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Qualitative and quantitative phytochemical analysis of *Costus igenus* leaf extract

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Abstract
Costus igneus, is a medicinal plant and capable of having magic cure for diabetes. leaf of this herbal plant helps to build up insulin by strengthening β–cells of pancreas in the human body thus popularly known as "insulin plant" in India. In the present study qualitative analysis, leaf extract of *Costus igenus* were analysed for the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoids and glycosides were screened in methanolic solvent extracts. Methanolic extract of the leaf showed maximum amount phytochemicals was screened in *Costus igenus* Quantitative estimation of *Costus igenus* showed the presence alkaloids, saponins, phenols and flavonoids determined by using standard methods. From the results, it is evident that the plant *Costus igenus* leaves are found to have maximum amount of phytocomponents.

Keywords: Phytochemical constituents, *Costus igenus*, qualitative and quantitative analysis

1. Introduction
Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have define mechanism and protect from various diseases. Phytochemical studies have gained a lot of interest among the plant scientists due to the development of newer technology and sophisticated outcome. These techniques play a significant role in finding of important material for pharmaceutical industry [1]. Plants have substances that induce a great interest due to their versatile applications [2]. It is estimated that 14-18% of higher plant are used medicinally and related to 74% of pharmacologically active plant are discovered after following up on ethnomedicinal usage of the plants [3].
The plant *Costus igneus* belongs to the family Costaceae, which is found in tropical Africa, Asia, Australia, and North, Central and South America. In India, it is cultivated in coastal area, Uttar Kannada district of Karnataka. In this area, people take traditionally 2-3 leaves of this plant twice a day for the management of diabetes. It is a prostrate growing plant with spreading, rooting stems. Its leaves are slender and lance shaped with tooted, scalloped or lobed margins. They are grayish green stained with red purple above and darker purple beneath. The tiny white flowers grow intermittently throughout the year. This plant reaches a height of 6-inches and have an indefinite spread [4-7]. Considering the medicinal importance of these widely available plant species, the aim of the present study was to identify the Qualitative and Quantitative phytochemical analysis of *Costus igneus*.

2. Materials and Methods
2.1 Collection of plant material
Fresh *Costus igneus* leaves used in this experiment were collected in the surrounding areas of Lalgudi, Trichy.

2.2 Extraction of plant material
Healthy plant leaf *Costus igneus* were collected, washed thoroughly in tap water and dried in room temperature for 30 days. The dried leaves were powdered and 25 g leaf powder soaked in 225 ml of Methanol for 3 days. The extracts were filtered through whatmann No.1 filter paper. Similar process was repeated twice with fresh solvent and the filtrate was collected together. Similar procedures were followed for other solvents like Dichloromethane, Petroleum ether, hexane and benzene. The extract was stored at the refrigerator for further studies.

2.3 Qualitative analysis of primary and secondary metabolites from *Costus igneus*
The leaf extracts from *Costus igneus* were analyzed for the presence of alkaloids, carbohydrates, reducing sugar, saponin, protein, phenolic compounds, tannin and

glycoside according to the common phytochemical methods described by Harborne (1998).

2.4 Quantitative estimation of secondary metabolites from *Costus igneus*

2.4.1 Determination of Alkaloid by the method of Harborne (1973) [8]

5 gs of the *Costus igneus* leaves powdered was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solutions were allowed to settle and the precipitated were collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.4.2 Determination of Saponin by the method of Obadoni and Ochuko (2001) [9]

5 gs of *Costus igneus* leaves powder were put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hs with continuous stirring at about 55 ^{0}C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{o}C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant

2.4.3 Determination of total phenols by spectrophotometric method of Kim *et al*., (2003) [10]

A diluted *Costus igneus* leaves extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23 °C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The Total Phenolic content was expressed as milligrams of Gallic acid (GAE) equivalents per gram dried sample.

2.4.4 Determination of total Flavonoids by the method of Katasani (2001) [11]

Costus igneus leaves extract (0.5 ml) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions to concentrations 20 to 80 µg/ ml in methanol**.** The Total flavonoids content was expressed as milligrams of quercetin equivalents per gram of dried sample.

