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RESEARCH ARTICLE

Phytochemical Screening and Antioxidant Activity of Rhizome Extracts of *Costus speciosus* (Koen.) J.E. Smith

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Abstract

Costus speciosus is an erect perennial herb belonging to family Costaceae, an important medicinal plant widely used in several indigenous medicinal formulations. Presently, these plants could be collected from wild habitat only. Due to indiscriminate collection from natural habitat it has become endangered. In the present investigation, the phytochemical and antioxidant activity of the rhizome extracts of *Costus speciosus* were evaluated. Phytochemical screening indicated that, rhizomes are rich in a variety of primary and secondary metabolites such as carbohydrates, alkaloids, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins and minerals like Zn, Cu, Mn, Se and Fe. The DPPH antiscavenging activity of aqueous extract was 9.98% lower than those of methanol (78.03%), chloroform (18.33%) and acetone (30.02%) extracts. The present findings for flavonoids and minerals suggested that their contents are responsible for significant antioxidant activity in all extracts.

Keywords: *Costus speciosus,* phytochemicals, antioxidant, rhizome, secondary metabolites, flavonoids.

Introduction

Costus speciosus is a perennial rhizomatous herb with erect or spreading stems commonly called as crepe ginger or spiral flag in English (Gupta, 2010). It is an erect plant up to 2.7 m high with tuberous root stalk, a sub-woody stem at the base flowers are larger, white, in thick, cone like terminal spikes, with bright red bracts. *Costus speciosus* is native to the Malay peninsula of the south-east Asia. In India, the plant naturalizes in sub-Himalayan tract of central India and parts of Western Ghats of Maharashtra, Karnataka and Kerala (Sarin *et al.*, 1974). It is known as Keukand, Keu, Kust (Hindi), Pakarmula (Gujrathi), Penva, Pushkarmula (Marathi), Kustha (Sanskrit) and Kostam (Tamil) (Anonymous, 2001). The rhizomes are bitter and show anthelmantic, astringent, depurative and expectorant properties (Chopra *et al*., 2006; Anonymous, 2007; Bown, 2008; Nadkarni, 2009; Gupta, 2010). It has antioxidant, antifungal, antituberculosis and oestrogenic activity. The rhizome extract is used as tonic and useful in reliving burning sensation, constipation, asthma, bronchitis, leprosy, anaemia and other skin alignments (Shivarajan and Balchandran, 1994). The rhizome of *Costus speciosus* has hepatoprotective properties (Bhuyan and Zaman, 2008). Rhizome paste is used for treating boils and used as contraceptive (Warrier *et al.,* 1993-1995). Rhizome possesses antifertility, anticholinestrase, anti-inflammatory, antipyretic and antihelminthic activities (Bhattacharya *et al.*, 1972; Hussain *et al.*, 1992). Steroid saponins and sapogenins from *C. speciosus* exhibited antifungal activity (Singh and Srivastava, 1992). In south-east Asia, it is used to treat boils, constipation, diarrhoea, dizziness, headache, ear, eye and nose pain, and used to stop vomiting. Japanese used the rhizome extract to control syphilis (Jain, 1991). Pharmacological studies showed that the rhizomes of *Costus speciosus* possess cardiotonic, hydrochloretic, diuretic and CNS depressant activity (Mahato *et al.,* 1980). The demand of nutraceuticals is increasing day-by-day, so herbs can be a better option for the replacement of synthetic antioxidant agents. Keeping the above facts in view, this study deals with the comparative antioxidant activity of multi-solvent extracts of the rhizome of *Costus speciosus* based on their vitamin, flavonoids and other natural antioxidants.

