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Pharmacognostical study and establishment of quality parameters of aerial parts of *Costus speciosus*-a well known tropical folklore medicine

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PEER REVIEW

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Comments
The research carried out by authors on aerial parts of *C. speciosus* is genuine and valuable for the use of this plant as an ingredient in various formulations and treatment of several diseases because the authors have demonstrated all the quality control parameters, phytochemical analysis and microscopic characters which would be further authentic data in identification and authentication of this plant.
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ABSTRACT

Objective: To evaluate the diagnostic pharmacognostical characters of *Costus speciosus* (aerial parts) along with their physico-chemical parameters and fluorosence analysis.
Method: The pharmacognostical characters were determined in terms of macroscopy, microscopy, powder microscopy, leaf constant, fluorescence analysis and preliminary phytochemical investigation.
Results: The findings of macroscopy revealed that leaves elliptic to oblong or oblong-lancoelate, thick, spirally arranged, with stem clasping sheaths up to 4 cm, flowers large, white, cone-like terminal spikes, with bright red bracts. Transverse section of leaflet showed the presence of cuticularised epidermis with polygonal cells on adaxial surface and bluntly angled cells on abaxial surface of lamina, mesophyll cells differentiated in to single layered palisade cells on each surface and 2-3 layered spongy parenchyma, unicellular and uniseriate multicellular covering trichomes, paracytic stomata and vascular bundles surrounded by sclerenchymatous multicellular sheath. Preliminary phytochemical screening exhibited the presence of various phytochemical groups like alkaloids, glycosides, steroids, phenolic constituents. Further, the leaf constants, powder microscopy and fluorescence characteristics indicated outstanding results from this investigation
Conclusions: Various pharmacognostical and physico-chemical parameters have pivotal roles in identification, authentication and establishment of quality parameters of the species.

KEYWORDS
Costus speciosus, Quality control, Physico-chemical parameters, Microscopy, Fluorescence analysis

1. Introduction

Costus speciosus Koen., (*C. speciosus*) Costaceae, commonly known as keukand in Hindi; Kembuka in Sanskrit, is an erect, succulent, monocotyledonous perennial herb, up to 2.7 meters in height, arising from a horizontal rhizome, found in tropical region of India, and also cultivated for ornamental purposes[1]. It is ascribed to be bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative and febrifuge in Ayurveda[2]. Rhizomes are used in the treatment of pneumonia, rheumatism, dropsy, urinary diseases and jaundice, while

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the leaves are used to treat mental disorders. Bruised leaves are applied in fever treatments, while a decoction of the stem is used in the treatment of fever and dysentery[1]. The species has been validated for certain pharmacological activities namely; antidiabetic and hypolipidemic activity and in the treatment of bronchial asthma[3-6], anticholinesterase activity[7], hepatoprotective activity[8], antioxidant activity[9,10], antibacterial activity, antifungal activity[11], antifertility activity[12] and spasmolytic activity. Phytoconstituents isolated from this species include: Diosgenin[13], furostanol saponins-costusosides I and J[12], β-sitosterol-β-D-glucoside, prosapogenins A and B of dioscin, dioscin, gracillin, dihydrophytylplastoquinone, α-tocopherolquinone and 5α-stigmast-9(11) en-3β-ol; methyl hexadecanoate, methyl octadecanoate and tetracosanyl octadecanoate[12], 24-hydroxytriacontan-26-one and 24-hydroxytriacontan-27-one[12]. Other components identified are 31-norcycloartanone, cyloartanol, cycloartenol and cycloalaudenol[14].

Irrespective of the recent phytochemistry and pharmacological research, till now no pharmacognostic work has been carried out on this species. The present investigation used different pharmacognostic and phytochemical parameters to supplement the identification and standardization information.

