patil SS, Naikwade NS. Antidiabetic potential and Indian medicinal plants. J Herbal Med Toxicol,2013:2:45-50.
21. Worthington V. Alpha amylase, in: V. Worthington (Ed.), Worthington Enzyme Manual Freehold. Worthington Biochemical Corp NJ, 1993, 36-41.