Research Article

Comparative Pharmacognostical and Pharmacological Evaluation of *Costus speciosus* (Koen) J.E. Sm. Germplasm Collected from Eastern Ghats of India

Manish Kumar[1,2], Ankita Misra[1], Akanksha Srivastava[1], Pushpendra Kumar Shukla[1], L. M. Tewari[2], Sharad Srivastava[1,*]

Manish Kumar[1,2], Ankita Misra[1], Akanksha Srivastava[1], Pushpendra Kumar Shukla[1], L. M. Tewari[2], Sharad Srivastava[1,*]

[1]Pharmacognosy Division, CSIR-National Botanical Research Institute, Lucknow (U.P.) 226001, INDIA.
[2]Department of Botany, D.S.B. Campus, Kumaun University, Nainital-263002, Uttarakhand, INDIA.

Correspondence

Dr. Sharad Srivastava

Senior Principal Scientist, Pharmacognosy division, CSIR-NBRI, Lucknow, INDIA.

Phone no: 91-522-2297818

Fax: 91-522-2205836

E-mail: sharad_ks2003@yahoo.com

ABSTRACT

Background: *Costus speciosus* is an erect perennial herb belonging to family Costaceae, an important medicinal plant widely used in several indigenous medicinal formulations. **Objective:** A comparative evaluation of Pharmacognostical and Pharmacological potential of *Costus speciosus* for the validation of traditional claims and quality parameters for industry. **Materials and Methods:** Pharmacognostical studies were performed as per Ayurvedic Pharmacopeia of India and quantification of diosgenin was done through HPTLC. *In vitro* antidiabetic activity was evaluated by α-amylase inhibition assay based on starch iodine method and *in vitro* anti-inflammatory were done by using inhibition of protein denaturation assay. **Results:** The pharmacognostical standards were also laid down for each sample. Morpho-anatomical characters had no distinct variation in all the collected samples of Eastern Ghats. The quantification of diosgenin (without hydrolysis of samples) in the collected germplasm varies significantly from 0.002 to 0.076 % on dry weight basis. The maximum content was recorded in NBCS-06 from Patiya, Bhubaneswar and was identified as distinct chemotype with high metabolite content. IC_{50} value of *Costus speciosus* extract in starch-iodine assay was found to be maximum in NBCS- 6 (87.54 µg/ml) and inhibition of protein denaturation assay was found to be maximum in NBCS- 11 (73.91 µg/ml), respectively. **Conclusion**: The study suggests that the *Costus speciosus* germplasm possess potential anti-inflammatory and anti-diabetic activity and comparative pharmacognostical parameters will be useful in collection of location specific potential samples for industrial usage along with quality control of raw materials.

Key words: *Costus speciosus*, HPTLC, Diosgenin, Anti-inflammatory, Anti-diabetic.

INTRODUCTION

Costus speciosus (Koen.) Smith (family Costaceae), is a perennial rhizomatous herb commonly known as "Crepeginger".[1] It is widely distributed in central parts of India, Sub-Himalayan tract, Karnataka, Western Ghats of Maharashtra and Kerala.[2] 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[3] along with, tigogenin and saponin as other key ingredients.[4]

The anti-inflammatory and antipyretic properties were proved in the Rhizome and also possess significant hepatoprotective activity.[5] *C. speciosus* leaf is used as a bath for patients with high fever. Rhizome juice is given traditionally with sugar to treat leprosy and for headache relief.[6,7] Furthermore, its alkaloid extract is used as a muscle relaxant with an antispasmodic effect.[8] *C. speciosus* is also used as a plant food in Southeast Asia.[9]

Aqueous extract of Rhizome is shown to possess antimicrobial activity.[10] The leaves are also used to control diabetes.[11]

Ayurveda acclaimed variation in the quality due to impact of season, altitude, soil and period of harvesting on the content of active principles in the plants. Considering this fact, the study was aimed to evaluate the pharmacognostical parameters in *C. speciosus* germplasm collected from different locations of Eastern Ghats. The variability in diosgenin content was also recorded through HPTLC method.[12] The present work may be helpful in the identification and quality control of raw material used in various Ayurvedic formulations.