2. Materials and methods

2.1. Plant material

Fresh aerial parts (leaves) of *C. speciosus* Koen. (20 plants), were collected from the Bahadurpur forests of Kolkata and authenticated by *Dr. D.C. Saini*, Senior Scientist, Palaeobotany, Birbal Sahni Institute of Palaeobotany, Lucknow India. The voucher specimen no. BSIP 05 was deposited in Birbal Sahni Institute of Palaeobotany, Lucknow India. The leaves were dried under shade, mechanically reduced to moderate coarse powder and stored in amber colored air tight containers. Fresh leaves were used for the macroscopical and microscopical evaluation. Coarse form of drug was employed for determination of physicochemical parameters like moisture content, ash values, swelling index, foaming index, foreign organic matter, extractive values, qualitative and fluorescence analysis. The crude drug sample was also preserved in the department of Pharmacognosy, Teerthanker Mahaveer College of Pharmacy, Moradabad.

2.2. Macroscopic and microscopic analysis

The organoleptic characteristics such as shape, size, base and margin, venation of the leaves, taste and odour of the leaves of *C. speciosus* were determined. The macroscopy, microscopy and powder characteristics of aerial part of plant were studied according to the standard methods[15,16]. Leaf constants *viz.* stomatal index and stomatal number were also studied according to the standard procedures[15]. Stomatal indices of both adaxial and abaxial epidermis in 1 mm^2 leaf surface was determined and recorded by using camera lucida and stage micrometer.

2.3. Physico-chemical analysis

Air dried leaves was used for the quantitative determination of ash values, extractive values, moisture content, swelling index, foaming index and foreign organic matter, via standard methods[15-19]. The total ash value for a crude drug is not always reliable, since there is a possibility of presence of non-physiological substances such as earthy matters. So, the parameters such as acid insoluble, water soluble and sulphated ash values were performed. Extractive values with petroleum ether, chloroform, ethyl acetate, ethanol and water were also determined. The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Fluorescence analysis of crude powdered and various extracts was carried out by standard method[20].

2.4. Preliminary phytochemical screening

Preliminary phytochemical screening was performed by using standard procedures[15,16,21]. The extracts obtained from different solvents were subjected to identification tests for the detection of different phytoconstituents through organic and inorganic elements analysis, via the method of Khandelwal[15].

3. Results

3.1. Macroscopic characters

C. speciosus is an ornamental, perennial, erect, succulent herb, up to 2.7 m in height, arising from a horizontal rhizome. Leaves are alternate, spirally arranged, sub

sessile, elliptic to oblong or oblong-lancoelate, acuminate apex, thick, 15-35 cm×6-10 cm, glabrous above, silky pubescent beneath, with stem clasping sheaths up to 4 cm forming a complete tube around stem, flowers large, white, in thick, cone-like terminal spikes, with bright red bracts (Figure 1). Leaf lamina possessed 5 to 6 parallel veins on both sides of midrib No characteristic taste and odour was found (Table 1).

Figure 1. Aerial parts of *C. speciosus*.

Table 1

Organoleptic evaluation of leaves of *C. speciosus*.

Parameters	Leaves of *Costus speciosus*		
	Leaves	Stem	Flowers
Colour	Dark Green	Pale Brown	White
Odour	No characteristic odour	No characteristic odour	Characteristic odour
Taste	No characteristic taste	No characteristic taste	Characteristic taste

3.2. Microscopic characters

3.2.1. Leaf

Transverse section of leaflet (Figure 2a, 2b, 2c, 2d) shows adaxial epidermis composed of single-layered polygonal cells, covered by a thick cuticle; containing tanniferous substances and mucilage. Abaxial epidermis consists of bluntly angled cells with unicellular and multicellular uniseriate covering trichomes. Paracytic stomata are present on both adaxial and abaxial surface but more on abaxial surface. Mesophyll cells are distinguished between 2-3 layered loosely arranged spongy parenchyma cells and 2-layered palisade cells with shorter cells of lower layer than upper. Midrib is large and parenchymatous. The vascular bundles are amphicribal concentric *i.e.* phloem surrounds xylem; surrounded by double-layered sclerenchymatous cells.

