In Vitro Antidiabetic, Antioxidant and Antibacterial Activity of the Plant *Costus Igneus*

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Abstract

Diabetes mellitus (DM) is a major metabolic disorder causing morbidity and mortality worldwide. DM is treated by routine administration of drugs either orally or by injections. Since a hypoglycemic agent causes side effects, there is a growing interest in herbal remedies for the treatment of DM due to fewer side effects. In this research, the leaves extracts of the plant *Costus igneus* and are subjected to evaluate the anti-diabetic activity, α-amylase inhibition, antioxidant activity and antibacterial activity. The presences of phytochemicals in various extracts of *Costus igneus* are identified and the anti-diabetic activity was studied in dialysis membrane. Anti-diabetic activity was also carried by α-amylase inhibition study and the result showed that the plant can reduce the blood glucose level. The antioxidant activity was studied by FRAP & DPPH assay and the maximum antioxidant activity was shown by the phytochemicals present in the ethanolic extract. The antibacterial activity was studied by agar well diffusion method and the aqueous extract showed better activity among the extracts. Thus, the *Costus igneus* leaf extract exhibited good anti-diabetic, antioxidant & antibacterial property and can be used for the treatment of DM and the plant *Costus igneus* can be treated as herbal plant.

Keywords: anti diabetic; antioxidant; antimicrobial; herbal plant; *Costus igneus*

Introduction

Diabetes mellitus (DM) is a major metabolic disorder causing morbidity and mortality worldwide, characterized by elevated plasma glucose level due to insufficient insulin and insulin resistance, or both. Despite insulin deficiency, obesity and stress are the major causes of diabetes [1, 2]. Type-2 DM is more common, characterized by hyperglycemia, insulin resistance and relative insulin deficiency [3-5]. As per international diabetes federation, worldwide 415 million adults aged 20–79 year have DM in 2015 and it may be increased to 642 million in 2040. It is estimated that between 10 % to 12 % of the urban populations and 4.0 % to 6.0 % of the rural populations of India are affected by diabetes. Recent studies on geographical and ethical influences have shown that peoples of Indian origins are highly prone to diabetes.

DM may damage the eyes, heart & blood vessels, kidneys and leads to various complications like retinopathy, neuropathy, etc. [1]. Since oral hypoglycemic agents cause's side effects, there is a growing interests in herbals remedies for the treatment of DM [6, 7]. Plants having phytochemicals are capable of producing therapeutic effects and are non-toxic & safe. The trends of using herbal medicines are highly increasing due to its availabilities and cost effective. Nearly 25% of the world's population relies on traditional medicinal systems; these medicines are not only used by the rural, but also used in developed countries, where modern medicines are dominating [8].

In this research, the natural plant *Costus igneus* is taken and its therapeutic effects are investigated. *Costus igneus* is commonly known as fiery Costus, step ladder or spiral flag or insulin plant belongs to the family Costaceae [9]. It is strongly believed that consuming one leaf of *Costus igneus* per day can lower blood glucose level and diabetes. The invitro anti-diabetic activity was studied by glucose diffusion inhibitory method using a semi permeable dialysis membrane [10, 11]. α-Amylase inhibition study was carried out to check the anti-diabetic activity of the plants [12]. The antioxidant activity was studied by ferric reducing antioxidant power assay (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The antibacterial activity of the plant was studied by agar well diffusion method. The results of the analysis are compared with the literaturally available results.

Materials and Methods

The reagent ethanol, pet.ether, dinitrosalicylic acid, trichloroacetic acid, chloramphenicol, Mayer's reagent, acetic anhydride, Muller Hinton agar, α-amylase, phosphate buffer, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and dialysis bag were purchased from Himedia. The microorganism Pseudomonas fluorescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Aeromonas hydrophila and Bacillus cereus were purchased from MTCC -Institute of Microbial Technology- Chandigarh.

Collection of sample

Fresh leaves of the *Costus igneus* plants were taken from the backyard, washed under running water to remove the dust and pollutants. The clean leaves were dried in shade for 21days. The dried leaves were milled in an electric grinder and the fine powder obtained was used for preparing the extract.

