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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF COSTUS SPECIOSUS(Koening)

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Abstract

The aim of this study was to investigate the phytochemical composition and antioxidant activity of *Costus speciosus* collected from Courtallam regions of Tamil Nadu. The phytochemical screening was carried out on the leaf extracts of *Costus speciosus* using five different solvents. The phytochemical analysis revealed the presence of active ingredients such as steroids, saponins, phenols, flavonoids, terpenoids, cardiac glycosides, coumarins, alkaloids and tannins. Total phenol and flavonoid contents were quantitatively estimated. Total phenol and flavonoid content was quantitatively estimated which recorded maximum in acetone leaf extract of *Costus speciosus* was found with maximum total phenol (25.5 mg GAE /g) and flavonoids content (13.3 mg QE /g). The leaf extracts were evaluated for antioxidant activities by DPPH radical scavenging assay. Among the five different solvents used, maximum antioxidant activity was found in acetone leaf extract of *Costus speciosus* (94) followed by others. The powerful free radical scavenging effect is attributed to the greater amount of total phenol and total flavonoid compounds present in the acetone leaf extracts of *Costus speciosus*.

KEY WORDS: Antioxidant activity, phytochemical screening, *Costus speciosus*

Introduction:

Medicinal plants are of great importance to health of mankind. They are widely used in treatment and prevention of disease in recent years. The bioactive compounds of the plants are the main source of pharmaceutical and health care products. Knowledge of chemical constituents of plants contribute to the discovery of new drugs.From the numerous plants being researched *Costus speciosus* (Koening) is one such plants that has ethano medical importance. The plant belongs to family costaceae and commonly called as "crepe ginger". In tamil the plant is known as "kostum". It is found in sub Himalayan range and in Western Ghats of Maharashtra, Karnataka and Kerala (Sarin *et al*, 1979).It is also found in the tropical regions of India and cultivated for ornament. There are more than hundred species of costus. The different species of costus vary in the flower colour. *Costus speciosus* is a perennial, rhizomatous herb with erect or spreading stem (Gupta 2010). The plant reproduces vegetatively by rhizome or stem cutting. The leaves are large and spirally arranged on the stem. Several studies on different parts of *Costus speciosus* showed that plant antifertility, antiinflammatory, antipyretic and antihelminthic activities (Binny *et al*., 2010).

The leaf and the rhizome have been reported to possess steroid-diosgenin, which is antidiabetic in nature (Roy and Datta 1977). The leaf also possesses hypoglycemic properties and insulin potentiating action in addition to decreasing blood glucose (Eliza *et al* 20018). The present work is focused to reveal the quantitative photochemical studies to find out the bioactive compound and antioxidant activity of leaf extract of *Costus speciosus*.

Materials And Methods:

Costus speciosus was collected from the Coutrallam region of Tamil Nadu. The fresh leaves were collected and air dried under shade.After complete drying the dried leaves were ground into coarse powder and stored in air tight bottles for further use.

Preparation of the plant extract:

Preparation of the extracts was assessed by following method as described by Pizzale *et al*.,(2002) and Lu and Foo(2001). About 1g of fleshy dried powder of *Costus speciosus* plant materials were extracted with 20 mL ethanol 75%, acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 minute using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40 °C to a constant weight and then dissolved in methanol ethanol and water. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

Phytochemical Screening from leaf extracts of *Costus speciosus*

The phytochemical screening of leaf extracts were assessed by standard method as described by Brinda *et al.*, (1981); Siddiqui and Ali (1997) and Savithramma *et al.*, (2011). Phytochemical screening was carried out on the leaf extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides**,** coumarins and steroids. General reactions in these analysis revealed the presence or absence of these compounds in the leaf extracts tested.

Table.1. Phytochemical screening from leaf extracts of *Costus speciosus*

Phytochemicals	Leaf extract				
	Aqueous	Ethanol	Chloroform	Acetone	Petroleum ether
Tannins	-	+	-	+	-
Flavonoids	++	++	-	+	-
Quinones	+	+	-	+	-
Glycosides	+	++	++	++	++
Cardiac glycosides	+	++	+	++	+

Terpenoids	+	++	+	++	+
Phenol	++	++	+	++	-
Coumarins	+	+	-	+	-
Steroids	+	++	+	++	+
Alkaloids	+	+	-	+	-
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	+	+	-

+ + strong positive

\+ positive

\- negative

Quantitative Estimation of Phytochemicals

Estimation of Total phenolic content (TPC)

Total phenolic content in the acetone leaf extracts of ***Costus speciosus*** was determined by the Folin Ciocalteau colorimetric method (Lister and Wilson, 2001) with slight modification. For the analysis, 0.5 ml of dry powdered ethanolic leaf extracts were added to 0.1 ml of Folin-Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of Sodium carbonate (Na2CO3) 2% (w/v) was added. The blend was incubated in the dark at room temperature for 15 min. The absorbance of blue-colored solution of all samples was measured at 765 nm. The results were expressed in mg of Gallic acid equivalent (GAE) per g of dry weight of plant powders.

Estimation of Flavonoid Content

Total flavonoids content in the acetone leaf extracts was determined by the aluminium chloride colorimetric method (Mervat *et al*., 2009). 0.5 ml of leaf extracts of *Costus speciosus* at a concentration of 1mg/ ml were taken and the volume was made up to 3ml with methanol. Then 0.1ml aluminium chloride - AlCl3 (10%), 0.1 mL of 1M potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The

concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg Quercetin equivalent per g of sample.