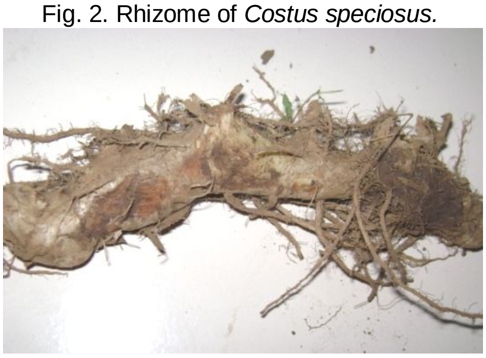
Materials and methods

Chemicals: All solvents were distilled prior to use. TLC was performed on silica gel 60 F254 (Merck). All reagents and solvents purchased from Merck Chemicals. Standard flavonoids were purchased from Sigma Aldrich. The UV-Vis spectra were recorded on a Shimadzu UV-1700 spectrophotometer. HPLC were recorded on Agilent Technologies 1200 series Quaternary. Detection of minerals was performed by using CEM Mars 6 microwave digester and Teledyne Leeman, ICP OES model Prodigy Dual View (Induction Coupled Plasma). HPTLC were recorded on CAMAG LINOMAT-5.

Sampling: Fresh samples of *Costus speciosus* bulbs were collected during monsoon (June 2012-September 2012) from Vajreshwari and Khandus village, Karjat regions of Western Ghats of Maharashtra (Fig. 1 and 2).

Fig. 1. Habit of *Costus speciosus* from Karjat.

Fig. 2. Rhizome of *Costus speciosus.*



The plants were identified and authenticated using herbarium collection at Botany research laboratory, DST-FIST School of Life Science, SRTM University, Nanded (MS) and the plantlist.org. Fresh rhizomes were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The shade dried material was ground into coarse powder using mechanical grinder (Panasonic make). This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in airtight containers at room temperature till further use.

Soxhlet extraction: Exhaustive soxhlet extraction was performed using a classical soxhlet apparatus with accurately weighed 10 g of the drug powder for 18-40 h. Extraction was performed with water, methanol, chloroform and acetone as the extracting solvent. The extraction was conducted for 6-8 h/d and finally all the extracts were evaporated under vacuum. The water, methanol, chloroform and acetone extracts of rhizome of these plants were prepared according to standard methods (Harbone, 1998). Nitrogen gas was purged through these extracts to prevent oxidation of secondary metabolites. These extracts were sealed in airtight containers and stored at -4°C.

Phytochemical screening: Phytochemical screening of active plant extracts was done by following standard methods (Khandelwal, 1999) for the qualitative analysis of various phytochemical studies such as alkaloids, carbohydrate, glycosides, saponins, flavonoids, phenols, vitamin C and vitamin E which could be responsible for antioxidant activity.

Antiscavenging activity: DPPH solution (0.1 mM) was prepared in methanol by dissolving 0.0394 g DPPH in 1000 mL methanol. The solution was kept in darkness for 30 min to complete the reaction. The free radicals scavenging activity of the crude extracts was determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH). Antioxidant activity was measured according to Brand-Williams *et al.* (1995). Wherein, the bleaching rate of stable free radical, DPPH was monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbed at 570 nm, but upon reduction by an antioxidant or radical species its absorption decreased. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [(ABS_{control}-ABS_{sample})/(ABS_{control})] \times 100$$

Where, $ABS_{control}$ is the absorbance of negative control and ABS_{sample} is the absorbance of the reaction mixture containing the sample extract.

Mineral analysis-Micro-scaled digestion: CEM-MARS 6 microwave oven was used for micro-scaled digestion. About 0.5 g of herbal samples were weighed and transferred to CEM-Xpress vessels and 8-10 mL of conc. HNO_3 was added to the samples. The samples were pre-digested for 10-15 min prior to capping the vessels. The CEM-Xpress vessels were assembled for microwave irradiation. The microwave program was adjusted with respect to the number of vessels and reference to the guidelines of CEM at 1000 W with 100% level. Twenty five minutes ramping period was used to reach the digestion temperature of 180°C which there upon was maintained for 15 min. The CEM-Xpress vessels were kept in fume hood for cooling and to release the pressure by uncapping. The contents were transferred to 50 mL volumetric flasks and volume was made with distilled water. The solutions were filtered prior to use. For calibration, Leeman and Thomas Baker Std. samples were used as the reference for the calibration range. The spray chamber, nebulizer and torch assembly was completely cleaned to eliminate any form of contamination. The plasma was stabilized for 15 min by flushing with distilled water. An instrument calibration was performed to check the wavelength shift and the same was successful with a minimum deviation of <10% with master scan. Diluted samples were used for further analysis by using Teledyne Leeman, ICP (Induction Coupled Plasma).