Preparation of plant extracts

10g of *Costus igneus* powder was taken in a beaker and 50mL of ethanol was added into it. The mixture was stirred continuously for 30 minutes and kept as such at room temperature for one day. It was filtered in a muslin cloth and again through Whitman filter paper to remove the residue and the extract of ethanol was stored at 4 ºC for further analysis. Similarly, the extracts of pet. Ether, water was prepared and used for further analysis.

Qualitative analysis of phytochemical constituents

The extract of *Costus igneus* were analyzed as per the procedure reported by Harbone et al. [13] and the results of the analysis are given in Table-1.

Invitro anti-diabetic activity by glucose diffusion inhibitory assay

Glucose diffusion inhibitory assay is an invitro method used to study the effects of plant extracts on glucose movements [8]. A solution of 0.15M sodium chloride containing 0.22M D-glucose was prepared. 1.0 mL of this solution was taken in dialysis bag sealed at each end and was considered as control. 1.0 ml of ethanol extract & 1.0 ml of 0.15M sodium chloride containing 0.22M D-glucose were added into another dialysis bag and was the test solution. The dialysis bag was kept in a sodium chloride solution and was incubated at room temperature. The diffusion of glucose from the bag into an external sodium chloride solution was tested calorimetrically. 1.0 ml of external solution was pipetted out into 1.0 ml of dinitrosalicylic acid, boiled for 15 min; the absorbance was measured at 540 nm. The concentration of the glucose in the external solution was obtained by colorimetric method.

The test was carried out over the incubation period of 1hr, 3hr, 5hr, 24hr and 27hr. Similarly, the above analysis was carried out for the extract of pet.ether, aqueous and the results are presented in Table- 2.

α-amylase inhibition assay

α-amylase is an enzyme that hydrolyses the polysaccharides such as starch, glycogen into glucose and maltose. α-amylase inhibitors bind to α- bond of polysaccharide and prevent the breakdown of polysaccharide into mono and disaccharide. By means of inhibiting the α–amylase, the glucose formation from the glycogen and starch will be reduced [8]. A mixture containing 500µL of ethanolic extracts (20µg/ml), 500µL of sodium phosphate buffer and 500µL of α-amylase enzyme solution were incubated at 25ºC for 10 min. After the incubation, 500µL of 1% starch solution was added and again incubated at 25ºC for 10min. The reaction was stopped by adding 1mL of dinitro salicylic acid, the whole system was once again incubated in boiling water bath for 15min, cooled to room temperature and subjected to colorimetric analysis at 540 nm for the estimation of glucose obtained through hydrolysis of starch by the α-amylase. Similarly, the above analysis was carried out for pet.ether and aqueous extracts. A mixture containing 500µL of sodium phosphate buffer, 500µL of α-amylase enzyme and starch was used as control. Ascorbic acid was used as standard α-amylase inhibitor and the % of inhibition are given in Table-3.

Antioxidant assay

The antioxidant properties of this plant are measured by ferric reducing antioxidant power assay (FRAP) [14] and 2, 2-diphenyl-1-picrylhydrazyl assay (DPPH) [15].

FRAP assay

The antioxidant activity of the plant depends on the capacity of the phytochemical constituents in reducing the ferric to ferrous ion. The formation of coloured ferrous complex was analyzed at 700 nm calorimetrically. The 0.2 mL of ethanolic extract of *Costus igneus* was mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was vortexed well and incubated at 50ºC for 20 minutes. 2.5 ml of trichloroacetic acid was added into the tubes and centrifuged at 3000 rpm for 10 minutes. Into the 2.5mL of supernatant, 0.5 mL of ferric chloride was added, the coloured complex formed was analyzed and the reducing power of the extract was calculated. The buffer & potassium ferricyanide were used as a blank and ascorbic acid was used as a standard reference. Similarly, the above analysis was carried out for ethanolic, pet.ether and aqueous extract at various concentrations (4, 8, 12, 16 and 20 mg/mL). The analyses were performed in triplicates and the results are presented in Table-4.