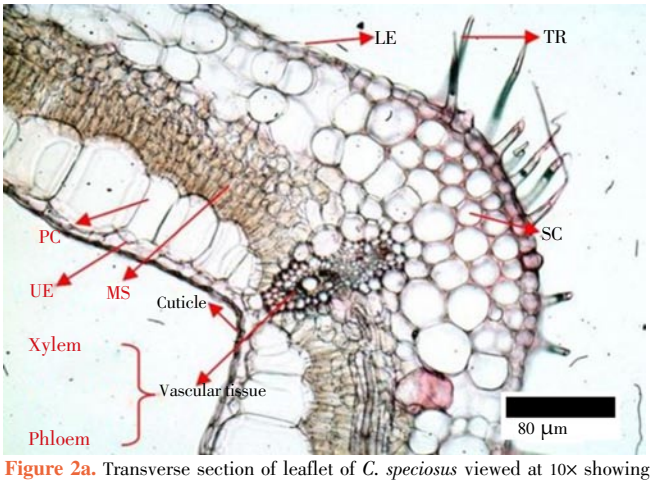

Figure 2a. Transverse section of leaflet of *C. speciosus* viewed at 10× showing adaxial epidermis (UE) with thick cuticle followed by single layer of palisade cells (PC), abaxial epidermis (LE) with multicellular uniseriate trichomes (TR), Multilayered mesophyll cells (MC), vascular tissue with sclerenchymatous cells (SC).

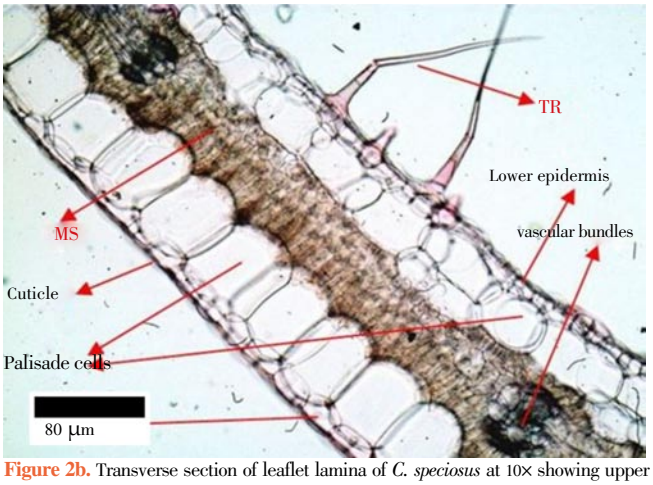

Figure 2b. Transverse section of leaflet lamina of *C. speciosus* at 10× showing upper epidermis (UE) with cuticle, lower epidermis having multicellular uniseriate trichomes (TR), palisade cells, multilayered mesophyll cells (MS) and vascular bundles.

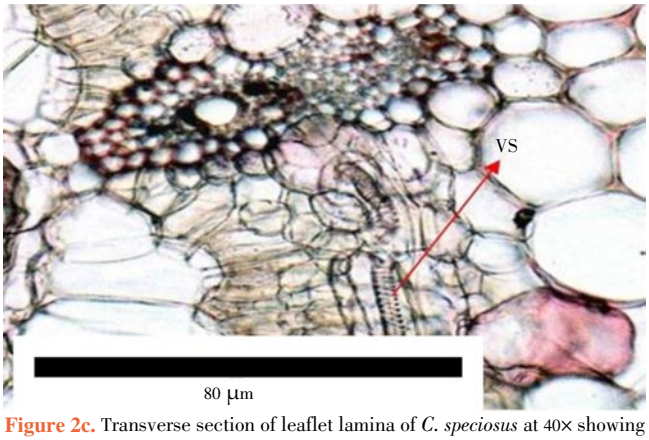

Figure 2c. Transverse section of leaflet lamina of *C. speciosus* at 40× showing vascular strands (VS) when treated with safranine.

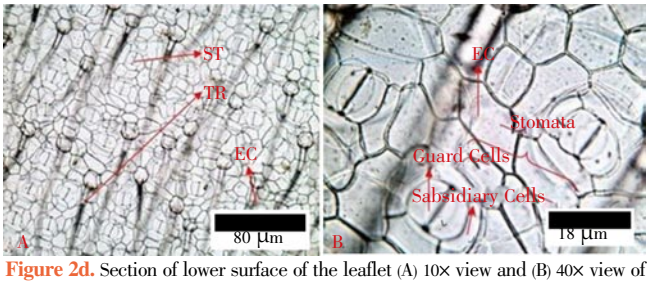

Figure 2d. Section of lower surface of the leaflet (A) 10× view and (B) 40× view of trichomes (TR) and stomata (ST) clearly depicted the structure of an anomocytic stomata showing guard cells and subsidiary cells along with epidermal cells (EC).