Vitamin E analysis by High performance liquid chromatography: Standard dl α-tocopherolacetate (96%) (Vitamin E) manufactured by Merck was used for calibration of standard curves. About 1 mg of dl α-tocopherolacetate was dissolved in 1 mL in HPLC grade methanol. The dilutions of 100, 50, 25, 15 and 10 µg/mL was prepared. The pre-treated sample extracts and stock solutions were filtered through 0.45 µm syringe filters.

Reverse phase HPLC method: The concentration of α-tocopherol (Vitamin E) in the extracts was determined by Agilent Technologies 1200 series Quaternary system, equipped with auto sampler, quaternary pump, degasser, column oven and a DAD detector. The spectral data was collected at UV detection 220 nm. The solvent system of acetonitrile and water (95:5) was used a gradient mobile phase on Agilent ZORBAX 300 SB column (4.6 mm × 150 mm × 5 µm) at a flow rate of 1.0 mL/min, 10 µL injection volume and detection was optimized at 220 nm with 15 min separation time.

Vitamin C: About 0.025% ethanolic solution of DCPI (2,6-dichlorophenol-indophenol sodium salt) was prepared for the detection of Vitamin C. To the 0.5 mL of sample extracts, 2 drops of DCPI indicator was added. The blue coloration changed to red confirmed the presence of vitamin C. The test was carried out for all the extracts.

Flavonoids analysis by HPTLC: The standards Quercetin, Kaemferol, Catechin gallate, Rutin hydrate and Hesperdin were procured from Sigma Aldrich USA. All the standard solutions were prepared in ethanol where as hesperdin in water. Chromatography was performed on silica gel $60F_{254}$ (10 cm X 10 cm; 25 mm layer thickness; Merk) with aqueous, methanolic, chloroform and acetone extracts of *C. speciosus* rhizome. The fraction residues were collected and (10 µL) subjected for HPTLC (CAMAG, Switzerland) analysis. The fractions were impregnated on silica gel $60F_{254}$TLC plate. The plate was air-dried and then inserted in CAMAG-twin through lass chamber containing solvent system of composition with ethyl acetate, acetic acid, formic acid and water (100:11:11:27) as a gradient mobile phase for 20 min. The well eluted TLC plate was then dried at 105°C for 15 min and scanned using Scanner 3 at 254 and 366 nm using Win Cat 4 software.

Results and discussion

Optimization of extraction method: In order to extract the phytochemicals from herbal samples efficiently, variables involved in this procedure were optimized, including extraction solvent (water, methanol, chloroform and acetone, 100%), extraction method (Soxhlet, reflux, percolation) and extraction time (18-40 h). The extraction time in water was 40 h. The biomass was refluxed for 40 h and then it was dried naturally for 2-3 d. To the dried biomass, 100% methanol was added and the reaction was percolated for phytochemicals. The methanolic fraction was collected in amber colored bottle under nitrogen atmosphere. The material was dried for 5-6 h. The procedure was repeated for chloroform and acetone. The extraction time was optimized for all the samples. All the extracts were preserved under nitrogen atmosphere in amber colored bottle.

Phytochemical screening: It is known that plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins and volatile oils. It is necessary to identify the phytochemical components employed by herbalists in the treatment of diseases (Banso and Adeyemo, 2007). The presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts. For example, saponins are a special class of glycosides which have soapy characteristics and have been reported to be active antifungal agents. Antimicrobial properties of a number of tannins, flavonoids, alkaloids have been reported. The rhizomes are the major source of diosgenin. The major chemical constituents are diosgenin, curcumin and curcuminoids. Tubers and roots of Costus contain 5α-stigmasten-3b-ol, sitosterol-β-D-glucoside, dioscin, prosapogenins A and B of dioscin, gracillin and quinines (Bhattacharya *et al.*, 1973). Saponins were also reported from rhizomes, including seeds and roots. Saponins isolated from seeds were reported to possess hypotensive and spasmolytic effect. Tigogenin and diosgenin (2.6%) have been isolated from rhizomes (Gupta *et al.*, 2008). Phytochemical screening of the rhizome extracts of *C. speciosus* revealed the presence of different phytochemicals, indeed phytochemical investigations of this plant have resulted in occurrences of carbohydrates, alkaloids, glycosides, saponins, flavanoids, phenols, vitamin E and vitamin C. Table 1 illustrates the results of phytochemical screening of all the extracts of *C. specious*. The qualitative analysis of carbohydrates (Benedict's reagent test) and glycosides (Borntranger's reagent) were carried out in all extracts i.e. aqueous (S1), methanol (S2), acetone (S3) and chloroform (S4) extracts.