MATERIALS AND METHODS

Chemicals and reagents

Solvents and chemicals *viz.* soluble starch, iodine, aluminum chloride, sodium carbonate, folin's reagent, methanol, ethyl acetate, n-Hexane and diethyl ether were procured from SD Fine Chemicals, Mumbai, India and HPTLC precoated silica gel 60 f_{254} (20 x 20 cm) plates were procured from Merck, India. Marker compound diosgenin was purchased from ChromaDex Inc, USA for quantification studies. Bovine Serum Albumin (BSA flakes, >99.8 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals and reagents used in study were of analytical grade purity.

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Plant material and extraction protocol

Rhizomes were collected during the month of September from Eastern Ghats region covering Orissa (India). Eleven samples were collected from varied condition(s); specimens were authenticated and deposited in repository of CSIR-NBRI with individual voucher number. GPS information of the collected samples was recorded. The samples were washed with water, chopped, and shade dried. The dried tubers were coarsely powdered by passing through 40 mesh sieve (up to 500 mm). The material was then macerated (cold method) with methanol (25 ml) for 24 h at room temperature (25°C ± 2°C) for 3 consecutive days. Extraction was repeated thrice, filtered (Whatman no. 4), and the pooled filtrate was dried in a rotatory evaporator (Buchi, USA) under standard conditions of temperature (55°C ± 2°C) and pressure (40 mbar). The nature and yield (%) of extract obtained in each germplasm was also documented.

Morpho-anatomical studies

The freshly collected samples were preserved in 70% ethanolic solution for macro-morphological studies. The anatomical studies were performed as per standard method.[13] Free hand sectioning was done to obtain thin sections so that cellular details are clearly visible. Sections were stained with 0.1 % toluidine blue 'O' solution for 30 second to visualize the secondary anatomical structures. The stained sections were mounted with glycerine on the glass slide and then observed under light microscope. Photomicrographs were taken with Nikon Advance Research Microscope, Eclipse 80i, Nikon instruments Inc., USA.

Physicochemical studies

Various physicochemical parameters *viz.* Extractive values by cold maceration method in different solvents (hexane, alcohol and water) and LOD (loss on drying) were estimated as per the protocol of Ayurvedic Pharmacopeia of India.[14,15] In addition, sugar, starch[16,] tannin[17,] phenolics, flavonoids[18] were also analyzed through spectrophotometric methods as per standard protocols.

High performance thin layer chromatography

Sample preparation

The coarsely powdered rhizome (2 gm) was macerated with methanol for 24 h at room temperature (25 ± 2°C). Extraction was repeated thrice, filtered and pooled filtrate was dried in rotatory evaporator (Buchi, USA) under standard conditions of temperature (55 ± 2°C) and pressure (40 mbar) and finally lyophilized (Labconco, USA) to dry residue. The extractive yield of collected sample was calculated (%).

Preparation of standard and sample solutions

The stock solution of standard Diosgenin (1.0 mg/ml) and plant samples (10 mg/ml) were freshly prepared in methanol. A working solution of 0.1 mg/ml diosgenin were diluted from the stock in same solvent to obtain dilutions in the concentration, ranging from 0.1 – 0.9 μg/ml. The solutions were filtered through a 0.45 μm Millipore membrane filter (Pall, USA) before application. The working dilutions were prepared freshly on the day of analysis.

HPTLC conditions

High performance thin layer chromatography was used for separation of the components present in extract, both quantitatively as well qualitatively. For quantitative analysis 10 μl sample solution (10 mg/ml) was applied using 100 μl sample syringe (Hamilton, Switzerland) on pre-coated plates with silica gel 60 F_{254} of 0.2 mm thickness as 6 mm-wide bands positioned 10 mm from the bottom and 15 mm from side of the plate, using CAMAG Linomat V automated TLC applicator with nitrogen flow providing a delivery speed of 150 nl/s from application

syringe. HPTLC plate was developed in a CAMAG twin trough glass chamber pre-saturated with mobile phase. After development, the chromatogram was air-dried and then derivatized with anisaldehyde-sulphuric acid reagent. The marker compounds were quantified using a CAMAG TLC Scanner equipped with CAMAG vision CAT software at 440 nm. The following scan conditions were applied: slit width, 4 mm × 0.45 mm and absorption–reflection mode.[19]

Pharmacological evaluation

Antidiabetic activity

Starch–iodine assay

Starch–iodine test was carried out according to the standard method.[20] Inhibition of enzyme activity was calculated as following:

Inhibition of enzyme activity (%) = [(C-S)/C] x 100.

