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Phytochemical, Histochemical and *in-vitro* Antimicrobial Study of Various Solvent Extracts of *Costus speciosus* (J.Koenig) Sm. and *Costus pictus* D. Don

Costus speciosus (J.Koenig) Sm.'nin Çeşitli Çözücü Ekstraktlarının Fitokimyasal, Histokimyasal ve In vitro Antimikrobiyal Çalışması. ve Costus pictus D. Don

Sana Saffiruddin Shaikh*, Dr AbubakarSalam Bawazir, Barrawaz Aateka Yahya
Y.b. Chavan College Of Pharmacy, Dr. Rafiq Zakaria Campus Aurangabad-431001 (ms) India

Corresponding Author Information
Sana Saffiruddin Shaikh
sana.shaikh310194@gmail.com
8793127276
orcid.org/0000-0001-8517-7531
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ABSTRACT:
Objectives: Costaceae family comprises many ornamental and medicinal plants used for different diseases. The present investigation includes the Phytochemical, Histochemical, and *in-vitro* antimicrobial study of *Costus speciosus* (J.Koenig) Sm. (CS.) and *Costus pictus* D. Don (CP).
Materials and methods: Solvents such as methanol, ethyl acetate, and hexane were used to extract leaves and rhizomes of both plants. The antibacterial study was executed by using the agar well diffusion technique.
Results: Phytochemical study confirms alkaloids, flavonoids, quinones, saponins present in solvent extracts of both plants. The macro-morphological studies, including size, shape, texture, surface characters, color, were analyzed. *Salmonella typhi, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* were used for the antibacterial study. Agar well diffusion and agar disk diffusion method determined the susceptibility of bacterial strains against various solvent extracts.
Conclusion: Histochemical analysis reveals alkaloids, proteins, and phenols in vascular bundles, cortex, and epidermis of stem, root, and leaf of the plants. Inhibition zones achieved by methanol and hexane extracts show better antibacterial activity compared to other extracts. Future work on the isolation, purification, characterization of the active constituents, and the elucidation of possible mechanisms can be executed.

ÖZ:
Amaç: Costaceae familyası, farklı hastalıklar için kullanılan birçok süs ve tıbbi bitkiyi içermektedir. Mevcut araştırma, Costus speciosus (J.Koenig) Sm.'nin Fitokimyasal, Histokimyasal ve in vitro antimikrobiyal çalışmasını içerir. (CS.) ve Costus pictus D. Don (CP).
Gereçler ve yöntemler: Her iki bitkinin yapraklarını ve rizomlarını çıkarmak için metanol, etil asetat ve heksan gibi çözücüler kullanıldı. Antibakteriyel çalışma, agar kuyusu difüzyon tekniği kullanılarak gerçekleştirilmiştir.
Sonuçlar: Fitokimyasal çalışma, her iki bitkinin çözücü ekstraktlarında bulunan alkaloidler, flavonoidler, kinonlar, saponinleri doğrulamaktadır. Boyut, şekil, doku, yüzey karakterleri, renk gibi makro-morfolojik çalışmalar analiz edildi. Antibakteriyel çalışma için Salmonella typhi, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus kullanıldı. Agar kuyu difüzyonu ve agar disk difüzyon yöntemi, bakteri suşlarının çeşitli solvent ekstraktlarına karşı duyarlılıklarını belirlemiştir.
Sonuç: Histokimyasal analiz, bitkilerin gövde, kök ve yaprağının damar demetleri, korteks ve epidermisindeki alkaloidleri, proteinleri ve fenolleri ortaya koymaktadır. Metanol ve hekzan ekstraktları ile elde edilen inhibisyon bölgeleri, diğer ekstraktlara kıyasla daha iyi antibakteriyel aktivite gösterir. Aktif bileşenlerin izolasyonu, saflaştırılması, karakterizasyonu ve olası mekanizmaların aydınlatılması ile ilgili gelecekteki çalışmalar yürütülebilir.

Keywords: Costaceae*, Costus pictus, Costus speciosus,* Histochemical, Antibacterial

anahtar kelimeler: Costaceae, *Costus pictus, Costus speciosus*, histokimyasal, antibakteriyel

INTRODUCTION

Plants have been used as medicines since the start of the human race.[1] These medicines initially were used as poultices, tinctures, teas, powders, etc.[2] Medicinal plants are familiar sources of medicine. Substantial evidence can be cited favoring herbs being used to treat diseases and restoring and fortifying body systems in the ancient system of medicines such as Ayurvedic, Unani, and Chinese traditional medicine.[3] Antimicrobial activity is one of the most eyed usefulness in the field of herbal medicines. One of the measures of determination of antibacterial activity is the zone of inhibition. There is a proportionate relationship between the zone of inhibition and antibacterial activity.[4] Many plants have shown profound antimicrobial activity. The family of Zingiberaceae comprises about 1300 species and 52 genera spread all over Asia, tropical Africa, and the Americas.[5] In a country like India, the plant propagates in the Sub-Himalayan region, central India, Maharashtra, Karnataka, and Kerala.[6] The *Costus* spp. from the family Costaceae (Zingiberaceae) are commonly grown as medicinal and ornamental plants.[7,8] The *Costus* spp. additionally used as a dietary supplement to manage many diseases throughout the world.[9] *Costus speciosus* [CS], commonly known as crepe ginger[10], is an essential plant grown in India.[11] The name *Costus speciosus* was changed very recently to *Hellenia speciosa* (J.Koenig ex Smith) S. Dutta.[12,13] The pharmacological activities reported for *C. speciosus* are antioxidant, antibacterial, analgesic, anti-cholinergic activity, antidiabetic, anti-inflammatory,