Table 1. Preliminary phytochemical screening of rhizome extracts of *Costus speciosus.*

Constituents	Test	Observation	Inference			
			S1	S2	S3	S4
Carbohydrates	Benedicts Reagent	Red precipitate	+	+	+	+
Alkaloids	Mayer's Reagent	White precipitate	-	+	-	-
Glycosides	Borntranger's Reagent	Pink coloration	+	+	+	+
Saponins	Foaming	Frothing persisted for 10-15 min	+	+	-	-
Flavonoids	Shinoda	Pink-Red colouration	+	-	-	-
Phenols	Ferric chloride	Dark brown coloration	-	+	+	+
Vitamin C	2,6-dichlorophenol-indophenol sodium salt	Red coloration	+	-	-	-
Vitamin E	HPLC method	-	-	-	+	-

S1=Water, S2=Methanol, S3=Acetone, S4=Chloroform.

The solutions turning red and pink confirmed the presence of carbohydrates and glycosides respectively. The hydrophilic carbohydrates and glycosides were present in water (S1) whereas, hydrophobic carbohydrates and glycosides were detected in rest of the organic solvents (S2-S4). The Mayer's test of extract S2 displayed appearance of white turbidity for alkaloids. The alkaloids were absent in S1, S3 and S4 extracts. The dark brown coloration test for phenols was observed in S2-S4 extracts. The water soluble phenols were absent in all the extracts. The extracts S1-S4 were shaken with distilled water. The persistence of froth in S1, S2 was observed, indicated the presence of saponins. The hydrophilic flavonoids were detected in extract S1. The water soluble vitamin C was found in S1 and vitamin E was qualitatively analyzed by HPLC method in extracts S3 of *C. speciosus.*

Antiscavenging activity: The phytochemical screening of the crude rhizome extracts showed positive reactions for alkaloids, flavonoids, phenolic compounds, saponins, glycosides, carbohydrates, vitamin C, vitamin E and minerals. The scavenging ability assayed the ability of extracts to react rapidly with DPPH radicals and reduce most DPPH radical molecules. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.,* 1997). Vijayalakshmi and Sarada (2008) investigated different parts of *C. speciosus* for their polyphenol content and antioxidant activity. Chakraborty (2009) showed the antioxidant activity of chloroform extract of *C. speciosus* leaves for its free radical scavenging activity. Plant derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins could delay or provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage and DNA strand breaking (Ghosal *et al.*, 1996), because of their redox properties, which allow them to act as hydrogen donors, reducing agents, free radical scavengers (Robards *et al.*, 1999; Govindarajan *et al.*, 2005). The antioxidant capacity *C. speciosus* rhizome extracts was measured by antiscavenging activity method and the results are shown in Table 2. Antiscavenging activity of aqueous extract was 9.98% lower than those of methanolic (78.03%), chloroform (18.33%) and acetone (30.02%) extracts. However, the antiscavenging value of methanolic extract is four times higher than any other extracts. These results might suggest higher medicinal suitability of alcoholic extracts in various antioxidant applications.

Mineral analysis-Optimization and calibration of C. speciosus rhizome extracts: After optimization, a new calibration method was done using Cu 324.754, Mn 257.610, Se 196.090, Fe 259.940 and Zn 213.856 nm wavelengths for calibration (Table 3).

Table 2. Antiscavenging activity of rhizome extracts of *Costus speciosus.*

Extract	Antiscavenging activity (%)
Water	9.98
Methanol	78.03
Chloroform	18.33
Acetone	30.02

Table 3. Instrumental characteristics and setting for ICP-OES: Spectrometer Leeman lab's simultaneous ICP-OES Prodigy XP dual system.

