



## Phytochemical and Antimicrobial Studies of Medicinal Plant *Costus Speciosus* (Koen.)

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**Abstract:** The present paper deals with the phytochemical and antimicrobial screening of therapeutic importance from *Costus speciosus* (Koen.), an important medicinal plant. The study involves the preliminary screening and qualitative HPTLC separation of secondary metabolites from the rhizome of *Costus speciosus* (Koen.). The *in vitro* antibacterial activity was performed against a few pathogens viz. *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The generated data has provided the basis for its wide use as the therapeutic both in traditional and folk medicine.

**Keywords:** Phytochemical, Antimicrobial, *Costus speciosus* (Koen.).

### Introduction

Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12000 have been isolated, a number estimated to be less than 10% of the total<sup>1</sup>. These substances serve as molecules of plant defense against predation by microorganisms, insects and herbivores and at the same time also exhibit medicinal properties for treating several ailments<sup>1</sup>. The steroidal saponin, diosgenin has been reported from the rhizome of *costus speciosus* (Koen.). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action<sup>2-5</sup>. In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in treatment of infectious diseases<sup>6</sup>. In addition to this, antibiotics are sometimes associated with adverse effects on the hosts like hypersensitivity. Therefore, there is a need to develop alternative antimicrobial drug for the treatment of infectious disease from other sources, such as plants<sup>7</sup>. Natural products of higher plants may be a new source of antimicrobial agents possibly with novel mechanism of action<sup>5</sup>. The storage organs of higher plants show great biological activities. Chemical substances that produce definite physiological actions on human bodies accumulate in storage organs of the plants. The most important of these bioactive compounds are alkaloids, flavanoids and phenolic compounds<sup>8</sup>.

In the present study was concentrated on the preliminary screening, qualitative screening of metabolites and antibacterial activity from the rhizomes of *Costus speciosus*.

## Experimental

Rhizome of *Costus speciosus*, was collected from Arey Colony, Goregaon, Mumbai and authenticated by comparison with herbarium specimens and electronic herbarium at Department of Botany, The Institute of Science, Mumbai. These rhizomes were thoroughly washed under running tap water and with the help of a brush; the mud was scrubbed off from the surface of the rhizomes. Air-dried rhizomes were subsequently cut with a scalpel into slice of 0.5 mm thickness. The pieces were dried in an oven at 110 °C for 4 h. The dried material was cooled and powdered in a blender. The powder was stored in a cool and dry place and was used for extraction and further analysis.

### *Preliminary screening of secondary metabolites*

The shade dried plant material was powdered using mixer grinder and subjected to soxhlet extraction with petroleum ether, chloroform, 95% ethanol and distilled water for 18 h in the order of increasing polarity of solvents. The condensed extracts were used for preliminary screening of phytochemicals such as alkaloids (Wagner, Mayers and Dragendorff's tests), flavonoids (Shinoda and NaOH tests), cardiac glycosides (Keller-Kiliani, conc.H<sub>2</sub>SO<sub>4</sub> tests), saponins (foam and haemolysis tests), sterols (Lieberman-Burchard and Salkowski tests) and tannins (gelatin test) were carried out<sup>9-10</sup>.

### **Separation of secondary metabolites by HPTLC**

#### *Chromatographic conditions*

Chromatography was performed on silica gel 60 F<sub>254</sub> HPTLC plates (10 x 20 cm; 0.25 mm layer thickness). Samples and standard compounds were applied to the layer as 8 mm wide bands positioned 10 mm from the bottom of the plate, using an automated TLC applicator Linomat IV (Camag, Muttex, Switzerland) with nitrogen flow providing delivery from the syringe at a speed of 10 s/μL.

#### **HPTLC study**

##### *Anthra-glycosides, arbutin, bitter principles and flavanoids*

Powdered drug (1 g) was extracted by heating on a water bath for 15 minutes with 5 mL methanol. 2 μL and 10 μL of the filtrate were applied to the chromatogram. The bitter principles spots were separated using solvent system: 5% acetic acid and anisaldehyde H<sub>2</sub>SO<sub>4</sub> as spray reagent<sup>11</sup>.

##### *Alkaloids*

The powdered rhizome were wet with a half diluted NH<sub>4</sub>OH and lixiviated with EtOAc for 24 h at RT. The organic phase is separated from the acidified filtrate and basified with NH<sub>4</sub>OH (pH 11- 12).It was extracted with chloroform (3X), condensed by evaporation and used for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). The colour and R<sub>f</sub> values of the separated alkaloids were recorded both under ultraviolet (UV 254 nm) and visible light after spraying with Dragendorff s reagent<sup>11</sup>.