antidiuretic, antifungal, larvicidal, estrogenic activities, and anti-stress.[14,15] *Costus pictus* [CP] is another ornamental plant from the family of Costaceae. *C. pictus* is also called as fiery *Costus,* insulin plant, spiral flag, and step ladder.[16,17] The Rhizome and leaves show antidiuretic, bacterial, anti- anthelmintic, and antitumor activities.[18] It also possesses hypoglycemic and anti-inflammatory action.[19,20] The main purpose of the current study was to carry out phytochemical and histochemical analysis and evaluate the antibacterial activity of various solvent extracts of *Costus speciosus* and *Costus pictus* on selected bacterial pathogens. add antibacterial activity.

MATERIALS AND METHODS

Analytical grade chemicals such as hexane, methanol, ethyl acetate, and nutrient agar were procured from Sigma–Aldrich, Germany. Other chemicals such as phloroglucinol, safranin were obtained from Loba Chemie, Sudan red III and iodine from Qualigens Fine chemicals. Other chemicals such as sulphuric acid, hydrochloric acid, sodium hydroxide, ferric chloride, ammonium hydroxide, acetic acid were also used

Collection, identification, and authentication of plant material

Healthy plants of *Costus speciosus* and *Costus pictus* were collected from Usha nursery, Mallapuram district, Kerala. Both the plants have been deposited vide accession number 722 and 723 in Herbarium, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India. The fresh leaves [21] from the plant's *C. pictus* while the Rhizome of *C. speciosus* [22] was collected, washed thoroughly, and shade dried. The sample was powdered using a laboratory mixer grinder at high speed for 5 min and was stored in a tightly-closed container for one day before being used for analysis.

Preparation of the plant extract

2.3.1 Aqueous extraction: Preparation of the extracts was done by following the method. 2 g of powdered material was extracted with 50 mL of water by the maceration process. The mixture was filtered by Whatman filter paper. The filtered solution was reduced to one-fourth of its original volume [50 mL] by vacuum in a rota-evaporator at 40˚C to a constant weight until the volume gave the concentration of 160mg/mL. The solution obtained was autoclaved at 121˚C and 15lb pressure and stored at 4˚C for further studies.[23]

Solvent extraction (Cold maceration)

2g of dried, powdered material (Rhizome and leaves) was weighed accurately. The powder was macerated separately with ethyl acetate, hexane, and methanol sequentially with occasional stirring for 48 h.[24] The mixture was filtered and then reduced to one-fourth volume at 40˚C with rota-evaporator and stored for further studies. [25,26]

Macro-morphological study

Macroscopic observation of the plant was carried out.[27,28] The qualitative analysis was performed based on morphological and sensory properties such as size, shape, texture, surface characters, taste, color, odor, etc., was recorded. [29,30,31]

Histochemical study

Freehand sections of leaves, stem, rhizome and root materials were taken and was treated with a respective reagent to localizes the chemical constituents in the tissues. The stained sections were

compared with the fresh unstained sections. The sections were mounted on the slide to be observed under a compound microscope. The mounted sections were observed under the compound microscope and were studied for various phytochemicals such as Alkaloids, Phenols, Tannins, Proteins, etc.[32,33,34]

Physio-chemical tests

Various parameters such as total ash value, acid insoluble ash, water-soluble ash, sulphated ash, moisture content (loss on drying), water content, Foreign organic matter, extractive values (Methanol, hexane, ethyl acetate, and water) was studied.[35, 36,37,]

Phytochemical screening:

a. Test for tannins: To about 2-3 mL of extract, a 2-3 drops of 5% $FeCl_3$ solution. With the formation of green or bluish-black color, the presence of tannins is indicated.[38]

b. Test for saponins (Foam formation test): To about 2 to 3 mL of extract, 5 mL de-ionized water was added. Vigorous shaking results in persistent foam formation. It was allowed to stand for 15 min and kept for honeycomb froth, which shows the presence of saponins.[39]

c. Test for flavonoids (Shinoda test): To 1 mL of extract, a few magnesium ribbon fragments and 4-5 drops of conc. HCl were added. The flavonoid is confirmed by the formation of the pink or red color.[40]

d. Test for terpenoids (Salkowwski Test): In 0.5 mL of extract, 2 mL of chloroform along with conc. H_2SO_4 was added. The red-brown color at the interface indicates the presence of terpenoids.[41]

e. Test for carbohydrates (Molisch's Test): 1 mL of extract in addition with 1 mL of conc.H_2SO_4, gives a red to violet zone which is visible at the interphase of the oil-water layers. The presence of carbohydrates and glycosides is indicated.[42]

f. Test for anthraquinone (Bontrager's Test): To 1mL of extract, 5 mL of benzene was added. Further, it was shaken and filtered. 5 mL of ammonium hydroxide (10%) was added, followed by shaking of the contents. A red, pink, or violet color in the lower ammoniacal phase confirms the presence of anthraquinones.[43]

