



PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ACTIVITY AND ANTIOXIDANT ACTIVITY OF *COSTUS IGNEUS*

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ABSTRACT

Costus igneus belongs to the Costaceae family. *Costus igneus* common name is 'Fiery Costus' or 'Spiral Flag', is a species of herbaceous plant in the Costaceae family. The study of antimicrobial, antioxidant activity, preliminary phytochemical screening of the different fractions of dried stem and root extracts revealed the presence of alkaloids, tannins, safonin and terpenoids. For antimicrobial activity, methanol extracts obtained from *Costus igneus* stem and root showed mild to moderate activity against most of the tested bacteria. The antioxidant and radical scavenging activities of *Costus igneus* the following methods; root extract showed more potent antioxidant activity compared to stem extract.

INTRODUCTION

Costus igneus (Insulin plant) is native to south East Asia, especially on the greater islands in Indonesia. It is especially on the greater new entrant to Kerala and India. The plant is characterized by large fleshy looking leaves. The maximum height of these plants is about two feet. The flowers are orange in color and beautiful to 1.5- inch in diameter. Flowering occurs during the warm months [1,2]. *Costus igneus* plant grows very quickly, propagation of this plant is by stem cutting, it needs sunshine but it also grows in slightly shady areas.

The plant grows in all variety of soils. It ensures that soil can drain water easily and roots grow. The plants can be propagated through cutting or roots. It is cultivated in India for its use in traditional medicine and elsewhere as an ornamental. The catchphrase of this plant is 'a leaf a day keeps diabetes away'. In ayurvedic treatment diabetes patients are advised to chew down the insulin plants leaves for months. The patients have to take two leaves per day in the morning and evening for one weak which helps to keeps the blood sugar level normal [3].

According to International Journal of Ayurvedic Research a new study on Insulin plant (*Costus igneous*) was published recently. The leaves of insulin plant (*Costus igneus*) reduced the fasting and postprandial blood sugar levels, bringing them down towards normal, in dexamethasone -induced hyperglycemia in rats. Reduction in the fasting and the postprandial blood sugar levels with leaves of insulin plant was comparable with that obtained with Glibenclamide 500 µg/kg at 250 mg/kg/day and 500 mg/kg/day of powdered leaves of the insulin plant (*Costus igneus*).

The present study was planned to evaluate the effect of the leaves of *Costus igneous* on dexamethasone-induced hyperglycemia in male Wistar rats. Four groups of male Wistar rats (n= 6) were treated with 10 mg/kg/day of dexamethasone subcutaneously for 20 days. From day 11 to day 20, different groups received 100, 250 or 500 mg/kg/day of powdered leaves of *Costus igneus* in distilled water orally or Glibenclamide 500 µg/kg orally [4]. On the 20th day, after overnight fasting, a retro-orbital puncture was performed for obtaining blood samples to estimate the fasting blood glucose level, and the same procedure was followed on the other eye 1 hour after a glucose load of 2.5 g/kg orally for estimation of post-glucose load blood glucose levels.

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Fasting blood sugar and post glucose load blood sugar levels were raised in the group that received dexamethasone when compared to normal controls ($P < 0.001$), whereas 250 and 500 mg/kg powdered leaf of *Costus igneus* and Glibenclamide 500 µg/kg decreased the dexamethasone induced hyperglycemia ($P < 0.01$). The leaves of *Costus igneus* reduced the fasting and postprandial blood sugar levels, bringing them towards normal, in dexamethasone-induced hyperglycemia in rats.

MATERIALS AND METHODS

Root, stem and leaves part of insulin plant of *Costus igneus* plant were used for phytochemical screening, antimicrobial activity and antioxidant activity using Hi-media Nutrient Broth and Nutrient agar and the test organisms *Escherichia coli*, *Enterobacter aerogens*, *Staphylococcus aureus*, *Bacillus Subtilis*, *Klebsiella oxytoca*, *Pseudomonas fragi*, *Streptococcus pyogens*, *Bacillus cereus* and *Proteus vulgaris*.

Methods

Plant Extraction

Fresh roots and stem of *Costus igneus* were collected from the nursery. The stem and roots were washed and were shade dried at room temperature for 4-5 days, after which it is grinded to a fine powder.

Extraction

30g of root and stem sample were taken in two separate beaker. 150 ml of methanol were added to both the beakers and kept it for stirring in magnetic stirrer for 24hrs at 35-40°C, after 24hrs of shaking the extracts were taken and filtrated using watt man filter paper. The solvents were evaporated under pressure and were stored at refrigerated condition until use

Preparation of Standard for Antimicrobial activity

50 mg of Root extract was dissolved in methanol using 10 ml volumetric flask.

Antioxidant Assay

Preparation of Standard for Antioxidant Activity

50 mg of Stem extract was dissolved in methanol using 10 ml volumetric flask.

The purpose of the present study was to evaluate the in vitro antioxidant activity of different fractions from extracts of *Costus igneus* by performing following tests and assays...

- Poly phenol estimation
- Preparation of DPPH Assay
- Vitamin C Assay
- Reducing Assay

Phytochemical Screening

Various tests were carried out to test the presence of different phytochemicals in the samples viz., terpenoids, flavanoids, saponins, tannins and alkaloids.

RESULTS AND DISCUSSION

In this study of *antimicrobial*, antioxidant activity, preliminary phytochemical screening of the different fractions of dried stem and root Extracts revealed the presence of alkaloids, tannins, saponin and terpenoids and the results are tabulated.

Antimicrobial activity of extract

Methanol extracts obtained from *Costus igneus* stem and root showed mild to moderate activity against most of the tested bacteria.