3.2.2. Leaf constants

The results of leaf constants evinced that abaxial surface has stomatal number 0.15 and adaxial surface shows 0.06. Thus stomatal number are found to be more in abaxial surface than adaxial surface as shown in Table 2.

Table 2

Leaf constants of *C. speciosus* Koen.

S. No.	Parameters	Values
1.	Stomatal number	Abaxial Surface-0.15 Adaxial Surface-0.06
2.	Stomatal index	Abaxial Surfac -14.11 Adaxial Surface-5.47

3.2.3. Powder microscopy

Powder is dark green to brown, with no specific odour or taste. It is easily flowable with a fine to coarse texture. Diagnostic microscopic powder features include prismatic and clusters crystals of calcium oxalate; starch granules are mostly simple, but rarely compound; unicellular and uniseriate multicellular covering trichomes; parts of epidermis in surface view showing straight-walled polygonal epidermal cells, paracytic stomata and spiral xylem vessels (Figure 3).

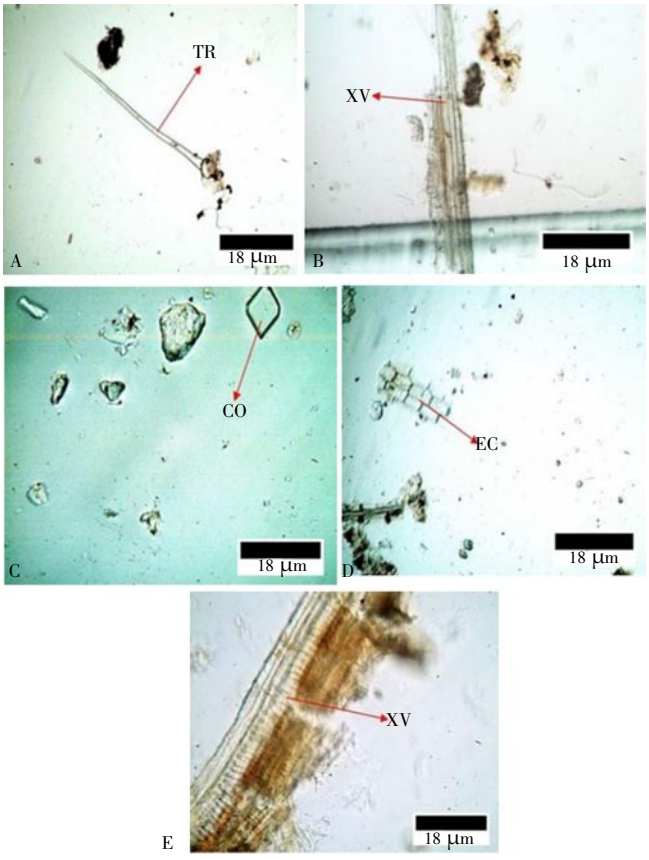

Figure 3. Powdered characteristics of the aerial parts of *C. speciosus* showing A: Trichomes (TR) B: Xylem vessels (XV) C: Tetragonal Calcium oxalate crystals (CO) D: Epidermal cells (EC) E: Spiral xylem vessels (XV)

3.3. Physico-chemical analysis

The results of physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash and sulphated ash are shown in Table 3. Sulphated ash value (12.5%) was lower than the total ash value (13.4%). The acid insoluble and water soluble ash values were 2.5% and 9%, respectively. Further, the results also showed that moisture content, swelling index and foaming index were found to be 1.43%, 0.7 cm and less than 100 respectively while foreign organic content was found to be nill (Table 4). The extractive values for various solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water were found to be 5%, 3%, 2%, 4% and 11% respectively (Table 5).