g. Test for cardiac glycosides (Keller-Kilani Test):, A mixture of 2 mL of acetic acid added with1-2 drops of 2% ferric chloride solution was mixed with 1 mL of extract. This mixture was then introduced into another test tube that had 2 mL of conc. H_2SO_4. The appearance of a brownish-colored ring at the interphase and cardiac glycosides are indicated in the sample.[44]

h. Test for coumarins: A test tube with filter paper moistened in dilute NaOH, about 1 mL of sample extract was taken. The sample was heated for 3-5 min. Further, the filter paper was examined under UV (365 nm) for yellow-colored fluorescence which confirms the test for coumarins.[45]

i. Test for steroids (Liebermann-Burchard Test): About 2 mL of acetic acid was added in 1 mL of extract. After cooling the solution on an ice bath conc. H_2SO_4 was added carefully. The development of violet to blue or bluish-green color confirms the test for steroids.[46]

j. Test for alkaloids: In 1 mL of extract, 2 mL of 1% HCl was added, and the solution was heated. Further, 4 to 5 drops of Mayer's reagent was added. A precipitate white or cream in color formation confirms the test for alkaloids.[47]

Antimicrobial activity studies

Micro-organisms

Reference bacterial strains were obtained from the Master of Science Department Maulana Azad College, Dr. Rafiq Zakaria Campus, Aurangabad (MS.) India. The strains comprise *Bacillus subtilis* (ATCC 19659), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027)*, Escherichia coli* (ATCC 8739), and *Salmonella typhi* (ATCC 14028). The bacterial isolates were kept at 4˚C on an agar slant. The sample was sub-cultured for 24 h in nutrient agar at 37˚C before any susceptibility test.[48]

Media preparation

About 23 g of nutrient agar was dissolved in distilled water (1000 mL). The mixture was autoclaved for 15 min at 121˚C. Further, it was left to cool at room temperature. After cooling (about 45˚C), it was transferred into Petri dishes. Each Petri dish was left for cooling for about 30–35 min until completely set.[49,50 ,51]

Agar disk diffusion method

Each bacterial sample was spread on sterile agar plates using a clean and pre-sterilized cotton swab. Pre-calibrated Whatman filter paper disks of about 5 mm diameter and 2.5 µl infused capacity were prepared and sterilized. Each disk was infused with different crude extracts of concentration 40 mg/disk and then sited on bacterial pre-swabbed agar plates. Streptomycin was used as a positive control. The samples were allowed to diffuse at RT for 30 min. The diffused Petri plates were kept for incubation at 35 ± 0.5˚C for 24h. After incubation, the microbial growth was determined by measuring the diameter (mm) for inhibition zones.[52,53,54]

Agar well diffusion test

Each bacterial inoculum was swabbed on the sterile agar plate using a clean and a sterilized cotton swab. Wells of about 7 mm diameter with 50 µl capacity were made into the agar plates. The initial concentration of crude extract used for the agar well diffusion method was 40mg/ml. Due to the higher concentration of the crude extract, it was found that the zone of inhibition merged with each other. Henceforth the concentration of the crude extracts was reduced to 20mg/ml. Positive control (Streptomycin) 30µg/µl, negative control (various solvent extracts), and sample solution (all crude extracts) were added to each well and allowed to diffuse for 30 min at RT. Plates were incubated for 24 h at 35 ± 0.5˚C to determine the inhibition zones (mm) as a measure of antibacterial activity.[55,56]

RESULTS

Macro-morphological study

Costus speciosus: *C. speciosus* is an erect succulent herb up to 3 meters in height. The leaves are elliptical to oblong-lanceolate silky beneath, subsessile, thick spirally arranged with stem-clasping sheaths up to 4 cm. The Rhizome of *C. speciosus* is tuberous, having 10-15 cm length and 1-3 cm diameter. Rhizomes are usually sub-cylindrical unbranched and covered with a brownish epidermis or cork. Small circular scars of about 2 to 4 mm in diameter are present on the upper and lower surfaces at specific intervals. Flowers are white in color, large, thick, cone-like terminal spikes, with bright red stripes, lip with the yellowish throat; red capsules, fruits globosely trigonous, the diameter of about 2 cm, black colored seeds, with white aril. Upper surface marked with nodal scars circular in shape with residue of leaf bases, lower and lateral surfaces exhibits small circular spots of roots, or few thin rootlets fracture fibrous and fractured

yellowish-brown surface. Stems more or less woody at base, unbranched, spirally twisted in the upper part. No characteristic of taste or odor (Figure 1).
Costus pictus: *Costus pictus* is a perennial herb. Typically, multi-trunked or clumping stems. The Stems hirsute and green near apex, glabrous and purple toward the base, with spiral light green leaves and airy, the tissue paper-like flowers yellow with orange-red stripes. The leaves are alternate, simple, entire, smooth surface, pinnate parallel venation, conspicuously ligulate (red-colored) large fresh-looking spirally arranged, oblong-lanceolate being dark green above when mature, and lighter green below. The leaf's shape is narrowly elliptical with a length of 10 to 25 cm and a width up to 6 cm. Leaves have characteristic taste and odor (Figure 2).