DPPH assay

DPPH is a stable free radical reacts with antioxidant and will be reduced to DPPHH, correspondingly the colour changes from purple to yellow, the degree of colour changes indicates the scavenging potential. If the plant extracts have the antioxidant property, it will also reduce the DPPH and correspondingly the colour changes from purple to yellow. Ascorbic acid was used as standard and extracts of *Costus igneus* was used as test samples. A stock solution of 1.0 mM of DPPH was prepared by dissolving 3.94 mg in 10mL of methanol. 1.0 mL of stock solution was diluted with methanol to 10mL and was used for further analysis. Extracts of *Costus igneus* was taken in a test tube, into this 2.0 mL of DPPH solution was added. The reaction mixture was shaken well and incubated at dark for 20 minutes. The colored solution obtained was subjected to colorimetric analysis at 517nm. The analysis was performed in triplicates and the values were recorded and a blank was carried out at same conditions. Similarly, the above analysis was carried out for pet.ether, aqueous extract at a concentration of 20, 40, 60, 80 and 100 mg/mL. The antioxidant activities were expressed as % scavenging of DPPH, and are presented in the Table-5.

Antibacterial assay

The antibacterial activity was studied by agar well diffusion method. The antimicrobials present in the plant extracts diffuse out and interact with the freshly seeded test organism present in the medium. As a result of interaction between anti-micro & organism, a zone will be formed and the resulting zone of inhibition's diameter is a measure of antibacterial activity. The medium was prepared by dissolving 3.39 g of the Muller Hinton agar in 100mL of distilled water; the medium was autoclaved at 121ºC for 15 minutes and poured on the petriplates. 0.35 g of nutrient medium was dissolved in 25 mL of distilled water, boiled to dissolve completely and was sterilized by autoclaving at 121ºC for 15 min. Bacterial strains were seeded on petriplates containing Muller Hinton agar. Four wells were made on each petriplates and 100 µL of the plant extracts (aqueous, ethanol and pet.ether extracts) were added into it. Petriplates were incubated at 37 ºC for one day and the antibacterial activity was assayed by measuring the diameter of the zone of inhibition. Standard antibacterial agent chloramphenicol disc was used as a positive control and compared with the antibacterial activity of the extracts. The antibacterial effect of various extract of *Costus igneus* against the microorganism Pseudomonas fluorescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Aeromonas hydrophila and Bacillus cereus are given in Table-6.

Result and Discussion

Phytochemicals analysis

The preliminary phytochemical screenings of various extracts of *Costus igneus* leaf are presented in Table-1. The result indicated presence of most of the phytochemicals in the extract of the *Costus igneus* leaf. (Table 1)

The results of the phytochemicals analysis were consistent with our present phytochemical analysis [16]. J.R.Pisari et al. [16] studied the *Costus igneus* and reported the presence of carbohydrates, proteins, tannins, alkaloids, phlobatanins, saponin, flavanoids etc., consistent with our present results.

Evaluation of anti-diabetic activity

Anti-diabetic activity was studied by using dialysis membrane. Dialysis bags containing glucose and the extract were kept in a sodium chloride solution. The glucose diffuse into the external solution, the concentration of glucose was determined and the results are presented in Table-2 and the comparison chart is given Fig-1. (Table 2) (Figure 1)

The glucose movement was checked for the control (without plant extract) and a mean glucose concentration of 82±0.5021µg/mL was reached in the external solution at the end of 27 hr. Ethanol and aqueous extracts has a mean glucose concentration of 65±0.1789 µg/mL and 78±0.4593 µg/mL respectively at the end of 27 hrs. in the external solution. The phytochemical

Table 1: Preliminary phytochemical screening of extracts.