Table 3

Ash values of *C. speciosus* Koen.

S. No.	Parameters	Values
1.	Total Ash	13.4%
2.	Acid Insoluble Ash	2.5%
3.	Water Soluble Ash	9.0%
4.	Sulphated Ash	12.5%

Table 4

Moisture content, foreign organic matter, foaming index and swelling index of *C. speciosus* Koen.

S. No.	Parameters	Values
1.	Moisture Content	1.43%
2.	Foreign Organic Matter	Nil
3.	Foaming Index	Less than 100
4.	Swelling Index	0.7cm

Table 5

Extractive values of *C. speciosus* Koen.

S. No.	Solvent	Values% (w/w)
1.	Petroleum ether	5
2.	Chloroform	3
3.	Ethyl acetate	2
4.	Ethanol	4
5.	Water	11

The fluorescence characteristics of crude powdered drugs and various extracts were determined under UV radiation of long and short wavelengths and ordinary visible light. When the powdered drug and extracts were treated with different reagents and observed under UV and ordinary light, they emitted various colour radiations. The colour change for the crude powder and individual extract were distinctive and reproducible revealing the solvent properties to the phytoconstituents and data is presented in the Table 6.

3.4. Preliminary phytochemical screening

The phytochemical profile of the plant revealed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, phenols, tannins, glycosides and steroids as organic phytoconstituents (Table 7a). Qualitative analysis of various inorganic elements revealed the presence of iron, sulphates, phosphates and chlorides (Table 7b).

Table 6

Fluorescence studies of *C. speciosus* Koen.

S. No.	Materials/Treatment	Observations Under UV Cabinet		
		Visible	Short 254 nm	Long 365 nm
1.	Drug Powder	Light Brown	Light Yellowish-Brown	Yellowish-Brown
2.	Drug Powder rubbed on filter paper	Brown	Off white	white (Low fluorescence)
3.	Powder treated with 1N NaOH in methanol	Yellowish Brown	Yellowish	Brownish-Yellow
4.	Powder treated with 1N NaOH in water	Dark Brown	Brownish-Yellow	Dark Yellow
5.	Powder treated with 1N HCL	Light Brown	Off white	Light Yellow
6.	Powder treated with 50% HNO_3	Orangish Yellow	Dark Brown	Brown
7.	Powder treated with 50% H_2SO_4	Black	Dark Brown	Black
8.	Ethanolic Extract	Brown	Off white	Light Brown
9.	Water Extract	Light Brown	Light Brown	Brown
10.	Chloroform Extract	Light Brown	Creamish white	Creamish Yellow
11.	Petroleum Ether	Light Brown	Off White	Light Yellow
12.	Dragendorff's Reagent	Blackish Brown	No Fluorescence	No Fluorescence

Table 7a

Phytochemical tests of *C. speciosus* Koen.

Chemical Groups	Name of the Extract				
	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Water
Alkaloids	–	+	+	+	–
Carbohydrates	–	–	+	+	+
Proteins & Amino Acids	–	–	–	–	–
Fixed Oils & Fats	+	–	–	+	–
Flavonoids	–	+	+	+	+
Phenolic compounds and tannins	–	+	+	+	+
Glycosides	+	+	+	+	+
Saponins	–	+	–	+	+
Steriods	+	+	–	–	–
Gums & Mucilages	–	–	–	–	–

+ = Positive, – = Negative

Table 7b

Inorganic elements tests of *C. speciosus* Koen.

Tests	Filterate	
	Hydrochloric Acid	Nitric Acid
Test for Calcium	Negative	Negative
Test for Potassium	Negative	Negative
Test for Magnesium	Negative	Negative
Test for Iron	Positive	Positive
Test for Sulphate	Positive	Positive
Test for Phosphate	Positive	Positive
Test for Chloride	Positive (Silver Nitrate) Positive (Lead Acetate)	Positive (Silver Nitrate) Negative (Lead Acetate)

4. Discussion

The quality control of crude drugs and herbal formulation is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is non-availability of rigid quality control profile for herbal material and their formulations[22]. Standardization is an essential measurement for ensuring the quality control of the herbal drugs and also encompasses the entire field of study from birth of a plant to its clinical application.