Histochemical study

The histochemical study helps in the determination of chemical constituents in the cells and tissues. The method can identify cellular components such as carbohydrates, proteins, nucleic acids, lipids, etc.[57,58] Thin sections of Leaves, Roots, Rhizomes, and Stem were taken. Further, the sections were stained using various stains such as Sudan red III, Iodine, Phloroglucinol, and Ferric Chloride. The TS of rhizome leaves and stem of the plants show the presence of fibrovascular bundles. Plants are monocot commelinids (Figure 3). The leaves and stem show epidermis bearing simple, unlignified, thick-walled trichrome unicellular, simple, and multicellular glandular with pointed apex; central vascular bundles closed system with collenchyma on both sides. Leaves are dorsiventral on the upper epidermis. The parenchymatous cells encircle the vascular bundles with the presence of starch grains. The phytochemicals such as phenols, alkaloids, and proteins were found to be present in the epidermal tissue, cortex, and vascular bundles of root, stem, and leaves. The stem shows scattered lignified vascular bundles, thin-walled unlignified collenchyma, uniseriate multicellular trichrome central vascular pith with primary xylem. Rhizomes are monocot with cortex showing scattered vascular bundle, thin-walled parenchymatous cells. The roots show wide vessels parenchymatous thin cork with thin-walled medulla. Central cork/ pith in the epidermal region brownish with the presence of fibrous vascular bundles scattered. The primary xylem towards the centre shows brownish content in the cell. Cortex is occupied in 2/3 portion surrounded by the thin-walled parenchymatous cell. (Figure 4)

Physio-chemical and phytochemical testing

As there is an increase in antibiotic resistance in various diseases, the alternative system of medicine can be of interest. Many plants contain phytochemicals having antimicrobial properties, which could exhibit a crucial role in therapeutic treatments.[59] The physio-chemical properties such as Organoleptic properties, Ash value, extractive values, moisture content, Foreign organic matter, and swelling index are given (Table 1). The aqueous, methanolic, ethyl acetate and hexane extract (Table 2) shows various compounds identified. The different test indicates the presence of alkaloids, flavonoids, quinones, and saponins. The crude extracts from leaf and rhizome samples of CP and CS did show a positive test for terpenoid and steroids. The methanolic crude extracts showed a positive test for tannins. Hexane extracts from both plants show the presence of chemicals like steroids, alkaloids, flavonoids, and triterpenoids, while saponins and tannins were absent. Ethyl acetate crude extract from both plants showed a negative test for saponins, while CP ethyl acetate extract indicates the presence of alkaloids.

Antimicrobial activity studies

The investigational study for antibacterial activity against various pathogenic bacterial strains using the agar disk diffusion technique was carried out. The disk diffusion method was only used for qualitative evaluation of antibacterial activity for respective crude extracts. The optimization of the antibacterial activity was carried out by using the agar well diffusion test.

Agar disk diffusion method

The different solvent extracts for CS had a lower to negligible effect based on the agar disk diffusion method. However, the marketed extract of CS was highly effective against various bacterial strains. The solvent extracts of CP had efficient antibacterial activity; however, the marketed extract shows lesser activity comparatively. Concluding the overall results, the marketed extract of CS and different solvent crude extracts of CP were used for the agar well diffusion method. The zone of inhibition obtained for the agar disk diffusion technique is shown in (Table 3). The graphical representation for the zone of inhibition achieved by the agar disk diffusion technique is shown (Figure 5).

Agar well diffusion test

The methanolic extract shows the highest inhibition against *E.coli*. In contrast, the hexane and ethyl acetate extract shows comparatively low activity. A potential antibacterial activity was exerted by hexane and methanolic extract against *S. aureus* with inhibition zones of 38 and 17 mm, respectively (Figure 6). However, the ethyl acetate extract shows comparatively lower activity (9 mm). The standard extract shows maximum activity against *B. subtilis*. While the hexane, ethyl acetate and methanolic extracts, show comparatively lower activity. Against *P. aeruginosa,* the hexane extract shows the highest activity while the methanolic, standard and ethyl acetate extract show comparatively lesser activity. Best antibacterial activity against *S. typhi* was observed with the methanolic extract comparatively with other extracts (Table 4). The aqueous extract shows mild activity against *S. typhi,* while no activity was recorded against all the other bacterial pathogens tested. The graphical representation for the inhibition zones achieved by the agar well diffusion method is shown (Figure 7).

DISCUSSION:

The histochemical and phytochemicals investigation of *Costus speciosus* and *Costus pictus*, reveals the presence of alkaloids, phenols, proteins, saponins, tannins, anthraquinones, and flavonoids. The literature[60,61,62] reveals that the presence of these phytochemicals is responsible to have curative activity against several microbes. Therefore, *Costus speciosus* and *Costus pictus* plant extracts were evaluated for antimicrobial activity and ensure promising results as antimicrobial agents (Tables 3 and 4). The comparative study carried out gives an idea about the antibacterial potential of the plant crude extracts. The standard extract and plant crude extracts show inhibitory activity against the examined bacterial strains. *B. subtilis* was found to be more resistant in comparison with other bacterial strains. While *P. aeruginosa and S. typhi* were found to be more susceptible towards the crude and standard extracts. The negative control used in the study does not show any markable antibacterial activity against the selected pathogenic strains. Extracts of both the plants which show inhibition zone diameters of > 10mm were considered to be active. In pursuant to this, it is believed that the extracts from both the plants are better antibacterial agents and therefore using these plants as an antibacterial agent has been validated.