Table-2 Quantification of Total Phenol and Total Flavonoid Content

Sample	TPC mg GAE/g dry material	TFC mg QE/g dry material
Costus speciosus	25.5	13.3

Antioxidant Activity

After the completion of phytochemical analysis the acetone extract was taken for antioxidant studies.

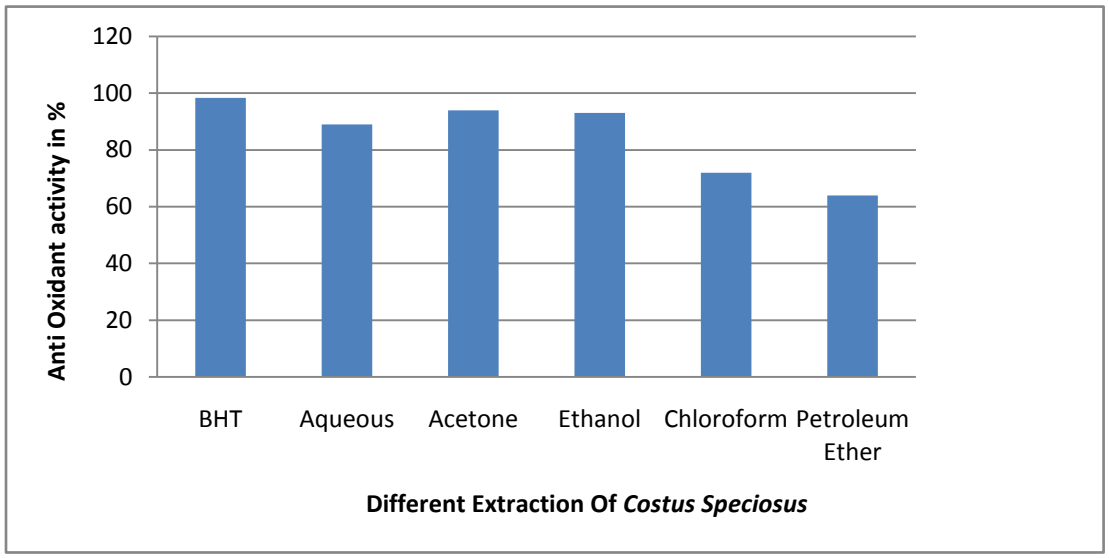
DPPH (2,2 di phenyl-1-picryl hydrozyl) Radical scavenging assay

The antioxidant activity of the leaf extract of *Costus speciosus* was estimated by DPPH radical scavenging protocol. Leaf extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2005). Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Antioxidant activity (%) of given samples at various time intervals

Costus speciosus Extract	Antioxidant activity (%) at various time intervals(time in minutes)						
	0	5	10	15	20	25	30
Petroleum Ether	62	64	63	63	64	64	64
Chloroform	57	66	69	70	71	71	72
Acetone	85	92	93	94	94	94	94
Ethanol	80	91	93	93	93	93	93
Aqueous	69	80	83	85	86	88	89



Results and Discussion

In the present syudy the phytochemical screening was carried out in the leaf extract of *costus speciosus* using five different solvents ethanol, chloroform, petroleum ether, acetone and aqueous. Table 1 shows that the acetone leaf extract of *costus speciosus* was rich in quinones, terpenoids, glycosides, cardio glycosides, coumarins, alkaloids, flavonoids, steroids, phenols, tannins and saponins followed by ethanol and other extracts. The curative properties of medicinal plants are perhaps due to the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, sapaonins, steroids etc (Britto and Sebastian, 2011). Thus the preliminary screening test may be useful in the detection of bioactive principles and subsequently may lead to drug discovery and development (Doss, 2009)

Table 2 shows the estimation of total phenol and flavanoid content in the acetone leaf extract of *costus speciosus*. The flavanoids are important plant antioxidants which exhibit considerable scavenging activity against free radicals. They prevent oxidative cell damage and carcinogenesis (Farquar 1996). The high amount of phenols indicate that they are used as antimicrobial agents (Iqbal Hussain 2011). Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing (Okwu *et al*., 2001)

DPPH radical scavenging activity of acetone leaf extract of costus speciosus are shown in table no 3. Many reports suggest that plants having more phenolic content show good antioxidant activity (Motlhanka *et al*., 2012) and there is a direct correlation between total phenol and antioxidant activity.The high potential of phenolic compounds to scavenge the free radical may be due to many phenolic hydroxyl groups they possess(Vaghasiya *et al.*, 2011).Hence antioxidant are protective against various diseases particularly cardiovascular diseases by inhibiting the oxidation of lipids(Kim *et al.*, 1994).

Conclusion

The result obtained in the present study indicated that the acetone leaf extract of *costus speciosus* has antioxidant potential and the presence of flavonoids and phenols may be the contributing factor for the free radical scavenging potential. Therefore the plant screened is considered as good source of natural product that has the potential to treat diseases associated

with oxidative stress. The results indicate that the plant material may become an important source of natural drug compounds with health protective potential and natural antioxidant of significant impact on the status of human health and disease prevention. Further studies are needed to isolate, characterize and elucidate structure of the bioactive compounds of this plant for industrial drug formulation.

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