	Parameters range		Actual parameters
	Min	Max	
Power	0.1	2.0	1.1 Kw
Coolant flow	5	20	18 L/Min
Auxiliary flow	0.0	2.0	0.2 L/M
Nebulizer flow	5	60	34 psi
Plasma torch	-	-	Dual
Spray chamber	-	-	Cyclonic
Nebulizer	-	-	Concentric
Sample aspiration rate	0.5	2.0	1.4 mL/min
Replicate read time	-	-	40 sec per replicate for Axial

Calibration STD solutions were measured 3 times one by one with an RSD <1%. Once all the calibration standards were finished, a necessary background correction was applied for each wavelength. The samples were measured with 3 reproductions. The average sum of the 3 measurements is tabulated in the analysis report. Quantitative multi-elemental analysis by inductively coupled plasma (ICP) spectrometry depends on a complete digestion of solid samples. However, fast and thorough sample digestion is a challenging analytical task which constitutes a bottleneck in modern multi-elemental analysis. Additional obstacles may be that sample quantities are limited and elemental concentrations are low. In such cases, digestion in small volumes with minimum dilution and contamination is required in order to obtain high accuracy data. We have developed a micro-scaled microwave digestion procedure and optimized it for accurate elemental profiling of plant materials. A commercially available 40-position rotor with 5 mL Polytetra flouro ethylene (PTFE) vials, originally designed for microwave-based parallel organic synthesis, was used as a platform for the digestion. The novel micro-scaled method was successfully validated by the use of various certified reference materials (CRM). The micro-scaled digestion procedure was applied on crude powder of dried plant material in small batches. The contents were transferred to 50 mL volumetric flasks and volume was made with distilled water. The solutions were filtered prior to use. Teledyne Leeman, ICP spectrometer was calibrated by using Leeman standard, National Institute of Standards and Technology (NIST), USA. Diluted samples were used for further analysis.

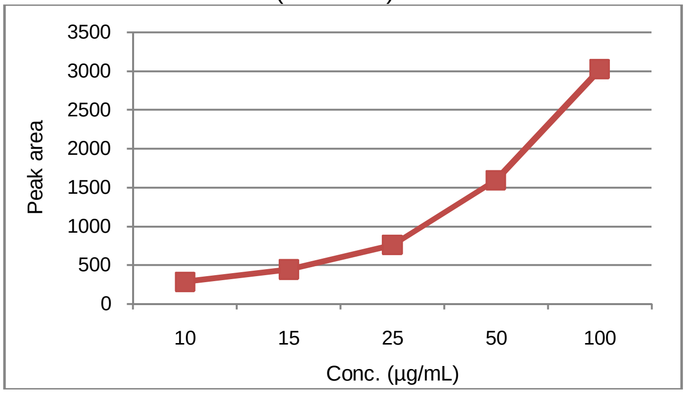
Iron and copper are of great importance for life. As redox-active metal they are involved in photosynthesis, mitochondrial respiration, nitrogen assimilation and hormone biosynthesis. Manganese is essential for plant metabolism and development and occurs in oxidation states II, III, and IV in approximately 35 enzymes of a plant cell. Zinc is important as a component of enzymes for protein synthesis and energy production and maintains the structural integrity of biomembranes. Most of the zinc enzymes are involved in regulation of DNA-transcription, RNA-processing and translation. Although the essentiality of Se to plants has not been established yet, Se is considered a beneficial element in promoting plant growth in some plant species. We have determined the 5 elements in coarse powder of rhizome of *C. speciosus* (Table 4). Thereby, the concentration of minerals in bulb extracts had different profiles and quantitative differences had been detected. The most abundant microelement was Fe in *C. speciosus,* whereas copper was found at the lowest concentration. The content of Fe was especially high followed by Mn, Zn and Cu whereas, Se was not detected.

