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Original Research Article

Qualitative and Quantitative Analysis of Phytochemicals of *Costus Speciosus*

Ramya R[1*], Dhamotharan R[2**]

[1]Assistant Professor, [2]Associate Professor,
[*]Department of Biochemistry, J.B.A.S College for Women, Teynampet , Chennai - 600018
[**]PG & Research Department of Botany, Presidency College, Triplicane, Chennai - 600005

Corresponding Author: Ramya R

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ABSTRACT

The aim of this study was to investigate the Qualitative and Quantitative analysis of bioactive compounds of medicinally important plant *Costus speciosus* collected from Chennai.
*Costus speciosus (*Koening) Smith belonging to family Costaceae is an Indian ornamental plant used medicinally in traditional system of medicine. In the study the leaf extract was prepared using different solvents and were subjected to phytochemical screening. The ethanol leaf extract of *Costus speciosus* indicate the presence of chemical constituents such as Alkaloids, Tannins, Saponins, Steroid, Terpenoids, Flavonoid and Total phenol. Quantitative analysis of the above active compounds was also carried out in the ethanol leaf extract. The results suggest that the presence of bioactive constituents may be used for curing various ailments and possess potential antioxidant, antimicrobial, anti-inflammatory and antidiabetic activity.

Key words: *Costus speciosus*; Phytochemicals; antioxidants; antimicrobial; anti-inflammatory; antidiabetic.

INTRODUCTION

Plants are the source of inspiration for novel drug compounds; as plant derived medicines contribute to human health and well being. The phytochemicals present in the medicinal plants play a vital role in the discovery of new pharmaceutical and health care products. [1] Thus it is important to characterize different types of medicinal plants for their antioxidant, antimicrobial, anti-inflammatory and antidiabetic activities.

Costus speciosus (Koening) Smith belongs to the family Costaceae, is an herbaceous plant. It is commonly called "Crepeginger". [2] It is widely distributed in central parts of India, Sub-Himalayan tract, Karnataka, Western ghats of Maharashtra and Kerala. [3] In Ayurvedic literature it is mentioned as antidiabetic plant. There are more than 100 species of *costus*. The plant is a perennial, rhizomatus herb with erect or spreading stem. [4] The plant reproduces vegetatively by rhizome or stem cutting. Several studies have been carried out in the Rhizome part of plant. The rhizome is useful to treat fever, cough, indigestion, asthma, helminthiasis, bronchitis and skin diseases. Diosgenin is the principle constituent of the rhizome used in the commercial production of steroidal hormones. [5] The plant is main source of diosgenin, tigogenin and saponin. [6]

The anti-inflammatory and antipyretic properties were proved in the Rhizome and also possess significant

hepatoprotective activity. [7] Aqueous extract of Rhizome is shown to possess antimicrobial activity. [8] The leaves are also used to control diabetes. [9] Several studies were carried out on Rhizome for its antimicrobial, anti-inflammatory, anti diabetic activities; but the studies on the medicinal properties of the leaf is lacking. In this paper, the phytochemical analysis of crude extract of the leaf has been studied as part of the exploration for bioactive compounds.

MATERIALS AND METHODS

Healthy plants of *Costus speciosus* were collected from Chennai. 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 done by following the method. [10,11] One gram of dried powder of plant materials were extracted with 20 ml of ethanol (75%), acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40°C to a constant weight and re dissolved in the same solvent namely aqueous, ethanol, chloroform, petroleum ether and acetone for extraction. The solution was stored at 18°C for further studies.

Phytochemical analysis

Chemical test for screening and identification of bioactive compounds in the leaf extract of medicinal plant was assessed by using the standard method. [12-14]

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS CONSTITUENTS

Test for Tannins

For tannin identification to 1 ml of the plant extract, 1 ml of ferric chloride (5%) was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins

For saponin identification to 2 ml of plant extract, 2 ml of distilled water was added and shaken in graduated cylinder for 15 minutes, length wise formation of 1 cm layer of foam indicates the presence of saponins.

Test for Quinones

For quinone identification to 1 ml of plant extract, 1 ml of concentrated sulphuric acid (H_2SO_4) was added. Formation of red colour indicates the presence of quinones.

Test for Flavonoids

For flavonoid identification to 2 ml of plant extract, 1 ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Alkaloids

For alkaloid identification to 2ml of plant extract, 2ml of concentrated hydrochloric acid (HCl) was added. Few drops of Mayer's reagent were added. Formation of green colour or white precipitate indicates the presence of alkaloids.