##### *Saponins*

Two grams of powdered rhizome were extracted with 10 mL 70% EtOH by refluxing for 10 min. The filtrate was condensed, enriched with saturated *n*-BuOH, and thoroughly mixed. The butanol was retained, condensed and used for chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and R<sub>f</sub> values of these spots were recorded by exposing chromatogram to the iodine vapours<sup>11</sup>.

### *Cardiac-glycosides*

Powdered drug (1 g) was mixed with 5 mL of 50% methanol and 10 mL of 10% lead(II) acetate solution and then heated for 10 minutes on water bath. The cooled filtrate was extracted with 2 separate 10 mL quantities of dichloromethane (DCM). The combined DCM extracts were completely evaporated. The residue was dissolved in DCM: methanol (1:1). 2  $\mu$ L and 10  $\mu$ L of this solution was applied to the chromatogram. The cardiac-glycosides spots were separated using solvent mixture. Solvent system of ethyl acetate: methanol: water (10:1.4:1) and methanolic H<sub>2</sub>SO<sub>4</sub> as spray reagent<sup>12</sup> were used.

### *Essential oils, coumarins, phenols carboxylic acids and valepotriates*

Powdered drug (1 g) was extracted by heating under reflux for 15 minutes with DCM. The filtrate was evaporated to dryness and the residue was dissolved in 1 mL toluene, 2  $\mu$ L and 10  $\mu$ L of the filtrate were applied to the chromatogram. Best solvent system used was toluene: ethyl acetate (9.3:0.7) and 5% vanillin in H<sub>2</sub>SO<sub>4</sub> as spray reagent<sup>11</sup>.

### *Anthraquinones*

The anthraquinones spots were separated using solvent mixture. Toluene: ethyl acetate: formic acid (15:5:1) and 10% methanolic KOH as spray reagent<sup>11</sup>.

### *Steroids*

Air dried plant powder was extracted with pet ether (40-60 °C). Hot methanol was added to the green coloured extract which was obtained. This was then evaporated to reduce the volume. The steroids spots were separated using solvent mixture. DCM:diethyl ether: methanol: water (7.7:1.5:0.8:0.12) and methanolic H<sub>2</sub>SO<sub>4</sub> as spray reagent<sup>11</sup>.

### *Flavanoids*

One gram powdered rhizome were extracted with 10 mL methanol on water bath (60 °C / 5 min). The filtrate was condensed by evaporation, a mixture of water and EtOAc (10:1 mL) was added and mixed thoroughly. The EtOAc phase thus retained was used for chromatography. The flavanoids spots were separated using chloroform and methanol (19:1) solvent mixture anisaldehyde-sulphuric acid reagent. The colour and R<sub>f</sub> values of these spots were recorded under ultraviolet (UV 254 nm) light<sup>11</sup>.

### *Sterols*

Two grams of powdered rhizome were extracted with 10 mL methanol in water bath (80 °C /15 min).The condensed filtrate was used for chromatography. The sterols were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and R<sub>f</sub> values of these spots were recorded under visible light after spraying the plates with anisaldehyde-sulphuric acid reagent on heating at 100 °C for 6 min<sup>11</sup>.

### *Antimicrobial screening*

Extraction of plant material was prepared by cold percolation method. The air-dried and powdered plant material 5 g of each was soaked in 50 mL methanol and kept for 48 h with intermittent shaking. The plant extract were filtered through Whatman no. 1 filter paper. The filtrated were dried until a constant dry weight of each extract was obtained. The remaining part of the plant residue was dried and soaked in 50 mL distill water for 48 h and extract was collected as described earlier. Each extract was dissolved in 1 mL dimethyl sulfoxide (DMSO) and 20  $\mu$ L of each sample was taken for experiment.