The plates were then made to check the antimicrobial activity compared with those of "Gentamycin" as a standard antibiotic using methanol extract of root showed strong activity against *Klebsiella Oxytoca*, *Pseudomonas Fragi*, *Enterobacter aerogens* and using the agar diffusion assay was observed.

The antioxidant and radical scavenging activities of *Costus igneus* the following methods were used in which both Stem extract and Root extract exhibited antioxidant activity. root extract showed high inhibition than stem extract. We considered root extract has more potent antioxidant activity compared to stem extract

In this estimation methanol extract of *Costus igneus* of stem exhibit high activity (mg/g equivalent of Gallic acid) and the phenol contents were increased in Root extract. Among the stem and root extracts of *Costus igneus*, the total phenolic contents were found to be greater for roots extracts rather than stem. Root extraction from methanol is Suitable for total phenolic extraction. Root extract of *Costus igneus* from have high amount of vitamin E.

Total Reducing Power Assay

This method is used to total reducing power in the extracts in reducing power assay root extract seemed to have high reducing activity, compared to stem extract. The reducing power assay of different extract is given in table

Root extract exhibited increasing concentration ranging than stem extract which did not inhibit the growth of tested organism used in the study at any concentration ranging from 100 µg/mL -500 µg/mL

Phytochemical screening

Phytochemical screening of the different fractions of dried stem and root extracts revealed the presence of alkaloids, tannins, flavonoids, and terpenoids.

Costus igneus plants contain positive result of stem extracts are flavonoids, and tannins.

Root extract are terpenoids, tannins and alkaloids and are important to treat various diseases. The comarision between stem extract and root extract in which root extract showed more activity which are beneficial for the treatment of various diseases which are caused due to the oxidative stress like cancer and diabetes. If we isolate these phytochemicals then it will be very helpful for the



herbal drug industries to make new drugs and ultimately it helps the human beings [5].

Based on the results of antimicrobial testing methanol used for the Insulin leaves can inhibit *E. aerogens*, *Klebsiella oxycota*, *Pseudomonas fragi* showed better than the extract viscous of methanol. This is marked by the diameter of the inhibition of the four microbial tests.

Extract of insulin roots towards the bacterial test based on the result of the antimicrobial testing, we can conclude that n-butanol fraction has the best activity in inhibiting fungi or bacteria [6].

Antioxidant activity of insulin plant significantly increase with the presence of high concentration of total phenol content. Flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [7]. The extracts are very complex mixtures of many different compounds with distinct activities.

Polyphenol and antioxidant scavenge off this radical and hence inhibit cellular damage. It is apparent from the present study that the polyphenols and antioxidant

not only scavenge off the free radicals but also inhibits the generation of the free radical. It was already reported that naturally occurring phenolic compounds have free radical scavenging properties due to their hydroxyl groups. Further phenolic compounds are effective hydrogen donors which make them antioxidant [8]. The entire fraction showed free radical scavenging activity in different extracts of this study the antioxidant activity of these might be due to inactivation of free radical or complex formation with metal ions, or combinations of both.

The extracts of scavenging the stable DPPH radical are a widely used method to evaluate the scavenging ability of various samples. It was found that the radical scavenging activities of all extracts increased with increasing concentrations. The high phenol content of root and stem of *Costus igneus* may cause high antioxidant activity of this plant. Phenols and phenolic compounds such as flavonoids are widely found in foods products which are derived from plant sources, and they have been shown possess significant antioxidant property [9].

Table 1. Antioxidant Activity

Sl. no	Organisms used	Result	Zone formation
1	<i>Enterobacter aerogens</i>	+ve	1.4cm
2	<i>Streptococcus pyogens</i>	-ve	
3	<i>Bacillus cereus</i>	-ve	
4	<i>Klebsiella oxycota</i>	+ve	1.6cm
5	<i>Pseudomonas fragi</i>	+ve	1.2cm

Table 2. DPPH Assay

Sl. no	Concentration of root sample(mg/ml)	% of inhibition of root	% of inhibition of stem
Control	-	-	-
1	40	30%	5%
2	80	40%	20%
3	120	59%	22%
4	160	80%	30%
5	200	83%	43%

Table 3. Standard Assay

Sl no	Concentration of Gallic acid Solution (mg/ml)	Optical density at 760nm	% of inhibition
Blank	-	-	-
1	10	0.192	0.2
2	20	0.288	0.4
3	30	0.538	0.6
4	40	0.718	0.8
5	50	1.117	1.0

Table 4. Polyphenol Assay

Sl. No	Concentration of Extract In Mg/MI	OD At 760nm Root	OD At 760nm Stem
Control	-	-	-
1	0.1	0.213	0.075
2	0.3	0.231	0.186
3	0.5	0.362	0.250



Table 5. Total Reducing Power Assay

Sl.no	Concentration of extract in (mg/ml)	OD at 700nm of root sample	OD at 700nm of stem extract
Control	-	-	-
1	100	0.162	0.027
2	200	0.194	0.084
3	300	0.266	0.100
4	400	0.406	0.147
5	500	0.593	0.186

Table 6. Phytochemical screening

Sl no.	Test	Result of Stem	Root
1	Terpenoids	+ve	+ve
2	Flavonoids	+ve	-ve
3	saponin	-ve	-ve
4	Tannins	+ve	+ve
5	Alkaloids	-ve	+ve

CONCLUSION

It may be concluded the fraction obtained from different extracts of *Costus igneus* have significant antioxidant activity. The antioxidant potential may be

attributed to the presence of polyphenolic compounds. These results are encouraging enough to pursue characterization of this fraction.

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