C. speciosus Koen. (Costaceae) has many medicinal and therapeutic actions that have been scientifically validated and documented. The present investigation deals with all the physico-chemical and pharmacognostical perspectives of its leaves. The organoleptic evaluation depicts that leaves are alternate, spirally arranged, sub sessile, elliptic to oblong or oblong-lancoelate with no characteristic taste and odour whereas the microscopical analysis shows that leaves consists of adaxial and abaxial epidermis with paracytic stomata and covering trichomes. Mesophyll cells constitute palisade cells and spongy parenchyma along with amphicribal concentric vascular bundles surrounded by sclerenchyma. The diagnostic features of power microscopy include prismatic and clusters crystals of calcium oxalate; starch granules simple and spiral xylem vessels.

It was concluded from physico-chemical parameters that total ash value was found to be the highest (13.4%) while acid insoluble ash was lowest (2.5%). Cold extractive values revealed that the percentage yield of aqueous extract was highest 11.0% followed by petroleum ether (5.0%), ethanol (4.0%), chloroform (3.0%) and the lowest percentage yield was that of ethyl acetate (2.0%).

The moisture content was calculated through loss on drying method and was found to be 1.43%. The swelling and foaming index values were recorded to be 0.7 cm and less than 100 respectively. The phytochemical investigation indicate the presence of the organic phytoconstituents such as alkaloids, carbohydrates, flavonoids, proteins, amino acids, phenols, tannins, glycosides, steroids and inorganic constituents like iron, phosphates, sulphates and chlorides.

It was concluded from current investigation of *C. speciosus* plant that the pharmacognostical data will provide the standards for its identification and authentication. The other parameters which are useful in the establishment of its quality control parameters are ash value, extractive values, moisture content, swelling and foaming index, leaf constants, fluorescence analysis and phytochemical parameters. The findings of the current research will help in the evaluation, identification and authentification of the plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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work. The authors are also thankful to Dr. D. C. Saini, Senior Scientist, Palaeobotany, Birbal Sahni Institute of Palaeobotany, Lucknow India for plants identification and its authentication.

Comments

Background

In recent years, the use of herbal medicines has increased remarkably with the global trend of people returning to natural therapies because these are considered harmless and without side effects. Despite of all, quality evaluation of herbal preparation is a fundamental requirement of industry and other organization to ensure the purity and quality of herbal medicines.

Research frontiers

The aim of present research work is to investigate the various pharmacognostical and physico-chemical properties of aerial parts of *C. speciosus*. Pharmacognostical studies includes macroscopy, microscopy, powder analysis and physico-chemical studies involved the assessment of ash values, moisture content, foaming index, swelling index, foreign organic content, extractive values and preliminary phytochemical screening.

Related reports

It is concluded from the literature survey that *C. speciosus* is a traditional medicinal plant consisting of various therapeutic constituents and used in various diseases.

Innovations and breakthroughs

C. speciosus commonly known as keukand in Hindi, is traditionally used as purgative, febrifuge, dysentery and as extensive acceptability as therapeutic agent for several diseases. Therefore, development of authentic analytical methods including pharmacognostical and physico-chemical parameters is established to ensure the safety, efficacy and purity of this important plant.

Applications

The research carried out by authors can be applicable in identification of plant and engraining the therapeutic safety and efficacy.

Peer review

The research carried out by authors on aerial parts of *C. speciosus* is genuine and valuable for the use of this plant as an ingredient in various formulations and treatment of several diseases because the authors have demonstrated all the quality control parameters, phytochemical analysis and microscopic characters which would be further authentic data in identification and authentication of this plant.

References

[1] Wealth of India Division, CSIR-National Institute of Science Communication and Information Resources. *The Wealth of India: A dictionary of India raw materials and industrial products: raw materials (Volume 2: Cl-Cy)*. New Delhi: National Institute of Science Communication and Information Resources; 2007. p. 211, 213.

[2] Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian medicinal plants*. New Delhi: National Institute of Science Communication and Information Resources; 2006. p. 79.

