

Phytochemical Analysis and Antibacterial Determination of *Costus Igneus* Leaves

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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. *Costus igneus* is traditionally known as insulin plant in Maharashtra. In India it is grown in garden as ornamental plant. This plant is becoming popular because of its anti-diabetic property. In the study is preliminary effort to provide basic analytical value for *Costus igneus* leaf powder. Identification of the secondary metabolites from *Costus igneus* plant in therapeutic application of diabetes is of growing interest as they contain many active phytochemical constituents against hyperglycaemic condition. The antibacterial potential of *Costus igneus* hot and cold aqueous extract was tested by using agar well diffusion method.

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KEYWORDS: *Costus igneus*; phytochemical; anti-bacterial

INTRODUCTION

Plant used in traditional medicine may comprise a substantial source of modern biologically active compounds. *Costus igneus* common name fiery costs or spiral slag is an herbaceous belong to cetacean family. It is a delicious perennial herb, enlarging to 2.7 m tall and having erect stem the plant reproduces vegetative through rhizomes or seeds dispel by bird (Vinayaka *et.al.*2011). They are greyish green stained with red purple atop darker purple below the teeny white flowers grow occasionally throughout the year. It is specially on the higher new entrant to Kerala and India (Ramya *et.al.*2015). The plant extracts have been developed and proposed for use as antimicrobial substance. The antibacterial activities of medical plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoid that are present in this plant. In the recent year, secondary plant metabolites (phytochemical), previously with unknown pharmacological activities have been intensively investigated as a source of medical plants. Thus, it is anticipated that phytochemical with adequate bacterial efficacy will be used for the bacterial infection (Vasantharaj *et.al.*, 2013).

MATERIAL AND METHOD

Collection of sample

Mature leaves of *Costus igneus* were collected from Titwala garden in Kalyan.

Processing of sample

All leaves were washed under tap water separately in batches and shade dried for seven to ten days. After drying leaves were processed in powdered using electric grinder and stored in airtight container.

Preparation of leaf extract with aqueous solution

For Cold extract, 2gm of leaf powder was added to 20mL of distilled water and stirred it constantly for half an hour. This mixture was kept at room temperature for 24 hours. Followed by filtration through Whatman filter paper no.1. For Hot extract, 1gm of leaf powder was added to 20mL of distilled water and stirred it constantly for half an hour. This mixture was kept at room temperature for 24hour. The solution was then boiled at 100°C to make hot extract followed by filtration through Whatman filter paper no.1

Phytochemical analysis of hot aqueous leaf extract of *Costus igneus*

Test for phenol

For qualitative determination of phenol 2.0 mL aqueous plant sample were taken. To these extracts, 0.5 mL of FeCl₃ solution was added. Formation of intense brown colour indicates the precipitate of phenol.

Test for flavonoid

For qualitative determination of flavonoid, 2.0 mL of aqueous plant sample were taken. To these extracts, 0.5 mL of NaOH solution was added. Formation of intense yellow

colour that become colourless on addition of few drop of diluted HCl indicated the presence of flavonoid.

Test for cardiac glycoside

For qualitative determination of cardiac glycoside 2.0 mL of aqueous plant sample were taken. To these extracts, 2 mL of chloroform solution was added. And add these extracts, 2 mL conc. H₂SO₄ solution was added. Formation of intense layer form and deep brown colour indicates the precipitate of cardiac glycoside.

Test for triterpenes

For qualitative determination of triterpenes, 2.0 mL of aqueous plant sample were taken. To these extracts, 0.5 mL of concentrated H₂SO₄ solution was added Formation of intense brown ring indicates the precipitate of triterpene.

Test for saponins

For qualitative determination of saponins, 2.0 mL of aqueous plant sample were taken. Addition of 20 mL distilled water was done along with extracts separately. The test tube was then shaken in graduated cylinder for 15 minutes. The formation of intense 1cm layer of foam indicated the presence of saponins.

Test for tannins

For qualitative determination of tannin, 2.0 mL of aqueous plant sample were taken. To these extracts, 0.5 mL of FeCl₃ solution was added. Formation of intense brownish green colour indicates the precipitate of tannin.

Test for carbohydrate

For qualitative determination of carbohydrate, 2.0 mL of aqueous plant sample were taken. To these extracts, 0.5 mL of H₂SO₄ solution was added. And these extracts, 2 mL of Molisch reagent was added. Formation of intense violet ring indicates the precipitate of carbohydrate.

Test for phlobatannins

For qualitative determination of phlobatannine 2.0 mL of aqueous plant sample were taken. To these extracts, 2 mL of 1% HCl solution was added and it was then kept for boiling in water bath. Presence of phlobatannine is indicated by intense red colour precipitate.

Test for alkaloids

For qualitative determination of alkaloids 2.0 mL of aqueous plant sample were taken. To these extracts, 2.0 mL of Wagner's reagent solution was added. Formation of intense brownish colour indicates the precipitate of alkaloids.