CONCLUSION:

The pharmacognostic study of *Costus speciosus* (J.Koenig) Sm. and *Costus pictus* D. Don gave important information concerning the morphology of crude drugs. They can be used for the

authentication of *C. speciosus* and *C. pictus* among all *Costus* spp. The adulteration and purity of these drugs can also be determined. The microscopic character, physicochemical, and phytochemical screening parameters studies help set standards for crude drugs. Significantly less data was available on the histochemical study of both the plants CS and CP; henceforth, it was carried out elaboratively. The histochemical studies reveal the presence of alkaloids, flavonoids, carbohydrates, and terpenoids in the leaf, stem, and roots. The phytochemical screening study also confirmed chemical components such as alkaloids, flavonoids, carbohydrates, phenols, glycosides, and terpenoids. To date, the antibacterial potential and comparative study against specified bacterial pathogens were not reported. Besides this, the novelty of current work is to differentiate the activity between gram-positive and gram-negative bacterial strains. The potential increase of 2 folds in the antibacterial activity for HE (CP) against *S. aureus* was observed however ME (CP) shows the equivalent activity as PC. The result indicates that both the plants show potential antibacterial activity ensuring bioactive compounds useful in primary healthcare. Further work is needed on the isolation, characterization, and purification of the active constituents and understanding the possible mechanism of action as an antibacterial agent.

Abbreviations:
TS- Transverse section
CS- *Costus speciosus*
CP- *Costus pictus*
UV-Ultra violet
PC-Positive control
AE-Aqueous extract
ME- Methanolic extract
HE- Hexane extract
EAE- Ethyl acetate extract

SE- Standard extract

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List of Tables:

Tests	Results	
	Costus speciosus (Rhizome)	*Costus pictus* (Leaves)
1)Organoleptic properties a) Appearance	Buff colored powder	Light green powder
b)Color	Buff Brown	Light green
c)Odor	No Characteristic odor	Characteristic odor
d)Taste	No taste	Sour in taste

e)pH	7.4 ± 0.06	6.5 ± 0.08
2) Moisture content (%)	4.5 ± 0.32	10.155 ± 0.032
3) Ash value (%) a)Total Ash value	4.5 ± 0.5	14.29 ± 0.15
b) Acid Insoluble Ash value	0.932 ± 0.03	3.20 ± 0.060
c) Water soluble Ash value	1.58 ± 0.07	8.70 ± 0.012
4) Foreign organic matter (FOM %)	0.7± 0.04	0.07 ± 0.063
5) Extractive values (%) a) Methanol	8.74 ± 0.99	17 ± 0.03
b)Ethyl acetate	5.32 ± 0.63	7.60 ± 0.02
c)Hexane	2.06 ± 0.02	6.50 ± 0.03
d) Aqueous	7.34 ± 0.63	10.25 ± 0.07
6)Swelling Index	Initial(ml)- 3.5 ± 0.32 Final(ml)- 5.6 ± 0.13	Initial(ml)- 2.5 ± 0.20 Final(ml)- 6.5 ± 0.130

Table 1*:* Physio-chemical test results for *C. speciosus* and *C. pictus*

Chemical constituent	Costus speciosus				Costus pictus			
	AE	ME	EAE	HE	AE	ME	EAE	HE
Tannins	-	+	-	-	-	-	-	-
Saponins	+	+	-	-	+	+	-	-
Flavanoids	+	-	-	-	+	+	+	+
Quinones	+	+	+	+	+	+	+	+
Glycosides	-	+	+	+	+	+	+	+
Cardiac Glycosides	+	+	+	+	+	+	+	+
Carbohydrates	-	+	+	+	-	+	+	+
Terpenoids	-	+	+	+	+	+	+	+
Phenols	+	+	-	-	+	+	+	+
Coumarins	+	+	-	-	+	+	+	+
Steroids	+	+	-	-	+	+	+	+
Alkaloids	-	+	-	-	+	+	+	+

Table 2: Phytochemical constituent of plants *C. speciosus* and *C. pictus*

Microorganism	Zone of inhibition (in mm)											
	Costus speciosus						*Costus pictus*					
	PC	AE	ME	HE	EAE	SE	PC	AE	ME	HE	EAE	SE
S. aureus	33	-	-	5	-	12	33	-	-	-	11	12
B. subtilis	30	-	-	-	-	15	3	-	80	-	12	13
P. aeruginosa	28	-		-	-	18	25	-	70	-	10	1
E.coli	26	-	-	-	-	9	26	-	80	-	80	9

S. typhi	-	-	-	-	-	9	-	-	14	60	60	9

Table 3: Disc diffusion method results. PC-Positive control, AE-Aqueous extract, ME-Methanolic extract, HE- Hexane extract, EAE- Ethyl acetate extract, SE- Standard extract.

Microorganism	Zone of inhibition (mm)					
	PC	*Costus speciosus*(SE)	*Costus pictus*			
			AE	ME	HE	EAE
S. aureus	31	12	-	17	38	9
B. subtilis	31	14	-	22	12	12
P. aeruginosa	35	12	-	12	13	12
E.coli	29	-	-	11	10	10
S. typhi	18	14	-	10	9	9

Table 4: Agar well diffusion method results. PC-Positive control, AE-Aqueous extract, ME-Methanolic extract, HE- Hexane extract, EAE- Ethyl acetate extract, SE- Standard extract.