3. Results and Discussion

3.1 Qualitative Phytochemical analysis of *Costus igneus* from leaf extract

The phytochemical screening of *Costus igneus* leaf extracts was done with Methanol, Dichloromethane, Petroleum ether, hexane and benzene The qualitative analysis of bioactive compounds for five extracts shown in (Table-1). Methanolic extract was found to have a wide range of bioactive compounds like alkaloids, carbohydrates, reducing sugar, protein, saponin, glycoside, flavonoids. Benzene extract was found to have a wide range of bioactive compounds like carbohydrates, saponin, glycoside and protein. Hexane being non-polar in nature was able to extract very less amount of bioactive compounds characterized like alkaloids, carbohydrates, tannin, glycoside and Phenolic compounds. The chloroform extract was positive for carbohydrate, Protein, reducing sugar, glycoside and Phenolic compounds. The Dichloromethane was found to have alkaloids, carbohydrate, reducing sugar and flavonoids.

Among the five different extracts, Methanolic extract of the leaves showed maximum amount phytochemicals was screened in *Costus igneus*. The presence of bioactive constituents indicates that the *Costus igneus* can be used in a multitude of ways for the beneficiary of population.

3.2 Quantitative Determination of secondary metabolites from Costus *igneus*

The amount of alkaloids, saponin, Total phenols and Total flavonoids was determined by using standard methods (Table-2)

Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.016x + 0.255$ $R^2 = 0.997$ (Figure: 1) where y is absorbance at 750 nm and x is total phenolic content in the extracts of *Costus igneus* expressed in mg/gm. The total phenolic content was 25.3±0.0027 mg/gm in the extracts, showed in (Table-2). The amount of total flavonoids was determined with aluminum chloride reagent. The calibration curve for quercetin is shown in (Figure: 2). Quercetin was used as a standard compound and the total flavonoid was expressed as mg/g quercetin equivalent using the standard curve equation: $y = 0.016x + 0.255$ $R^2 = 0.997$ (Figure: 2). Where y is absorbance at 510 nm and x is total flavonoid content in the extracts of *Costus igneus* expressed in 58.3±0.2837mg/gm. The total flavonoid content was mg/g in the extracts, respectively (Table- 2).

Alkaloids were determined by the method of Harborne (1973). The alkaloid content was 14.5±0.1124 mg/g in the extracts. Saponin was determined by the method of Obadoni and Ochuko (2001). The saponin content was 61.1±0.0823mg/g in the extracts. The phytochemical analyses of the medicinal plants is important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for the treatment of various diseases. Thus, we hope that the important phytochemical properties identified in the present study *of Costus igneus* leaves will be helpful in the treatment of various ailments.

Table 1: Qualitative phytochemical analysis of *Costus igneus* from leaf extract

Phytochemical Tests	Methanol	Benzene	Dichloromethane	Chloroform	Hexane
Alkaloids (Mayer's reagent)	+	-	+	_	+
Carbohydrates (Molisch's test)	+	+	+	+	+
Sugar (Benedict's reagent)	+	-	+	+	-
Saponin (foam test)	+	+	_	_	_
Protein (Millon's test)	+	+	_	+	_
Phenolic compounds and tannin (Ferric chloride test)	_	_	-	+	+
Flavonoid (Alkaline reagent test)	+	_	+	_	_
Glycoside (Legal's test)	+	+	_	+	_

(Presence of Phyto constituents = +) (Absence of Phyto constituent = -)