Test for Glycosides

For glycoside identification to 2 ml of the plant extract, 3 ml of chloroform and 10% of ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

Test for Cardiac Glycoside

For cardiac glycoside identification to 0.5 ml of the plant extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride solution were added. This was layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

Test for Terpenoids

For terpenoid identification to 0.5 ml of the plant extract, 2 ml of chloroform along

with concentrated sulphuric acid was added. Formation of red brown colour at the interface indicates the presence of terpenoids.

Test for Phenols

For phenol identification to 1 ml of the plant extract, 2 ml of distilled water followed by few drops of 10% ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenols.

Test for Steroids

For steroid identification to 0.5ml of the plant extract was treated with drops of chloroform, acetic anhydride and concentrated H_2SO_4. Formation of dark pink colour indicates the presence of steroids.

Test for Coumarins

For coumarin identification to 1 ml of plant extract, 1 ml of 10 % NaOH was added. Formation of yellow colour indicates the presence of coumarins.

Test for Anthocyanin and Betacyanin

For anthocyanin and betacyanin identification to 2 ml of the plant extract, 1 ml of sodium hydroxide (NaOH) was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

QUANTIFICATION OF PHYTOCHEMICALS OF *COSTUS SPECIOSUS*

Estimation of Tannins:

Tannins content of the given sample was estimated by following the method. [15] The ethanol extract (1 ml) was mixed with Folin-Ciocalteau's reagent (0.5 ml), followed by the addition of saturated Na_2CO_3 solution (1 ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Increasing concentrations of standard tannic acid was prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as mg tannic acid equivalent per gram of the sample.

Estimation of Total phenolic content (TPC)

Total phenolic content in the ethanolic leaf extract of *Costus speciosus* was determined by the Folin Ciocalteau colorimetric method. [16] 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% (wv) 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 gram of dry weight of plant powders.

Estimation of Flavonoid Content

Flavonoids content in the ethanolic leaf extract was determined by the aluminium chloride colorimetric method. [17] 0.5 ml of leaf extracts of *Costus speciosus* at a concentration of 1mg/ ml was taken and the volume was made up to 3ml with methanol. Then 0.1ml aluminium chloride – $AlCl_3$ (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 gram of sample.

Estimation of Total alkaloids:

The quantification method for alkaloids determinations has been used

with some modifications. [18] 100 ml of 10% acetic acid in ethanol was added to 1 gram of dry powdered plant and then the extracts were covered and allowed to stand for 4 h. After that, the extracts have been filtrated and concentrated on a water bath to 25 ml of its original volume. The droplets of concentrated ammonium hydroxide were added to the extract until the precipitation the whole solution was allowed to settle, and then the precipitates were washed with dilute ammonium hydroxide and then filtered using filter paper whatman. The residue was dried in the oven at 40 °C and weighed. The alkaloid content was determined using the following formula:

% alkaloid = [final weight of the sample / initial weight of the extract] × 100.

Estimation of Saponin content:

The determination of total saponin was done according to the method used with minor modifications. [19] 1 g of powdered plant has been added to 100 ml of 20% aqueous ethanol and kept in a flask on stirrer for half hour and then heated over a for 4 h at 45 °C with mixing. The mixture was filtered by using filter paper whatman and the residue again extracted with another 100 ml of 25% aqueous ethanol. The combined extracts were concentrated by using rotary evaporator in 40 °C to gets 40 ml approximately. The concentrate was transferred into separator funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was kept and then re- extracted with 30 ml n-butanol was added. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the samples were dried in the oven at 40°C to a constant weight and the saponin content was calculated.

Estimation of Steroids:

1ml of ethanol extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank. [20]

Estimation of Terpenoids:

100 g of plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether and the ether extract was treated as total Terpenoids. [21]

The experiments were carried out in triplicates and results are expressed as Mean ± Standard deviation.

RESULTS

The present was study carried out on the leaf extract of *Costus speciosus* revealed the presence of medicinal active constituents. The phytochemical active compounds of *Costus speciosus* were qualitatively analysed in the leaf extract and the results are tabulated in Table 1. Among the five different solvents used, the ethanol leaf extract of *Costus speciosus* was rich in Tannin, Quinones, Glycosides, Alkaloids, Phenols, Flavonoids, Coumarins, Steroids and Saponins followed by acetone extract.

Various phytochemical compounds detected are known to have beneficial important in medicinal science. The phytochemicals possess a wide range of activities which help in the protection against chronic diseases.