*Antimicrobial assay*

The methanolic and water extract of plant were screened against four bacteria. The test organisms *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* were obtained from Department of Microbiology, Institute of Science, Mumbai. Microorganisms were maintained at 4 °C on nutrient agar slants. The modified agar disc diffusion method<sup>13</sup> was used to screen the antimicrobial activity. Sterile filter paper discs of 6 mm diameter impregnated with 20 µL extract solution equivalent to 4 mg of the dried extract and after evaporation placed on the surface of the inoculated agar plate and the compound was allowed to diffuse for 5 minutes and plates were kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones around the disc were measured with transparent ruler in mm (Table 1). The study was performed in triplicate. Amikacin was used as control against all pathogens

**Results and Discussion**

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavanoids, glycosides, phenols, saponins, sterols *etc.* The successive extracts of rhizome have revealed the presence of alkaloids, flavanoids, cardiac glycosides, saponins, sterols and tannins (Table 2). Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds<sup>1</sup>.

**Table 1.** Inhibition zones (in mm) in accordance to test microorganisms by agar disc diffusion method

S. No	Plant Extract	<i>Staphylococcus aureus</i>	<i>E. coli.</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
1	Methanolic Extract	-	-	-	-
2	Aqueous Extract	15 mm	-	-	-

**Table 2.** Preliminary screening of secondary metabolites from rhizome of *Costus speciosus*

Secondary metabolites	Name of the test	Inference
Alkaloids	Mayers test	+
	Dragendorffs	+
	Wagner's test	+
Flavanoids	Shinoda test	+
	KellarKiliani test	+
Cardiac Glycosides	Molisch test	+
	Labat test	+
Saponins	Frothing test	+
Sterols	Libermann Burchard test	+
Tannins	Gelatin test	+
Anthraquinone Glycosides	With KOH, red colour formation	+

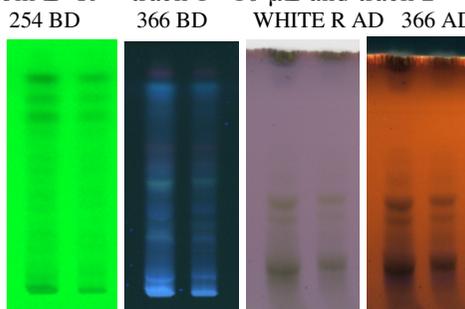
(‘+’: present, ‘-’: absent)

HPTLC screening showed the presence of different type of anthra-glycosides, arbutin, bitter principle, flavanoids, alkaloids, saponin, cardiac glycosides, essential oils, coumarins, phenols, carboxylic acids, valepotriates, anthraquinones, steroids and sterols in addition to the compound detected by preliminary screening (Plate No. 1- 8).

### *Bitter principle*

The HPTLC chromatogram can be observed best at any wavelength (254 nm, 366 nm). There is no necessary need for derivatisation since compounds are seen best separated before derivatisation. After derivatisation good band separation was seen under visible light and at 366 nm. The major compounds separated was seen at  $R_f = 0.03, 0.07, 0.26, 0.34, 0.48, 0.53, 0.63, 0.79, 0.89$  and  $0.95$  (Track 1 at 366 nm, Figure 1). Since the compound is highly polar in nature it can be seen just below the solvent front. Due its nature of polarity the chromatogram can further be used for preparative HPTLC. The sample concentration  $10 \mu\text{L}$  is found to be more than suitable as compared to  $5 \mu\text{L}$ .

(from L -R→ track 1 =  $10 \mu\text{L}$  and track 2 =  $5 \mu\text{L}$ )

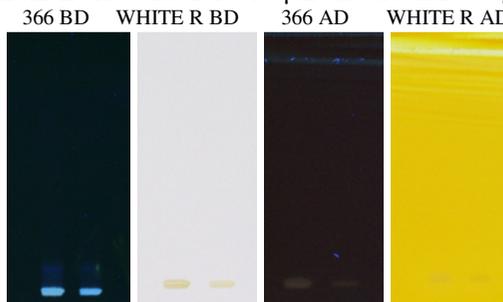


**Figure 1.** (Plate no 1) HPTLC of bitter principle  
(AD: After derivatisation; BD: Before derivatisation)

### *Alkaloids*

The HPTLC chromatogram can be observed best only in wavelength 366 nm, before derivatisation. There is no need to derivatise since compounds are not seen after derivatisation. A single band of alkaloid is seen to be separated before derivatisation at 366 nm. The compounds separated was seen at  $R_f = 0.02$  (Track 1 at 366 nm, Figure 2). However there is still need for improvements and modification in the process of extraction in order to optimize the concentration of separation of bands. The compound is non polar in nature. The sample concentration  $10 \mu\text{L}$  is found to be more than suitable as compared to  $5 \mu\text{L}$ .