phytochemical	aqueous	ethanol	pet.ether
flavanoids	+	+	–
alkaloids	+	+	+
saponin	–	+	+
tannin	+	+	–
terpenoids	–	+	+
cardiac glycosides	+	+	+
pylobatanin	+	+	–
anthraquinones	–	+	–
glycosides	+	+	+
proteins	+	+	+
steroids	–	+	+
+ indicates presence, – indicates absence			

Table 2: Glucose concentration in the external solution.

time hr	conc. of glucose µg/mL			
	control	pet.ether	aqueous	ethanol
1	35.97	30.57	24.30	24.15
3	47.83	40.34	39.31	38.25
5	62.49	52.47	54.38	50.13
24	70.77	66.27	64.47	60.33
27	82.43	80.17	78.33	65.14

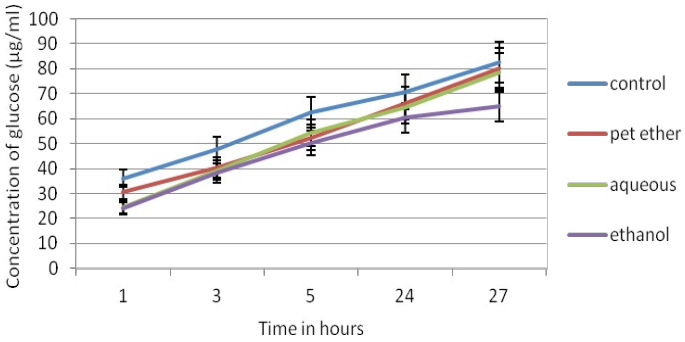

Figure 1: Antidiabetic activity of extracts of *Costus igneus* on dialysis membrane.

Table 3: Percentage inhibition of α-amylase by extract.

conc.of extract (mg/ml)	% of inhibition			
	standard	aqueous	pet.ether	ethanol
20	65.17	38.15	43.62	51.68
40	72.35	40.63	48.72	54.19
60	79.56	41.55	51.93	55.92
80	80.21	45.63	57.63	61.26
100	82.36	48.24	58.82	64.71

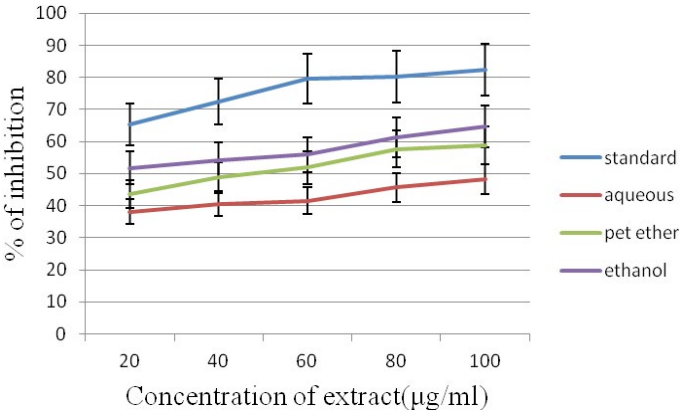

Figure 2: α - Amylase inhibitory activity of various extract of *Costus igneus.*

presents in the extract of pet.ether, ethanol and aqueous solution inhibit the glucose diffusion. Ethanol leaf extract was found to be more potent inhibitor when compared to aqueous and pet.ether extracts. The results of the analysis are comparable with the report of Amanpreet K.Sidhu et al., who studied the anti-diabetic activity by using dialysis membrane [8].

α-Amylase inhibition assay

The extracts of *Costus igneus* were tested for α-amylase inhibition activity; ascorbic acid was used as standard. The % of α -amylase inhibition are given in Table-3 and the comparisons of inhibition activity of extracts are given in Fig-2. (Table 3) (Figure 2)

The ethanolic extracts exhibited highest inhibition activity of 64.71% followed by pet.ether extracts which showed an inhibition activity of 58.82% and aqueous extract displays least inhibition activity of 48.24%. The α-amylase inhibition activity of the extracts of Costus igneus was comparable to the report of B. N.Joshi et al. who studied the inhibition activity of the plant Costus igneus leaf extracts and concluded that the extracts showed good inhibitory activity against α -glycosidase and α -amylase enzymes [17]. The result was also supported by V.Vinotha et al. [18]. The results and the literature study suggested that the plant could be used for the treatment of diabetes.