Qualitative analysis of vitamin E by HPLC: To meet the requirements for quantitative analysis, the following HPLC parameters were examined, including different columns (Agilent SB-C18 length 250 mm and 150 mm, width 4.6, particle size 5 µm), column temperature (25°C) and UV wavelength (220 nm). The best chromatographic resolution was obtained on Agilent SB-C18 length 4.6 X 150 mm, 5 µm column at 25°C. The UV detector was monitored at 200-380 nm for fingerprinting analysis because the peaks were observed under this wavelength. The high intense peak was observed at 220 nm. To obtain the calibration curve, working solutions of four concentrations containing vitamin E was analyzed in triplicate. The calibration curves were established by plotting peak areas versus the concentration of each analyte. In the regression equation $y = ax + b$, x refers to the concentration of pure dl α-tocopherol acetate (1 g/mL), y the peak area and r the correlation coefficient (Fig. 3). Vitamins are a diverse group of organic compounds essential in trace amounts for the normal growth and maintenance of life. To ensure the adequate intake of vitamins, the human diet can be completed with a high range of multi-vitamin tablets and food products enriched with vitamins, in other words, these compounds are usually administered as nutraceutical or functional ingredient. They are classified as either water-soluble or fat-soluble. Vitamin E is fat-soluble whereas, vitamin C is water-soluble. Vitamin E is a generic term for tocopherols and tocotrienols and it is fat-soluble antioxidant that block the production of reactive oxygen species formed when lipids undergoes oxidation. We employed reverse phase HPLC-analytical tool for qualitative estimation of vitamin E, in which HPLC has been coupled with UV detector.

Table 4. Accuracy of elemental conc. in *C. speciosus* after micro-scaled digestion expressed in ppm.

Minerals	Conc. (ppm)
Cu	3.994
Mn	336.440
Se	0
Fe	405.241
Zn	16.473

Fig. 3. Calibration curve of dl α-tocopherol acetate (96%) (Vitamin E).



The lipophilic vitamin E has been detected in the chloroform extract (Figs. 4, 5 and 6). Vitamin E is absent in methanolic and acetonic extracts (Figs. 7 and 8). The moderate antioxidant activity in organic extract suggests that the concentration vitamin E might be less as compared to that of the other antioxidants in aqueous extract. The organic extracts displayed significant antioxidant activity proposed that the concentration of vitamin E might be higher along with the other natural antioxidants.

Fig. 4. Vitamin E standard peak at 10 µL.

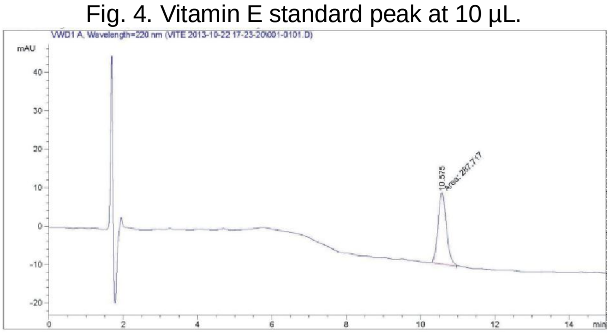


Fig. 5. Vitamin E standard peak at 20 µL.

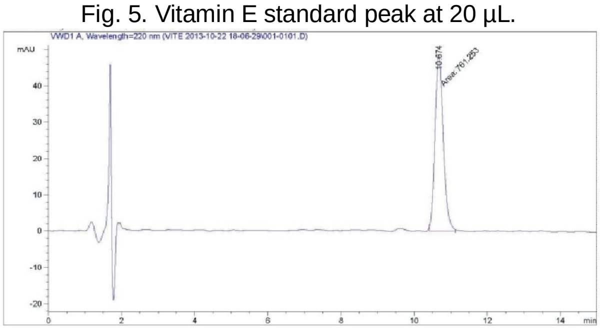


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Fig. 6. *Costus speciosus* rhizome contains vitamin E in chloroform extract.

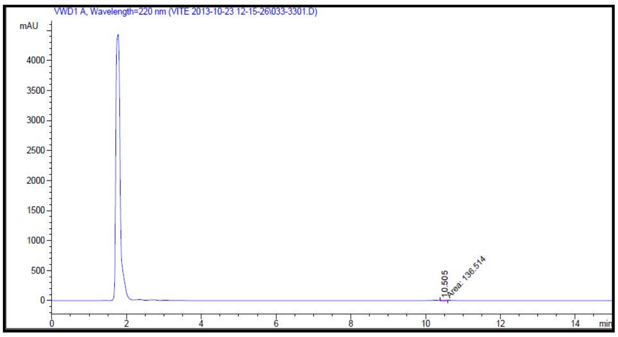


Fig. 7. *Costus speciosus* rhizome contains vitamin E in methanol extract.