Quantitative estimation of the percentage crude chemical constituents in the above plant was also studied and summarized in Table 2. The ethanol leaf extract of *costus speciosus* contained the highest % crude yield of tannin, phenol, flavonoid, alkaloid, saponin, steroid and terpenoids.

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

Phytochemicals	Leaf extract of *Costus speciosus*				
	Aqueous	Ethanol	Chloroform	Acetone	Petroleum ether
Tannins	-	++	-	+	-
Saponins	++	++	-	+	-
Flavonoids	+	+	-	+	-
Quinones	+	++	++	++	++
Glycosides	-	+	-	+	-
Cardiac glycosides	+	+	+	+	+
Terpenoids	+	++	+	++	+
Phenol	++	++	+	++	-
Coumarins	+	+	-	+	-
Steroids	+	+	+	++	+
Alkaloids	+	+	-	+	-
Anthocyanin	-	-	-	-	-
Betacyanin	+	+	+	+	-

++ = strong positive, + = positive, = negative

Table 2: Percentage of Phytochemcial of *Costus speciosus*

Bio Active Compounds	*mg/gm of Dry Material
Total Phenol	25.4 ± 0.4
Tannin	20.3 ± 0.62
Saponin	18.3 ± 0.66
Flavonoid	13± 0.79
Terpenoid	11.2 ± 0.5
Alkaloid	6.4 ± 0.45
Steroid	1.03± 0.15

*Values are expressed as Mean ± Standard deviation

DISCUSSION

Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties. They are non essential nutrients but the plants that produce these chemicals protect humans against diseases.

The phytochemical studies shows that the ethanolic leaf extract is rich in Flavonoid, Phenols, tannins, alkaloids, Steroids and Terpenoids. These secondary metabolites act as an effective antimicrobial substance against wide range of microorganisms. [22] Terpenoids acts as bronchodilator in humans and exhibit anti-inflammatory, antibacterial and antibiotic properties. Alkaloids posses numerous functions, among them the most important are their analgesic, antispasmodic and bactericidal effects. [23] One of the most important biological properties of alkaloid is their toxicity against the cells of foreign organism. [24] Tannins were reported to exhibit antiviral, anti bacterial and anti tumor activities. [25] Presence of coumarins contributes to the antifungal activity. [26]

Flavonoids, another constituent of leaf extract of *Costus speciosus* exhibited a wide range of biological activity like antimicrobial, anti-inflammatory, antiallergic and antioxidant properties. [27] Polyphenolic compounds like flavonoids and tannins are known anti oxidant and possess organ protective functions. Flavonoids are potent free radical scavengers, which prevent oxidative cell damage, have anticancer activity. [28]

The result also reveals that *Costus speciosus* is gratified with Phenols which mainly contributes to its antimicrobial potency. The results obtained were in accordance with previous papers. [29] Plant steroids are known for cardiotonic activities and also used in cosmetics, nutrition and herbal medicine. Steroids act as a precursor for the synthesis of sex hormones.

Thus, phytochemical screening may be useful for analyzing the bioactive principles, which are the potent source for drug discovery and development.

CONCLUSION

Costus speciosus is a medicinal plant which can be successfully used in many health problems. The present work explained the use of herbal plant as rich source of phytochemicals. Hence the plant can be used in the development and discovery of new drug molecules. The present study verified the traditional use of *Costus speciosus* as herbal medicine for various human ailments. Further studies are needed to isolate characterize and elucidate the structure of bioactive compounds.