Where S is the absorbance of the sample and C is the absorbance of blank (no extract).

Anti-inflammatory activity

Inhibition of protein denaturation method

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of plant extract at different concentration. The samples were incubated at 37°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. In control, 0.05 ml distilled water was used instead of extracts, whilst product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as per standard protocol, and the formula is given below. The control represents 100% protein denaturation. The results were compared with acetylsalicylic acid (250 mcg/ml)-treated samples.[21,22]

Percentage of inhibition = [(Abs $_{sample}$ - Abs $_{control}$)/ (Abs $_{sample}$)] x 100

RESULTS AND DISCUSSION

Microscopical description

On anatomical studies of collected 11 samples (rhizome), some common features were observed as follows: (1) single layered epidermis followed by 4-6 layered periderm, (2) Below this, a multilayered cortex is present having parenchymatous cells, (3) it contains scattered vascular bundle which is divided in to three zones i.e., cortical vascular bundle, endodermal vascular bundle and stellar vascular bundle, (4) xylem vessels were clearly seen and are surrounded by phloem cells, (5) Bunch of starch grains of varying sizes are seen scattered all around the cortex and pith region, (6) starch grains are medium to large in size, compactly arranged but sometimes distinctly placed. The starch grains are light colored, cylindrical in shape and showing clear hilum with concentric striations (Figure 1). There is no diagnostic difference observed among the anatomical structure of collected samples.
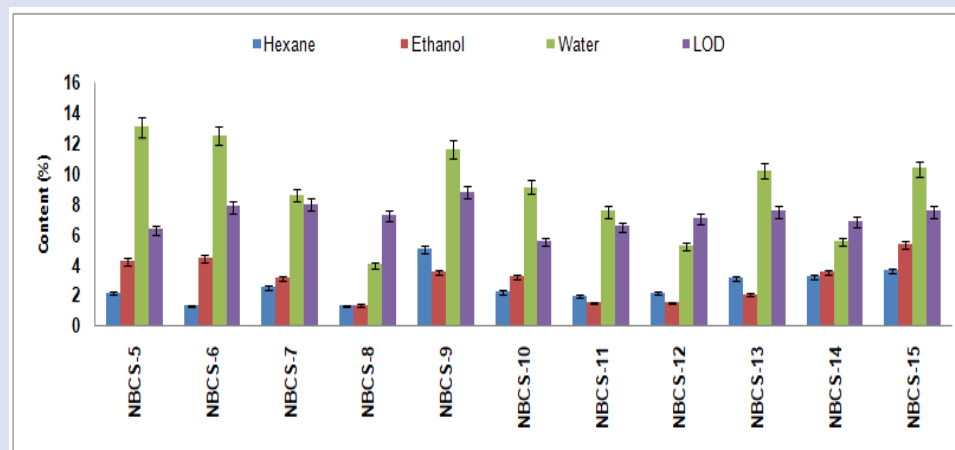
Physicochemical and phytochemical studies