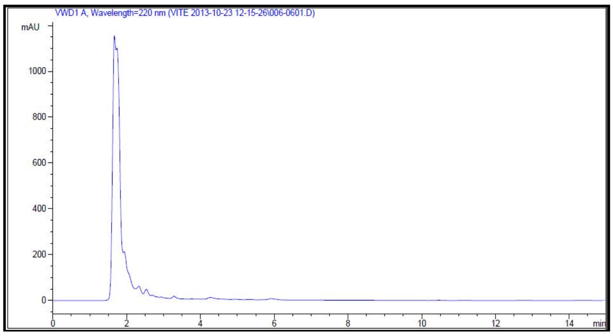
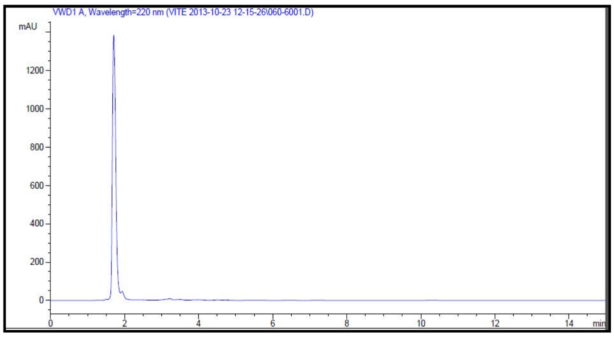


Fig. 8. *Costus speciosus* rhizome contains vitamin E in acetone extract.

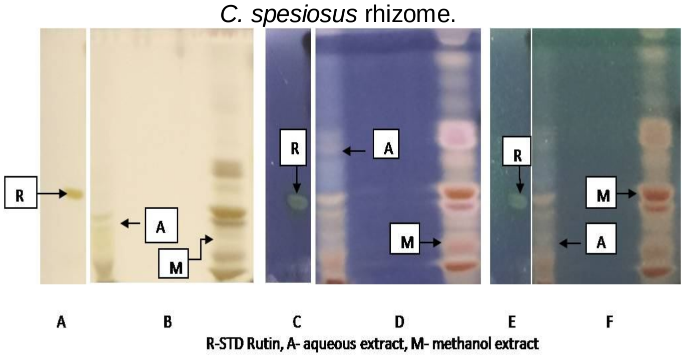


Vitamin C: Vitamin C (L-ascorbic acid or L-ascorbate) is an essential nutrient for humans and other animal species. Deficiency in this vitamin causes the disease known as scurvy in humans. This compound is also widely used as a food additive because of its antioxidant activity. The hydrophilic vitamin C has been detected in aqueous extract (Table 1).

Flavonoids analysis by HPTLC: Flavonoids, the most important and most diverse natural phenolics (Agarwal, 1989) have diverse chemical and biological activities including radical scavenging properties. Figure 11 and 12 shows the HPTLC profiles of aqueous and methanolic extracts of rhizome of *C. speciosus.* In HPTLC techniques, the flavonoids from methanolic extracts were determined by using methyl acetate, acetic acid, formic acid and water (100:11:11:27) as a gradient mobile phase.