List of Figures:

Figure 1: Costus speciosus plant (A) Flowers (B) Rhizomes (C) Complete plant of C. speciosus

Figure 2: Costus pictus plant (A) Flowers (B) Roots (C) Complete plant of C. pictus

Figure 3: Histochemical studies on (A) Leaves (B)Stem (C) Rhizome of Costus speciosus (1) Unstained section (2) Test for Fixed oils, Volatile oils (3) Test for lignified tissues (4) Test for proteins (5) Test for phenols.

Figure 4: Histochemical studies on (A) Leaves (B) Roots (C) Stem of Costus pictus (1) Unstained section (2) Test for Fixed oils, Volatile oils (3) Test for lignified tissues (4) Test for proteins (5) Test for phenols.

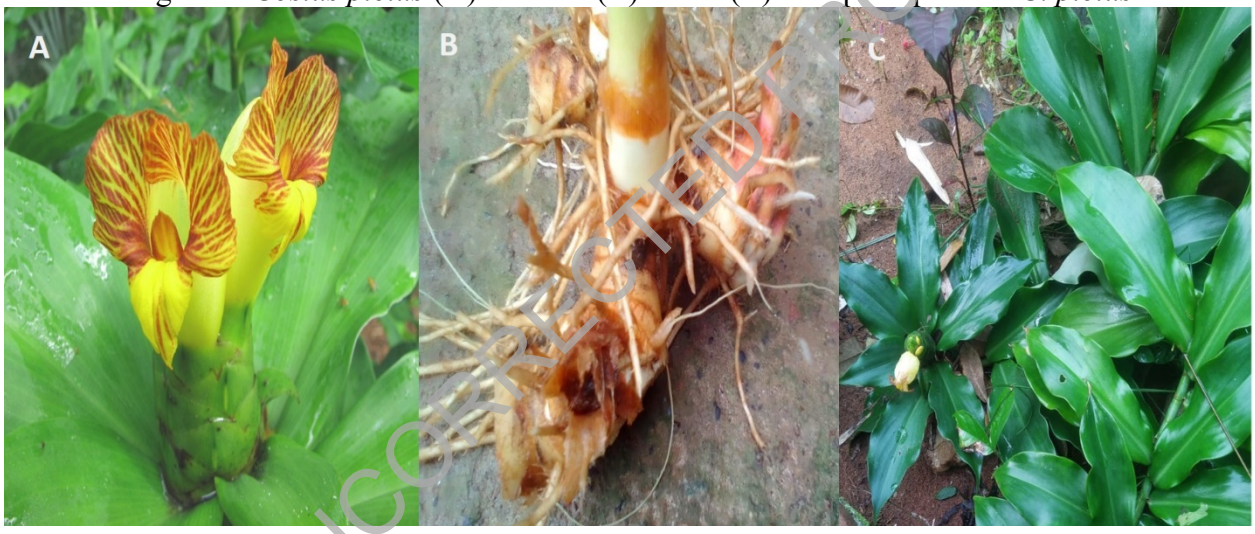
Figure 5: Graphical representation of the zone of inhibition achieved by Disc diffusion method (A) *Costus speciosus* (B) *Costus pictus*

Figure 6: Zone of inhibition for Agar well diffusion (A) *E. coli* (B) *P. aeruginosa* (C) *S. aureus* (D) *S. typhi* (E) *B. subtilis* (A: hexane extract, C: positive control, E: ethyl acetate extract M: methanolic extract, SE: standard extract, and W: aqueous extract)

Figure 7: Graphical representation of the zone of inhibition achieved by Agar well diffusion method

Figure 1: *Costus speciosus* (A) Flowers (B) Rhizomes (C) Complete plant of *C. speciosus*

Figure 2: *Costus pictus* (A) Flowers (B) Roots (C) Complete plant of *C. pictus*



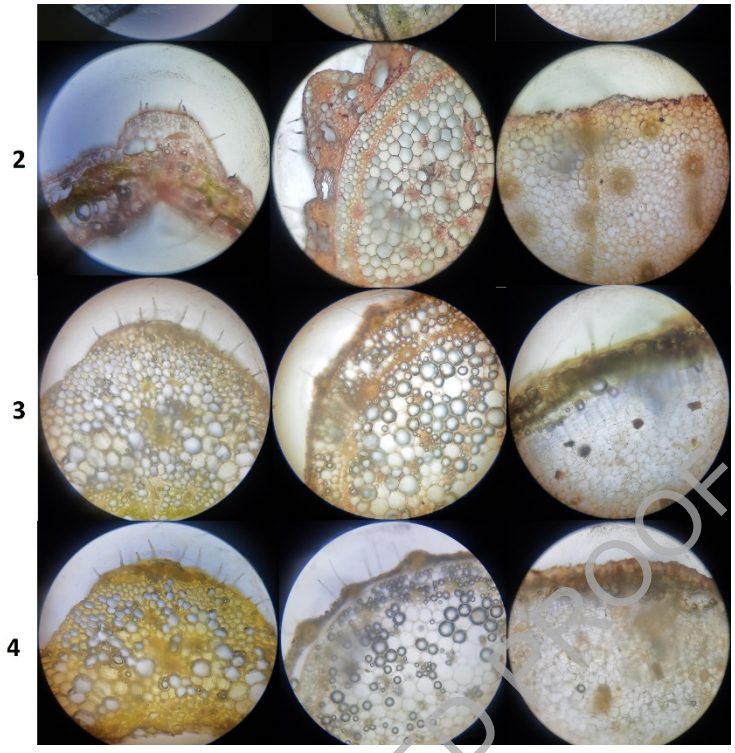


Figure 3: Histochemical studies on (A) Leaves (B)Stem (C) Rhizome of *Costus speciosus* (1) Unstained section (2) Test for Fixed oils, Volatile oils (3) Test for lignified tissues (4) Test for proteins (5) Test for phenols.