A comparative pharmacognostical studies of *Costus speciosus* samples (rhizome) revealed variability in hexane, alcohol and water-soluble extractive among the collected 11 samples from 1.3-3.6 %, 1.33-5.3 % and 4.0-13.1 % respectively. It is observed that within the samples, water soluble extractive was found to be maximum, followed by hexane and ethanol soluble extractives. These (extractive values) are primarily useful for determination of exhausted drug and was found to be within the limits of API. The LOD (%) of collected samples ranges from 5.5 to 8.8 %, maximum and minimum content was observed in NBCS-10 and NBCS-9 (Figure 2).
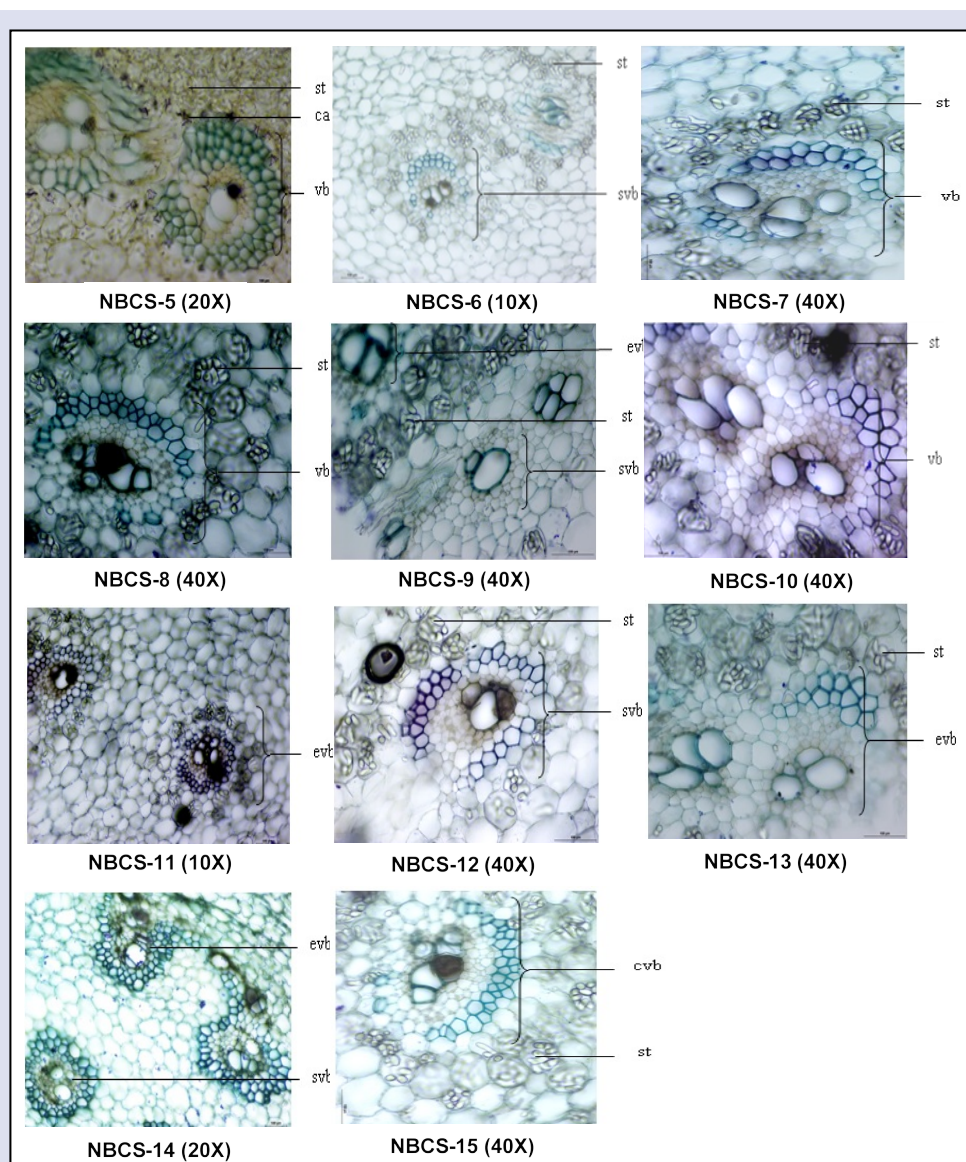
Figure 1: Microscopical description of *Costus speciosus* rhizome of the collected populations.

Abbreviations: vb - vascular bundles, ol - oil globule, st- starch granules, ca- calcium oxalate crystal, svb-stellar vascular bundle, evb- endodermal vascular bundle, cvb- cortical vascular bundle.

Figure 2: Physicochemical standards of *Costus speciosus* sample collected from Eastern Ghats. Values are mean ± S.E.