Antioxidant activity

Antioxidant suppresses the formation of reactive oxygen species and the activity was measured by FRAP & DPPH assay. The antioxidant activity of the extract was studied by FRAP and the % reducing powers are given in Table-4 and the comparative chart is given in Fig-3. (Table 4) (Figure 3)

The results indicated that the extracts of *Costus igneus* have the ability to reduce the ferric ion, indicating that the plant have antioxidant activity. The Fig-3 indicated that the reducing power of *Costus igneus* increased with increasing concentration of the extract. On comparing the reducing power of the extracts with the ascorbic acid a standard reducing agent (reducing power 95.33%) at a concentration of 20mg/ml, the ethanolic extract showed a reducing power of 66.66% while pet.ether and aqueous extract showed reducing power of 56% and 49% respectively.

The antioxidant activity of the plant was studied by DPPH assay and the % scavenging of DPPH by the extracts at different concentrations are given in Table-5 and the comparative chart is given in Fig-4.

The results indicated that the phytochemical present in the extract have the ability to scavenge the DPPH, and the plant have antioxidant activity. On comparing the scavenging activity of the extracts with the standard ascorbic acid (activity 77.27%) at a concentration of 20 mg/ml of extract, ethanolic extract showed scavenging activity of 65.9% while pet. ether and aqueous extract showed a scavenging activity of 54.55% and 31.81% respectively. The results of the analysis are consistent with literature report [19]. (Table 5) (Figure 4)

Both the DPPH assay and DPPH assay study indicated that the ethanolic extract showed high antioxidant activity while the aqueous extract showed least antioxidant activity. Thus, the ethanolic extract of the plants are rich in phytochemical and can be used as antioxidant to prevent or slow down the damage of the cells caused by free radicals.

Table 4: Reducing power of the extract *Costus igneus.*

conc.of extract (mg/ml)	reducing power in %			
	ascorbic acid	ethanol	pet.ether	aqueous
4	26.00	20.67	17.67	8.33
8	44.67	31.00	19.33	16.00
12	62.66	39.33	31.33	20.67
16	80.33	49.67	47.00	39.70
20	95.33	66.67	56.00	49.00

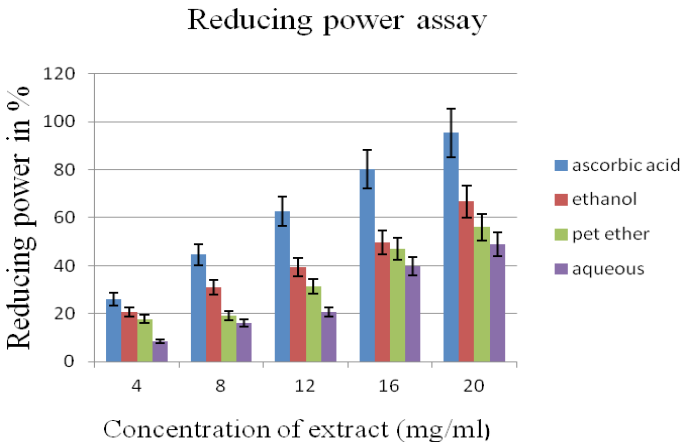

Figure 3: Reducing power assay of various extract of *Costus igneus.*

Table 5: % of free radical scavenging.

conc.of extract (mg/ml)	% of scavenging			
	ascorbic acid	ethanol	aqueous	pet.ether
4	58.13	18.18	2.27	15.90
8	68.18	31.81	9.09	27.27
12	70.45	36.36	20.45	31.81
16	72.72	54.54	27.28	43.18
20	77.27	65.90	31.81	54.55

Evaluation of antibacterial activity

The antibacterial activities of in the plant extracts was studied by agar well diffusion method, the result are given in Table-6 and the comparative chart is given in Fig-5. (Table 6) (Figure 5)