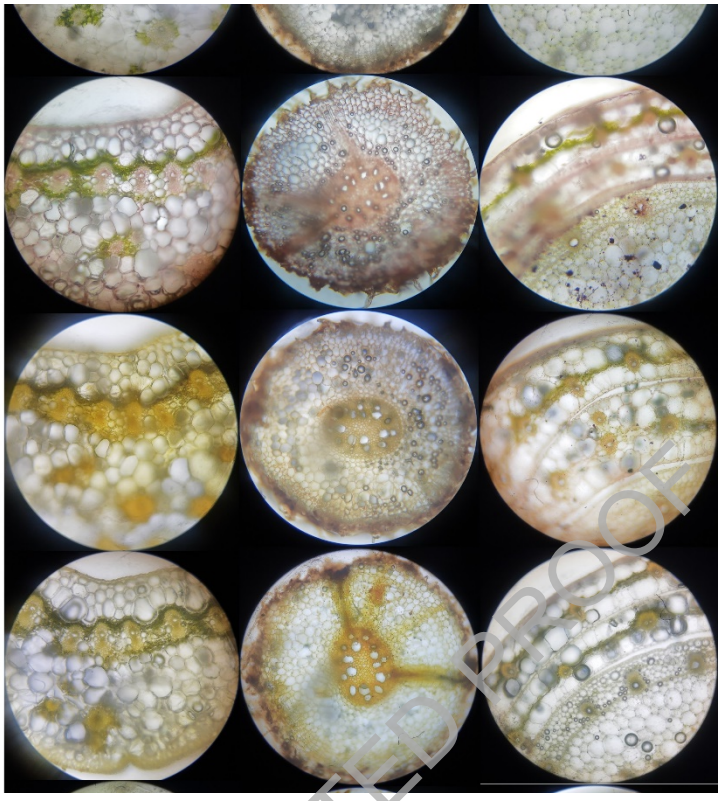


Figure 4: Histochemical studies on (A) Leaves (B) Roots (C) Stem of *Costus pictus* (1) Unstained section (2) Test for Fixed oils, Volatile oils (3) Test for lignified tissues (4) Test for proteins (5) Test for phenols.