The phytochemical estimation of various metabolites in the rhizome *viz.* total sugar, total starch, total phenolic and flavonoid through spectrophotometer method varies from 0.2 - 0.54 %, 0.38 - 0.6 %, 0.036 - 0.162 % and 0.073 - 0.322 % respectively (Figure 3). The starch content within each sample was found maximum than other metabolites, which is true in a sense that rhizome is vegetative part and serve as food storage unit of plant system.

HPTLC quantification of marker compound

The HPTLC study was done on the methanolic extract without hydrolysis with intent to provide a simple method for quality control. The extractive yield (methanolic) of *Costus speciosus* within the population significantly ($p<0.05$) varies from 1.48 to 8.2%. HPTLC condition(s), namely, selection of mobile phase, absorption maxima and slit dimensions was standardized to provide an accurate, precise, and reproducible method for the quantification of diosgenin. The method development was initiated with separation of diosgenin (extract) in various solvent systems by hit and trial method and finally a binary solvent system of n-Hexane: ethyl acetate (7:2 v/v) was selected based on separation of unknown markers from diosgenin. The absorption spectrum of diosgenin was observed at 440 nm after scanning the entire UV range of 200-800 nm. In sample, diosgenin was identified at R_f 0.23 ± 0.05. The saturation time for the development of chromatogram was optimized to 20 min for a good resolution of the diosgenin markers and the total run time was 30 min at room temperature (27 ± 2°C). Purity of the bands in the samples was confirmed by comparing band spectra of sample with the corresponding band spectra of standard at the start, middle and end position of the band peak.

The quantification of diosgenin without hydrolysis within the population revealed that the content varies from 0.002 - 0.076% on dry weight basis and the maximum content was recorded in NBCS – 06 from Patiya, Bubhneshwar. The minimum content was in NBCS-11 from Dubri, Jajpur (Table 1).

Anti-diabetic activity

In vitro anti-diabetic potential of *Costus speciosus* was assessed by starch–iodine colour assay. Data of starch–iodine show that activity increases linearly with increase in concentration, i.e. 20–100 µg/ml, IC50 value of *Costus speciosus* extract was found maximum in NBCS- 6 (87.54 µg/ml) respectively (Figure 4).

Anti-inflammatory activity

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation. Maximum inhibition i.e. 73.91 µg/ml was observed in NBCS-11 (Figure 5).
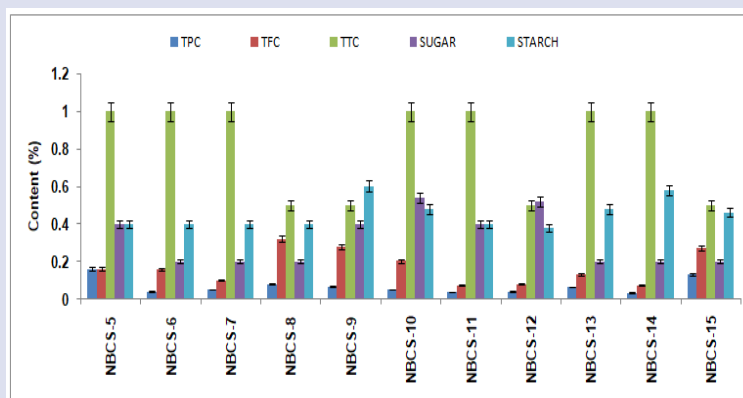
Figure 3: Phyto-chemical evaluations of *Costus speciosus* samples from Eastern Ghats. Values are mean ± S.E.
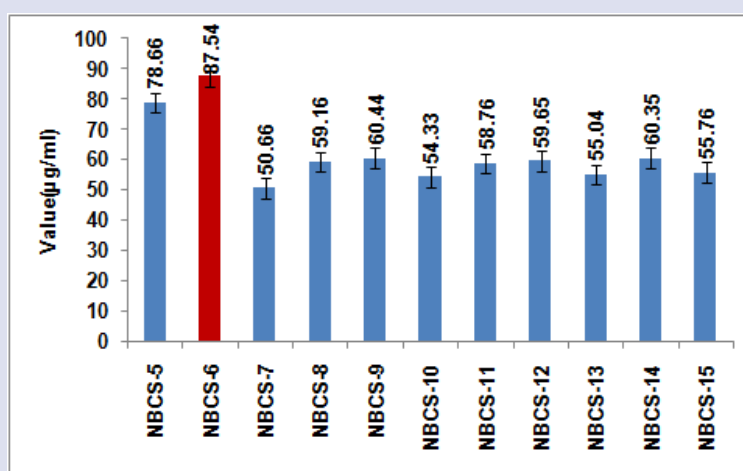
Figure 4: Anti-diabetic potential of *Costus speciosus* collected from Eastern Ghats. Values are mean ± S.E.