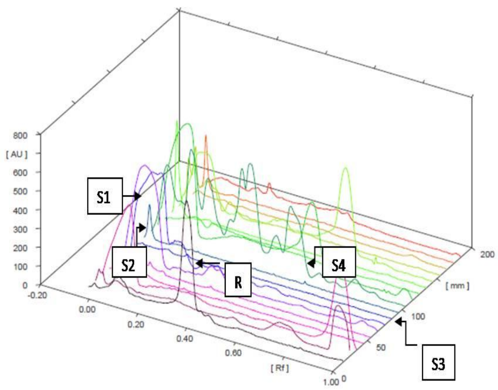
Figure 9A showed band of standard rutin under light, B showed different bands of flavonoids of aqueous and methanolic extracts under light. Similarly Fig. 9 (C, D, E and F) showed under UV 366 and 254 AD. The methanolic extracts showed maximum bands of flavonoids in clundingrutin. The aqueous extract showed different flavonoids, thus, HPTLC techniques could be considered as an accurate and precise method for the determination of flavonoids in rhizome extracts of *C. speciosus* (Fig. 10). There were eight different assigned substances in aqueous extract with range of Rf between 0.07 to 0.53. Same as in methanolic extract there were thirteen assigned substances including rutin. The Rf were ranging in between 0.06 to 0.90. The methanolic extract showed maximum flavonoids as compared to aqueous rhizome extract of *C. speciosus* (Table 5). The methanolic and aqueous rhizome extracts showed many flavonoids like Rutin, Kaemferol and Catechin (Table 6 and 7).

Fig. 9. HPTLC studies on the flavonoids of the *C. spesiosus* rhizome.



A. Standard Rutin under light AD.
B. Aqueous and methanolic extracts under light AD.
C. Standard Rutin under 366 AD.
D. Aqueous and methanolic extracts under UV 366 AD.
E. Standard Rutin under 254 AD.
F. Aqueous and methanolic extracts under UV 254 AD.

Fig. 10. HPTLC peaks of standard and extracts of *C. speciosus* rhizome.



All peaks at 366 nm wavelength (R - STD Rutin, S1-Aqueous, S2- Acetone, S3-Chloroform and S4-Methanol extract).

Table 5. HPTLC–Flavonoids profile of the aqueous rhizome extract of *C. speciosus* under UV 366 AD.

Peak	Rf	Height	Area	Assigned substance
1	0.07	442.5	24488.5	Unknown
2	0.12	92.2	11823.2	Unknown
3	0.22	0.3	4732.5	Unknown
4	0.26	25.8	517.3	Unknown
5	0.34	12.5	1679.3	Unknown
6	0.39	0.5	1518.6	Unknown
7	0.48	5.4	528.3	Unknown
8	0.53	21.4	480.7	Rutin

Table 6. HPTLC–Flavonoids profile of the methanol rhizome extract of *C. speciosus* under UV 366 AD.

Peak	Rf	Height	Area	Assigned substance
1	0.06	201.4	16002.6	Unknown
2	0.10	190.1	4001.5	Unknown
3	0.16	195.5	16514.7	Unknown
4	0.25	95.0	14394.3	Unknown
5	0.29	178.7	4023.7	Unknown
6	0.34	373.4	11683.9	Unknown
7	0.41	64.3	17196.1	Unknown
8	0.50	94.3	5830.6	Rutin
9	0.61	5.9	15948.1	Unknown
10	0.69	6.9	1092.5	Unknown
11	0.74	1.8	369.0	Unknown
12	0.85	17.8	2465.2	Catechin
13	0.90	1.8	552.7	Kaemferol

Table 7. Rf values of standard flavonoids.

Peak	Rf	Height	Area	Assigned substance
1	0.96	2.2	32581	Quercetin
2	0.53	17.9	17245.2	Rutin
3	0.87	1.3	28144.1	Catechin
4	0.93	20.0	66563	Kaempferol
5	0.65	2.7	447.6	Hesperdin

Conclusion

Costus speciosus have an ancient history of the multiple indigenous uses and is one of the most highly commercialized indigenous traditional medicines from India. Investigations of the phytochemicals and their biological activity have provided scientific support for many of its traditional uses. An improved RP-HPLC-UV-method has successfully applied for determination of dl α-tocopherol acetate in organic extracts. Similarly, the results obtained from phytochemical analysis illustrates the occurrences of various micronutrients i.e. carbohydrates, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins, alkaloids and minerals like Zn, Cu, Mn, Se and Fe. The present findings for microelements and minerals suggested that their contents are responsible for significant antioxidant activity in all extracts. The detection of flavonoids by HPTLC also revealed strong antioxidant activity in methanolic as well as in aqueous extracts. It showed the importance of rhizome in crude drug preparations as well as in traditional formulations. The structural characterization (FTIR, NMR studies) of isolated flavonoids from various extracts of *C. speciosus* are in progress.

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