The Fig 5 represents the antibacterial activity of *Costus igneus* extracts and chloramphenicol a standard antibiotics against bacterial organism Pseudomonas fluorescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Aeromonas hydrophilla and Bacillus cereus. The results indicated that all the extract have antibacterial effect against the microorganism. The aqueous extracts showed better antibacterial property in particular it was more effective against organism pseudomonas aeruginosa 28.409±0.05mm and klebsiella pneumonia 21.71±0.04mm. Pet.ether extracts showed least activity in particular it was not exhibiting any antibacterial activity against the microorganism bacillus cereus. In general all the extract showed least antibacterial activity against the microorganism pseudomonas fluorescens [20].

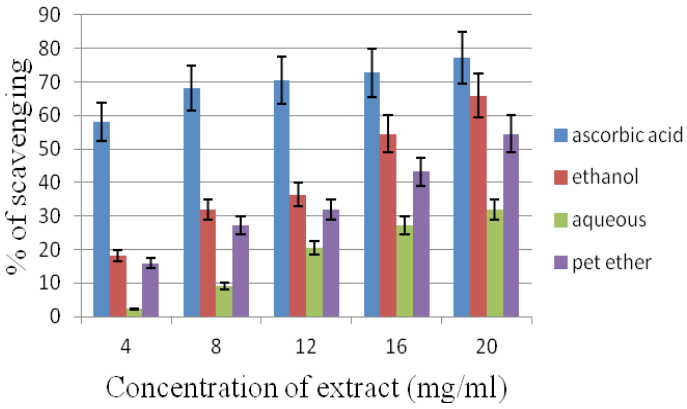

Figure 4: DPPH radical scavenging activity of various extract of *Costus igneus*.

Table 6: Zones of inhibition's diameter.

Organism	zone of inhibition (mm)			
	aqueous	ethanol	pet. ether	chloramphenicol
Pseudomonas fluorescens	7.08	2.76	0.11	7.84
Klebsiella pneumoniae	21.71	18.75	0.11	17.06
Pseudomonas aeruginosa	28.41	17.31	0.44	23.04
Aeromonas hydrophila	19.36	11.56	2.00	28.09
Bacillus cereus	13.40	7.08	0.00	20.79

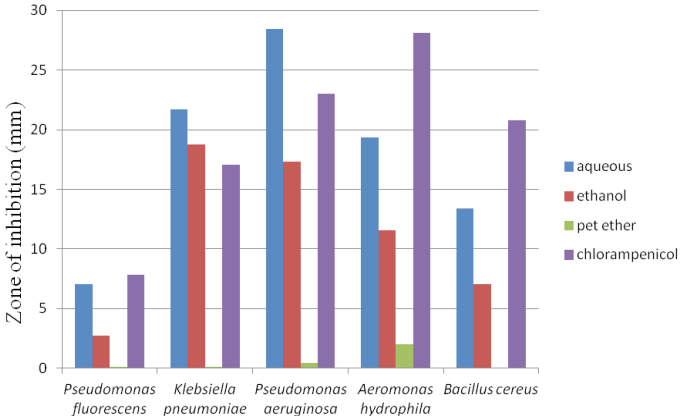

Figure 5: Antibacterial effect of extracts of *Costus igneus* on various organisms.

Conclusion

The presence of phytochemicals in various extracts of *Costus igneus* was identified. The dialysis membrane study indicated that the ethanolic extract exhibited better anti-diabetic activity. The α-amylase inhibition study supported that the ethanolic extracts exhibited high anti-diabetic activity. The FRAP & DPPH assay method indicated that the phytochemicals present in the ethanolic extract have high antioxidant activity. The antibacterial activity was studied by agar well diffusion method and the aqueous extract showed better antibacterial activity. Thus, the *Costus igneus* leaf extract exhibited good anti-diabetic, antioxidant and antibacterial property and *Costus igneus* can be considered as an herbal plant.

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Conflict of Interest

The authors declare no conflict of interest.

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