Figure 5: Anti-inflammatory potential of *Costus speciosus* collected from Eastern Ghat. Values are mean ± S.E.
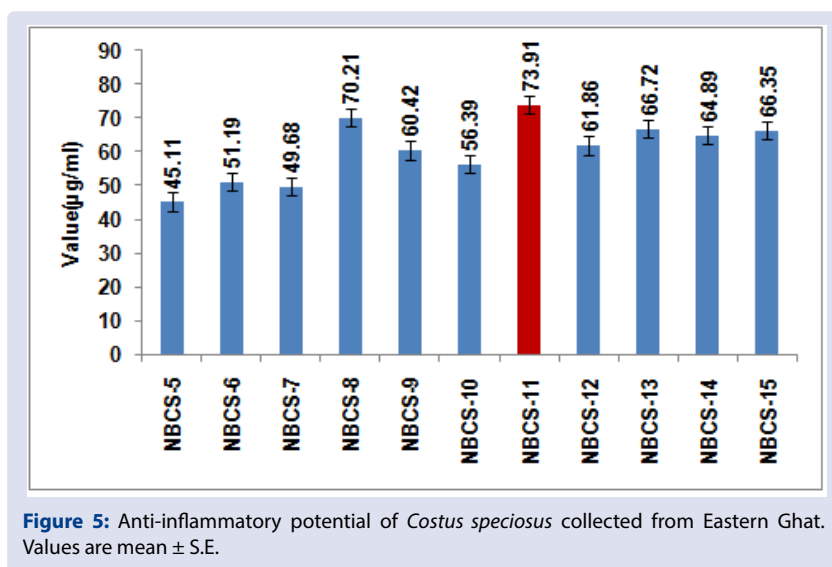
Table 1: Quantification of diosgenin in 11 different populations of *Costus speciosus*.

Sample code	Locations	Diosgenin content (% dry wt. basis)
NBCS – 05	Raigada	0.038%
NBCS – 06	Patiya, Bhubaneswar	0.076%
NBCS – 07	Kailashpurgati, Rayagada	0.039%
NBCS – 08	Bhubaneswar	0.048%
NBCS – 09	Madhopur	0.046%
NBCS – 10	Anandapur	0.007%
NBCS – 11	Duburi, Jajpur	0.002%
NBCS – 12	Sambalpur	0.017%
NBCS – 13	Sambhalpur university	0.022%
NBCS – 14	Attabira, Bargada	0.024%
NBCS – 15	Deogarh, Shambhalpur	0.01%

CONCLUSION

This study will help in promoting the site specific collection of high metabolite yielding germplasm to meet out the industrial demand of good quality raw material. The develop method will aid in regulation of quality standard and also batch to batch consistency raw materials in industry.

## ACKNOWLEDGMENT

CONFLICTS OF INTEREST

There is no conflicts of interest.