Test for steroids

For qualitative determination of steroid 2.0 mL of aqueous plant sample were taken. To these extracts, 2 mL of chloroform solution was added. And these extracts, 2mL of conc. H₂SO₄ solution was added. Formation of intense yellow with green fluorescence colour indicates the precipitate of steroid.

Test for terpenoid

For qualitative determination of terpenoid 2.0 mL of aqueous plant sample were taken. To these extracts, 2 mL of chloroform and 0.5 mL of concentrated H₂SO₄ was added. Formation of intense reddish-brown colour indicates the precipitate of terpenoid.

Test for reducing sugar

For qualitative determination of reducing sugar, 2.0 mL of aqueous plant sample were taken. To these extracts, 2 mL of Fehling A and Fehling B solution was added and boiled for 5 minutes. Formation of intense orange red colour indicates the precipitate of reducing sugar.

Test for protein

For qualitative determination of protein 2.0 mL of aqueous plant sample were taken. To these extracts, 2.0 mL of Ninhydrin reagent and 2.0 mL were added, and boiled for 5 to 10 minutes in boiling water bath. Formation of intense dark purple colour indicates the precipitate of protein.

IN VITRO DETERMINATION OF ANTIBACTERIAL ACTIVITY OF AQUEOUS PLANT EXTRACT FROM *COUSTUS IGNEUS* Qualitative test agar well diffusion method (Indian pharmacopoeia, 2007).

The colonies of the organism from overnight grown standard cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 were picked up with inoculating loop and suspended in 3.0-4.0mL of sterile saline. This was further adjusted to 0.5 McFarland's standard and used as inoculum for assay. Sterile muller nutrient agar was mixed with 1.0mL of bacterial inoculum and poured into sterile petri plate. The petri plates were poured on the level surface and allowed to solidify. Four wells were punched in each using sterile cork borer each of 8mm in diameter at equivalence 100µl of each dilution of cold plant extract and hot extract were added to the wells. Plates were incubated at 37° C for 24 hours.

RESULT AND DISCUSSION

Phytochemical Analysis of Aqueous Extract

Leaf powder was added to distilled water and stirred continuously for half an hour. This solution was kept at room temperature for 24 hours. The solution was then boiled at 100 to make hot extract followed by filtration through Whatman filter paper no 1. The result of Phytochemical analysis from hot leaves extracted from *Costus igneus* were as shown in table 1.

All phytochemicals were present in hot leaves extract of *Costus igneus* except for phlobatannine, reducing sugar, steroids and flavanoid. Similar results using aqueous extract of leaves were obtained by **Thiruchenduran et.al., 2017**

In vitro Determination of Antibacterial Activity of Hot and Cold Aqueous Plant Extract from *Costus igneus*

Qualitative study of hot and cold extract of *Costus igneus* was done by agar well diffusion method using different dilution of hot and cold extract of *Costus igneus* in the range of 10mg/ml to 100mg/ml on standard cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538. The results were as displayed in table 2 for hot and cold extract.

Both hot and cold aqueous extract leaves of grider powder of *Costus igneus* showed no zone of inhibition against standard cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 by agar well diffusion method. To compare result of antibacterial activity against test microorganism in the present study no literature and reported were available. Hence this kind of comparative study may be first reported and may serve as base line study to evaluated further by researcher in future

CONCLUSION

Costus igneus commonly known as fiery Costus, step ladder or Spiral flag or insulin plant is native to south and central America and used for its antidiabetic property and prevent the body from disease protect mind and which prolongs the longevity of life. The phytochemical analysis method in which identified phenol, triterpene saponins, tannins, carbohydrate, alkaloids, protein, terpenoids from hot extract *Costus igneus* plant. The antibacterial activity of cold and hot extract does not show the zone of inhibition around the well. The result of study has justified the to use different solvent extract for determine the antibacterial activity. These phytochemical constituents are of organic in nature. Bioprocess can convert simple compound to complex compound and it uses in several medicines and therapeutics.

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Table1: Phytochemicals from hot extract leaves of *Costus igneus*

Phytochemical test	Result
Phenol	+
Flavonoid	-
Cardiac glycoside	+
Triterpene	+
Saponins	+
Tannin	+
Carbohydrate	+
Phlobatannins	+
Alkaloid	+
Protein	+
Steroid	-
Terpenoid	+
Reducing sugar	-

Key: (+) Positive, (-) Negative

Table2: Determination of antibacterial activity by aqueous extract of *Costus igneus* leaves

Organism	Plant extract	Concentration of plant extract (mg/ml)									
		10	20	30	40	50	60	70	80	90	100
		Zone of inhibition (mm)									
<i>Escherichia coli</i> ATCC 25922	Cold	-	-	-	-	-	-	-	-	-	-
	Hot	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i> ATCC 6538	Cold	-	-	-	-	-	-	-	-	-	-
	Hot	-	-	-	-	-	-	-	-	-	-

Key: (-) No growth