(from L -R→ track 1 =  $10 \mu\text{L}$  and track 2 =  $5 \mu\text{L}$ )

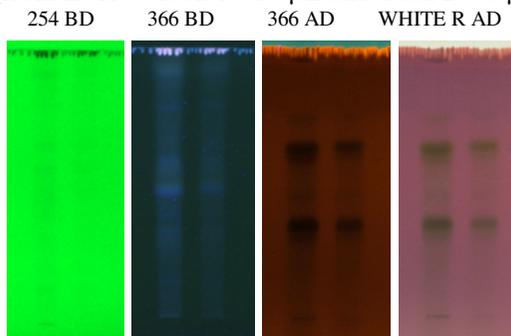


**Figure 2.** (Plate no 2) HPTLC of alkaloid

*Saponins*

Saponins was best observed at visible light after derivatisation but not seen under visible light before derivatisation. It is necessary to derivatise since compounds are seen well separated after derivatisation. The major compounds separated was seen at  $R_f = 0.30, 0.45, 0.49, 0.53, 0.59, 0.79, 0.91, 0.93$  and  $1.0$  (Track 1 at 366 nm, Figure 3). Since the compound is highly polar in nature it can be seen just below the solvent front. Due its nature of polarity the chromatogram can further be used for preparative HPTLC. The sample concentration  $10 \mu\text{L}$  is found to be more than suitable as compared to  $5 \mu\text{L}$ .

(from L -R→ track 1 =  $10 \mu\text{L}$  and track 2 =  $5 \mu\text{L}$ )

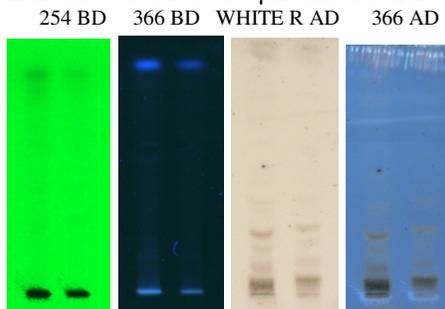


**Figure 3.** (Plate no 3) HPTLC of saponin

*Cardiac glycosides*

The HPTLC chromatogram for cardiac glycoside was best observed at 366 nm before derivatisation. There is no necessary need for derivatisation since compounds are seen best separated before derivatisation. After derivatisation good band separation is seen at 366 nm (Track 1 at 366 nm, Figure 4). Since the compound is highly polar in nature it can be seen just below the solvent front. The sample concentration  $10 \mu\text{L}$  is found to be more than suitable as compared to  $5 \mu\text{L}$ .

(from L -R→ track 1 =  $10 \mu\text{L}$  and track 2 =  $5 \mu\text{L}$ )



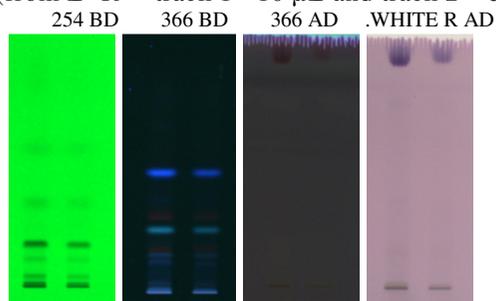
**Figure 4.** (Plate no 4) HPTLC of cardiac-glycoside

*Essential oils, coumarin, phenols and carboxylic acid*

Essential oils, coumarin, phenols and carboxylic acid was found to be present. The chromatogram can be observed at 254 nm and 366 nm before derivatisation and under visible light after derivatisation. No specific need for derivatisation, as they are seen to be best separated before derivatisation. The major compounds separated was seen at  $R_f = 0.63, 0.82$  and  $0.95$  (Track 1 at 366 nm, Figure 5). However there is still need for improvements and modification in the process of extraction in order to optimize the concentration of separation

of bands. Since the compound is highly polar in nature it can be seen just below the solvent front. Due its nature of polarity the chromatogram can further be used for preparative HPTLC. The sample concentration 10  $\mu\text{L}$  is found to be more than suitable as compared to 5  $\mu\text{L}$ .