REFERENCES

1. Gotep JG, Aganda Goa, Gibse DS, Chollam S. Antibacterial activity of ethanol extract of *Acalypha Wilkesiana* leaves growing in Jos, Plateau state, Nigeria. Malaysian Journal of Microbiology. 2010; Vol 6 (2): 62-74.
2. Bhogaonkar PY , Devarkar VD, Lande SK. Physical Characterization of *Costus speciosus* (Koenig Ex Retz.) smith_A well known Ayurvedic drug plant Life Sciences Leflets.2012;11:1-9.
3. Sabitha Rani A, Sulakshana G, Sudeshna Patnaik. *Costus speciosus*, An Antidiabetic Plant –review. FS.J.Pharm Res.2012; Vol 1/No 3.
4. Gupta R.K. Medicinal and Aromatic plants. CBS publishers and distributors.2010; 234, 499.
5. Jha MK, Alam MB, Hossan MS, Islam A. Invitro Antioxidant and Cytotoxic potential of *Costus speciosus* (Koen) Smith Rhizome. International Journal of Pharmaceutical Sciences and Research.2010; Vol 1 (10): 138-144.
6. Indu Sanadhya, Annika Durve, Meeta Bhot, Jossy Varghese and Naresh Chandra. Evaluation of medicinal activites of *Costus speciosus*. World Journal of Pharmaceutical Research. 2014; Vol 3, Issue 4: 623-633.
7. Binny K, Sunilkumar G, Thomas D. Anti-inflammatory and antipyretic properties of the rhizome of *Costus speciosus* (Koen). SM. J. Basic. Clin. Pharm.2010; 1: 177-181.
8. Saraf A. Phytochemical and antimicrobial studies of medicinal plant *Costus speciosus* (koen). E.J. Chem. 2010; 7:S405-S413.
9. Benny M. Insulin plants in gardens. Nat. Prod. Rad.2004; 3:349-350.
10. Pizzale L, Bortolomeazzi R, Vichi S, Conte LS. Antioxidant activity of *Sage* and *Oreganao* extracts related to their phenolic compound content. Journal of the science of food and agriculture.2002; 82: 1645-1651.
11. Lee Y, Foo Y. Antioxidant activities of polyphenols from *Salvia officinatis*. Food chem.2001; 75: 197-202.
12. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies of *Merugan Kizhangu*. Bull Med. Eth. Bot.1981; Res. 3: 84-96.
13. Siddiqui AA, Ali M. Practical Pharmaceutical chemistry. 1st ed. NewDelhi, CBS Publishers and Distributors.1997; 126-31.
14. Savithramma N, Linga RM, Bhumi G. Phytochemical screening of *Thespesia populnea* (L) Soland and Tridax procumbens L.J. Chem. Pharm.2011; Res. 3: 2834
15. Fagbemi TN, Oshodi AA, Ipinmoroti KO. Processing Effects on Some Antinutritional Factors and In vitro Multienzyme Protein Digestibility (IVPD) of Three Tropical Seeds: Breadnut (*Artocarpus altilis*), Cashewnut (*Anacardium occidentale*) and Fluted Pumpkin (*Telfairia occidentalis*). Pak. J. Nutr.2005; 4(4):250-256.
16. Lister E, Wilson P. Measurement of Total Phenolics and ABTS Assay for Antioxidant Activity (Personal Communication). Crop Research Institute Lincoln, New Zealand. 2001.
17. Woisky R, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J. Apic. Res. 1998; 37: 99-105.
18. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. 3 rd Ed. New Delhi: Springer Pvt. Ltd.
19. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the extracts of some haemostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl Sci. 2001; 8: 203–208.
20. Narendra Devanaboyina, RamaLakshmi N, Satyanarayana B, sudeepthi P, Hemachakradhar K, PavankumarRaju N. Preliminary phytochemical screening, quantitative estimation and evaluation of antimicrobial activity of *Alstoniamacrophylla* stem bark. International Journal of Science Inventions today.2013; 2(1): 31-39.

21. Ferguson, N. A textbook of Pharmacognosy, Mac Millan Company. 1956; 191.
22. Britto JD, Sebastian SR. Biosynthesis of silver nanoparticles and its Antibacterial activity against human pathogens. Int J Pharm Sci.2011; 5: 257-259.
23. Okwu DE and Josiah C. Evaluation of the chemical composition of two Nigerian Medicinal plants. African Journal of Biotechnology. 2006 5(4):357-361.
24. Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha Curcas*(Linn). African Journal of pharmacy and Pharmacology.2009; Vol.3 (2):058-062.
25. Shyamala Gowri S, Vasantha K. Phytochemical screening and Antimicrobial Activity of *Syzygium Cumini*(L) (Myrtaceae) Leaves Extract. International Journal of Pharm Tech Research. 2010; Vol.2, No2: 1569- 1573.
26. Saroj Kothari, Vaibhav Mishra, Savita Baharat, Shrinivas Tonpay D. Antimicrobial activity and Phytochemical screening of serial Extracts from leaves of *Aegle Marmalos* (Linn.). Natural Drugs. 2011; Vol 68 No.5: 687-692.
27. Ekundayo FO, Adeboye CA, Ekundayo EA. Antimicrobial activities and phytochemical screening of pignut (*Jatrophas Curcas Linn*.) on some pathogenic bacteria. Journal of Medicinal Plants Research.2011; Vol.5 (7):1261-1264.
28. Doss A. Preliminary Phytochemical screening of some Indian Medicinal Plants. Ancient Science of life.2009; Vol. 29, No2: 12-16.
29. Okwu DE, Okwu ME .Chemical composition of *Spondias mombin linn* plant parts. J. Sustain. Agric. Environ. 2004; 62 (2): 140-147.

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