[3] Khare CP. *Indian medicinal plants*: an illustrated dictionary. Berlin: Springer; 2007, p. 181-182.

[4] Rajesh MS, Harish MS, Sathyaprakash RJ, Shetty AR, Shivanandan TN. Antihyperglycemic activity of the various extracts of *Costus speciosus* rhizomes. *J Nat Remedies* 2009; **9**(2): 235-41.

[5] Bavara JH, Narsimhacharya AV. Antihyperglycemic and hypolipidemic effects of *Costus speciosus* in alloxan induced diabetic rats. *Phytother Res* 2008; **22**(5): 620-626.

[6] Eliza J, Daisy P, Ignacimuthu S, Duraipandiyan V. Normo-glycemic and hypolipidemic effect of costunolide isolated from *Costus speciosus* (Koen ex. Retz.) Sm. in streptozotocin-induced diabetic rats. *Chem Biol Interact* 2009; **179**(2-3): 329-334.

[7] Bhattacharya S, Nagaich U. Assessment of anti-nociceptive efficacy of *Costus Speciosus* rhizome in swiss albino mice. *J Adv Pharm Technol Res* 2010; **1**(1): 34-40.

[8] Verma N, Khosa RL. Evaluation of protective effects of ethanolic extract of *Costus speciosus* (Koenig) Sm. rhizomes on carbon tetrachloride induced hepatotoxicity in rats. *Nat Prod Radiance* 2009; **8**(2): 123-126.

[9] Vijayalakshmi MA, Sarada NC. Screening of *Costus speciosus* extracts for antioxidant activity. *Fitoterapia* 2008; **79**(3): 197-198.

[10] Chakraborty GS. Free radical scavenging activity of *Costus speciosus* leaves. *Indian J Pharm Edu Res* 2009; **43**(1): 96-98.

[11] Duraipandiyan V, Al-Harbi NA, Ignacimuthu S, Muthukumar C. Antimicrobial activity of sesquiterpene lactones isolated from traditional medicinal plant, *Costus speciosus* (Koen ex.Retz.) Sm. *BMC complement Altern Med* 2012; **12**: 13.

[12] Srivastava S, Singh P, Mishra G, Jha KK, Khosa RL. *Costus speciosus* (Keukand): a review. *Der Pharmacia Sinica* 2011; **2**(1): 118-128.

[13] Lijuan W, Kupittayanant P, Chudapongse N, Wray S, Kupittayanant S. The effects of wild ginger (*Costus speciosus* (Koen) Smith) rhizome extract and diosgenin on rat uterine contractions. *Reprod Sci* 2011; **18**(6): 516-524.

[14] Rastogi RP, Mehrotra BN. *Compendium of Indian medicinal plants*. New Delhi: National Institute of Science Communication and Information Resources; 2004. p. 215.

[15] Khandelwal KR. *Practical pharmacognosy techniques and experiments*. New Delhi: Nirali Prakashan 2002; p. 15-163.

[16] Kokate CK. *Practical pharmacognosy*. 1st ed. New Delhi: Vallabh Prakashan; 2005. p. 111.

[17] The British Pharmacopoeia Commission. *British pharmacopoeia. European commission*. New Delhi: Department of health, social services and public safety, medicine and health care predict regulations agency; 2010, A-2.88-2.89.

[18] Government of India, Ministry of Health and Family Welfare. *The Ayurvedic pharmacopoeia of India Vol-3, Part-1*. 1st ed. New Delhi: Ministry of Health and Family Welfare; 2001, p. 233-251.

[19] Trease GE, Evans WC. *Pharmacognosy*. 15th ed. Amsterdam: Elsevier; 2002, p. 95-105.

[20] Kumar D, Gupta J, Kumar S, Arya R, Kumar T, Gupta G. Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf. *Asian Pac J Trop Biomed* 2012; **2**(1): 6-10.

[21] Raman N. *Phytochemical techniques*. New Delhi: New India Publishing Agency; 2006, p. 19-24.

[22] Verma S, Singh SP. Current and future status of herbal medicines, *Vet World* 2008; **1**(11): 347-350.