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GRAPHICAL ABSTRACT

Anti-inflammatory

Anti-diabetic
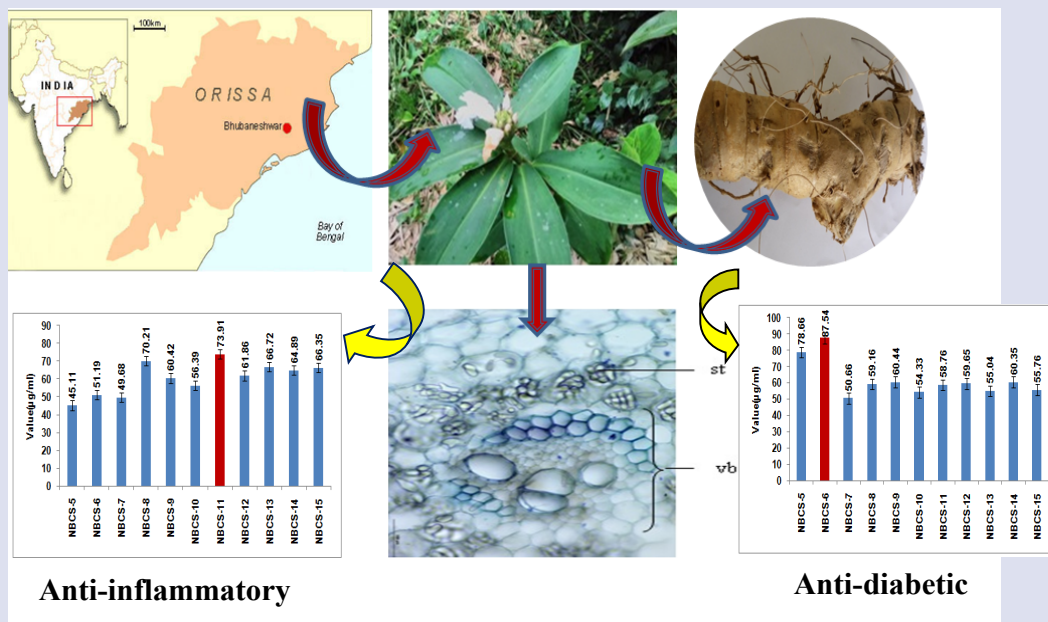
Graphical Representation of *Costus speciosus*

ABOUT AUTHORS

Mr. Manish Kumar: Senior Research Fellow (ICAR-SRF) in Pharmacognosy Division at CSIR-National Botanical Research Institute; Lucknow, INDIA. He is working in the area of analytical chemistry, anatomical study, molecular profiling and chemotaxonomy on medicinal plants. He has 9 publications in peer reviewed journals. Email id: mnubhu@gmail.com

Dr. Ankita Misra: Young Scientist in Pharmacognosy Division at CSIR-National Botanical Research Institute; Lucknow, INDIA. She is working in the area of analytical chemistry on medicinal plants, chemotaxonomy, bio-prospection and natural product development, handling major analytical instruments including HPLC, HPTLC, OPLC and column chromatography for quality control of herbal drugs. She has 32 publications in peer reviewed journals and 2 patented products are also to her credit. Email id: anku.mis@gmail.com

Mrs. Akanksha Srivastava: Senior Research Fellow (ICAR-SRF) in Pharmacognosy Division at CSIR-National Botanical Research Institute; Lucknow, INDIA. She is working in the area of *in vitro* pharmacological evaluation, and quality control of herbal drugs. She has 4 publications in peer reviewed journals. Email id: srivastavakn89@gmail.com

Dr. Pushpendra Kumar Shukla- Young Scientist in Pharmacognosy division, CSIR- National Botanical Research Institute, Lucknow. He is working in the area of analytical chemistry and chemotaxonomy, isolation of active metabolites and handling of sophisticated instruments like HPTLC, HPLC, OPLC, AAS, for identification and quantification of metabolites. He has 15 publications in peer reviewed journals Email id: itspushpendra85@gmail.com

Dr. Sharad Srivastava: Senior Principal Scientist in Pharmacognosy Division at CSIR-National Botanical Research Institute; Lucknow, INDIA. He has made significant contributions to quality control of crude drugs/products, chemotaxonomy, bio-prospection and natural product development and have developed quality parameters of single crude drugs (more than 70 medicinal plants) and also identified biomarkers for their quality control. He has contributed 30 monographs of single herbal drugs in Ayurvedic Pharmacopoeia of India. He has 145 publications in peer reviewed journals, 17 patents and developed some technologies/formulations, few has already been transferred to industry. Email id: sharad_ks2003@yahoo.com