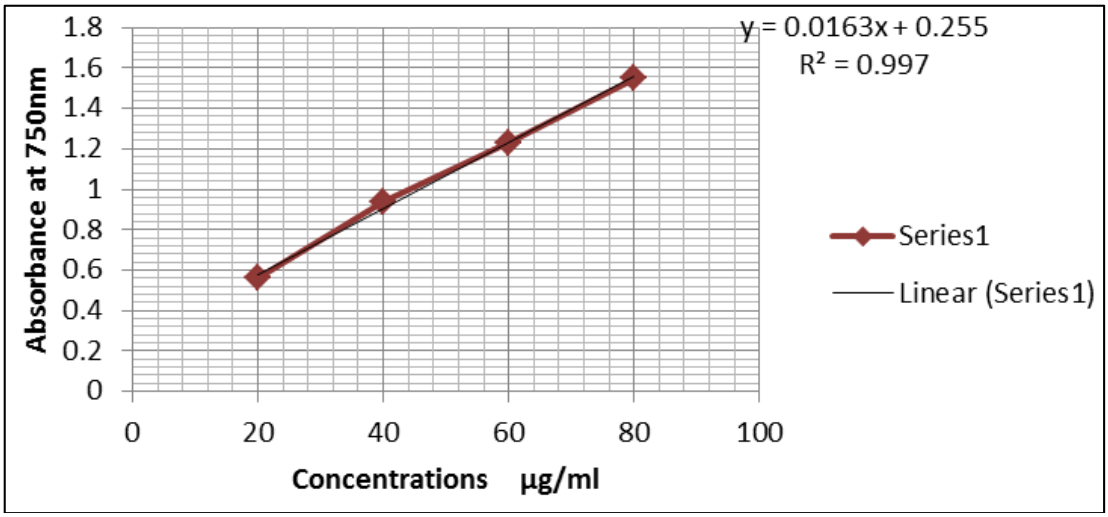

Fig 1: Standard Curve for Total Phenol using Gallic Acid

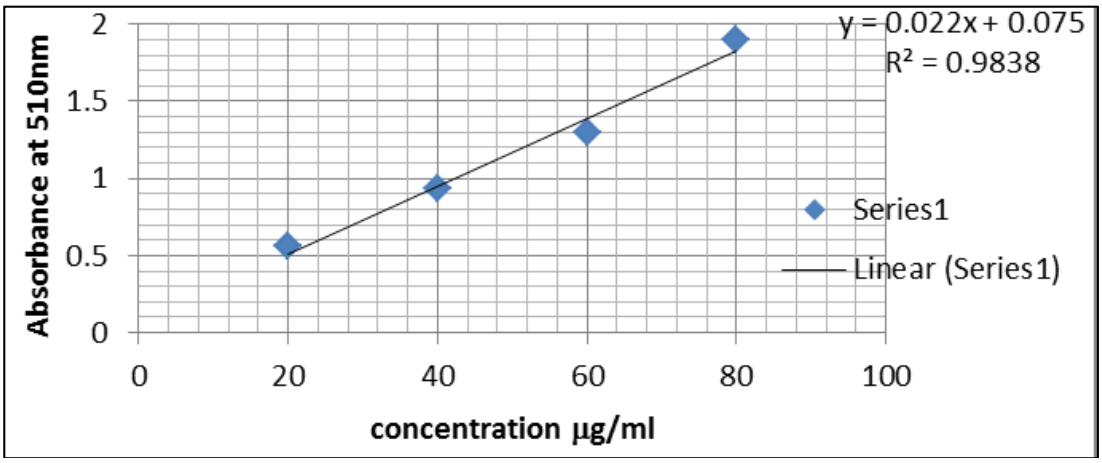

Fig 2: Standard Curve for Flavonoids using Quercetin

Table 2: Quantitative determination of Secondary metabolites from *Costus igneus* leaves.

S. No.	Name of the phytochemical constituents	Results (mg/gm)
1.	Alkaloids	14.5±0.1124
2.	Saponin	61.1±0.0823
3.	Total Phenols	25.3±0.0027
4.	Total Flavonoids	58.3±0.2837

Values are expressed Mean ± SD for triplicates

4. Conclusion

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The qualitative analysis of the five different leaf extract of *Costus igneus* reveals the presence of medicinally valued bio active components like, flavonoids, tannins, alkaloids, saponin, reducing sugar, phenolic compounds and glycosides. Quantitative estimation *Costus igneus* leaf contained higher content of saponins and total flavonoids, with lesser amount of phenols and alkaloids. *Costus igneus* are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs for treatment of various diseases. The research is in progress to discover its biological activity and enhance the pharmacological profile of it in the area of traditional medicine.

5. References

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