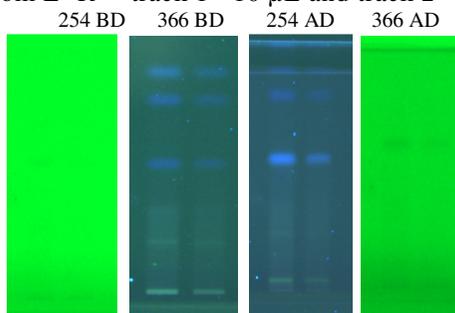
(from L -R $\rightarrow$  track 1 =10  $\mu\text{L}$  and track 2 = 5  $\mu\text{L}$ )



**Figure 5.** (Plate no 5) HPTLC of essential oil, coumarin, phenol and carboxylic

### *Anthraquinones*

The HPTLC chromatogram for anthraquinones was best observed at 366 nm before and after derivatisation. There is no necessary need for derivatisation since compounds are seen best separated before derivatisation. The major compounds separated was seen at  $R_f = 0.02, 0.05, 0.31, 0.33, 0.44, 0.54, 0.65, 0.66, 0.85$  and  $0.89$  (Track 1 at 366 nm, Figure 6). The sample concentration 10  $\mu\text{L}$  (from L -R $\rightarrow$  track 1 =10  $\mu\text{L}$  and track 2 = 5  $\mu\text{L}$ )



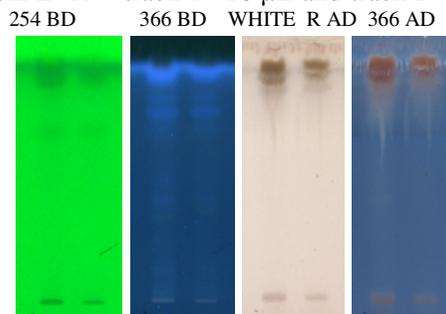
**Figure 6.** (Plate no 6) HPTLC of anthraquinone

### *Steroids*

The HPTLC chromatogram for steroids was best observed at 366 nm before and after derivatisation. There is need for derivatisation since the bands of the compound separated appears prominent after derivatisation. The major compounds separated was seen at  $R_f = 0.27, 0.47$  and  $0.68$  (Track 1 at 366 nm, Figure 7). However there is still need for improvements and modification in the process of extraction in order to optimize the concentration of separation of bands.

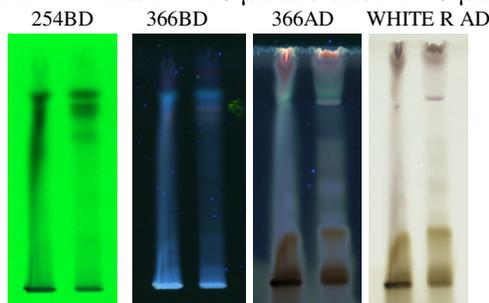
### *Flavanoids*

The HPTLC chromatogram can be best observed under fluorescence 366 nm before and after derivatisation. The sample concentration (5  $\mu\text{L}$ ) is sufficient to generate the compounds. The major compounds separated was seen at  $R_f = 0.56, 0.60, 0.74, 0.78, 0.84$  and  $0.88$  (Track 2 at 366 nm).

(from L -R→ track 1 =10  $\mu$ L and track 2 = 5  $\mu$ L)**Figure 7.** (Plate no 7) HPTLC of steroid

### *Sterols*

Sterols were found to be best observed at 254 nm and 366 nm before derivatisation and at 366 nm and under visible light after derivatisation with optimum sample application of 5  $\mu$ L. The major compounds separated was seen at  $R_f$  =0.02, 0.07, 0.20, 0.38, 0.75, 0.81 and 0.86 (Track 2 at 366 nm, Figure 8).

(from L -R→ track 1 =10  $\mu$ L and track 2 = 5  $\mu$ L)**Figure 8.** (Plate no 8) HPTLC of sterol

### *Antimicrobial studies*

The aqueous extracts appear to have antibacterial activity only against *Staphylococcus aureus*. This is interesting in that the traditional method of treating a bacterial infection was by administering a decoction of the plant or apart there by boiling it in water; our results are in accordance to the traditional system of administration. The methanolic extract did not show inhibitory activity against any bacteria, this may be because the active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed<sup>14</sup>. Lack of activity can thus only be proven by using large doses<sup>15</sup>. Alternatively, if the active principles are present in high enough quantities, there could be other constituents exerting an antagonistic effects or negating the positive effects of the bioactive agents<sup>16</sup>. With no antimicrobial activity, extracts may be active against other bacterial species, which were not tested<sup>17</sup>.

The data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. However, there is need to further carry out advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds. Furthermore, this data may be handy in probing of biochemistry of this plant in the future.

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