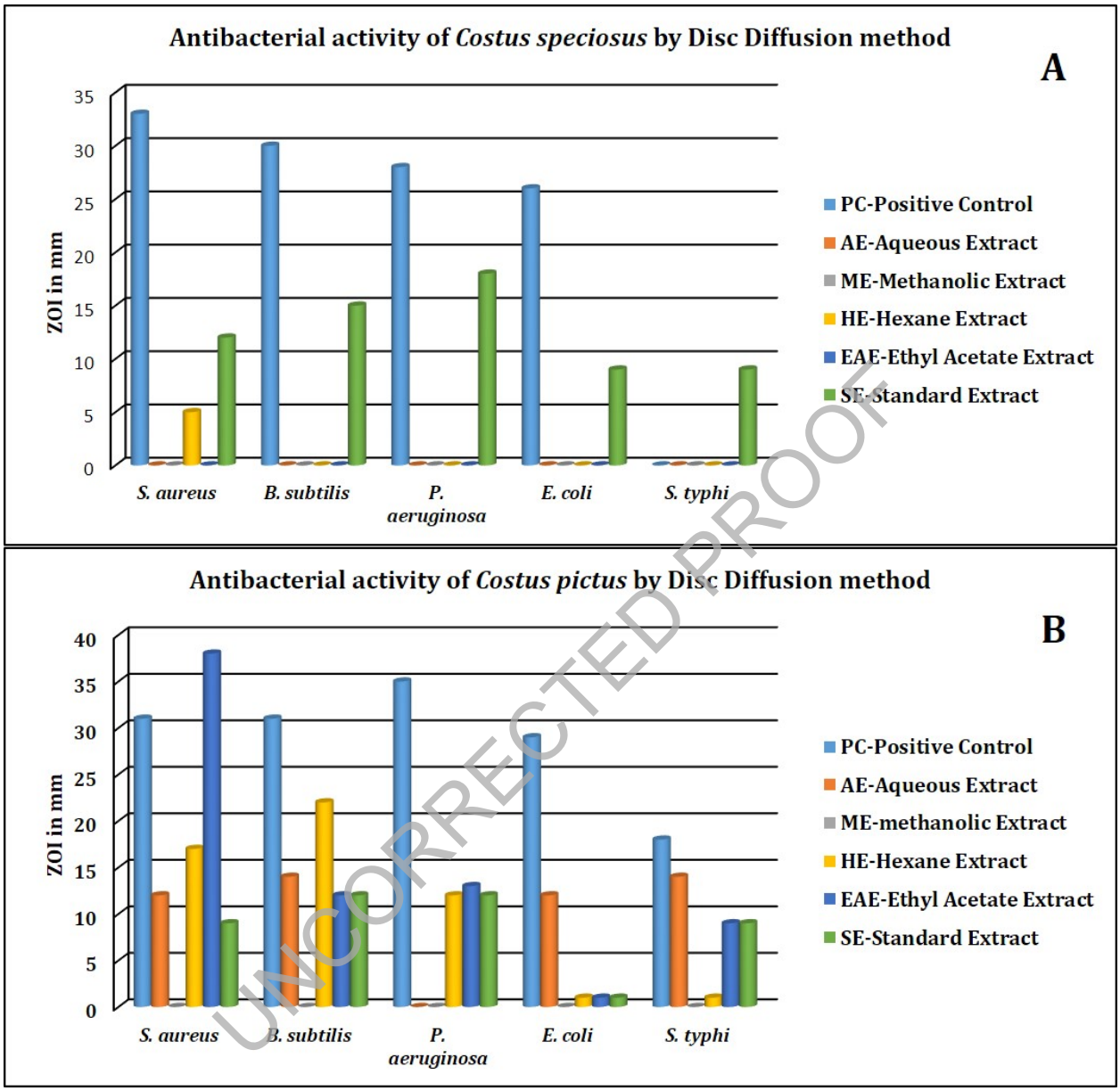


Figure 5: Graphical Representation of the Zone of Inhibition Achieved by Disc Diffusion Method

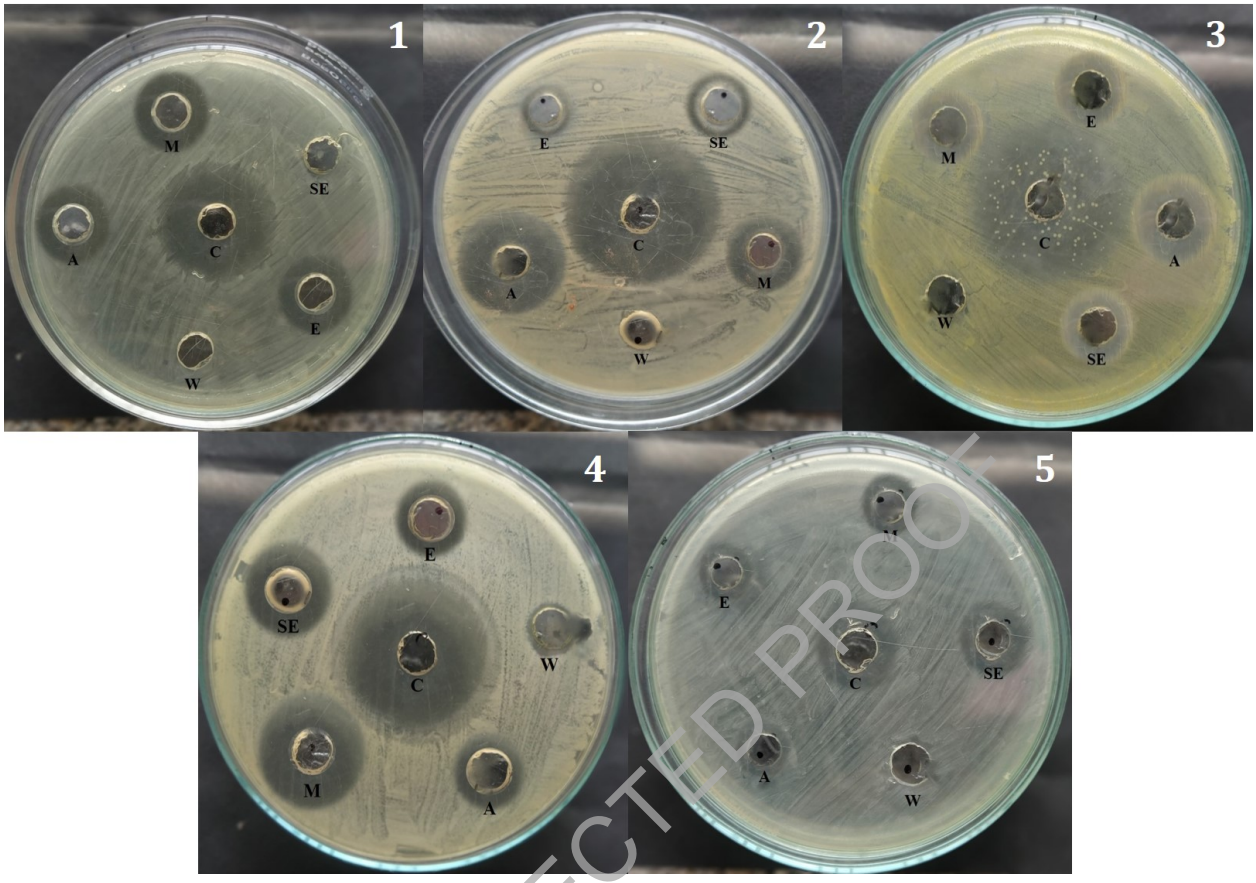


Figure 6: Zone of inhibition for Agar well diffusion (1) *E. coli* (2) *P. aeruginosa* (3) *S. aureus* (4) *S. typhi* (5) *B. subtilis* (A: hexane extract, C: positive control, E: ethyl acetate extract M: methanolic extract, SE: standard extract, and W: aqueous extract)

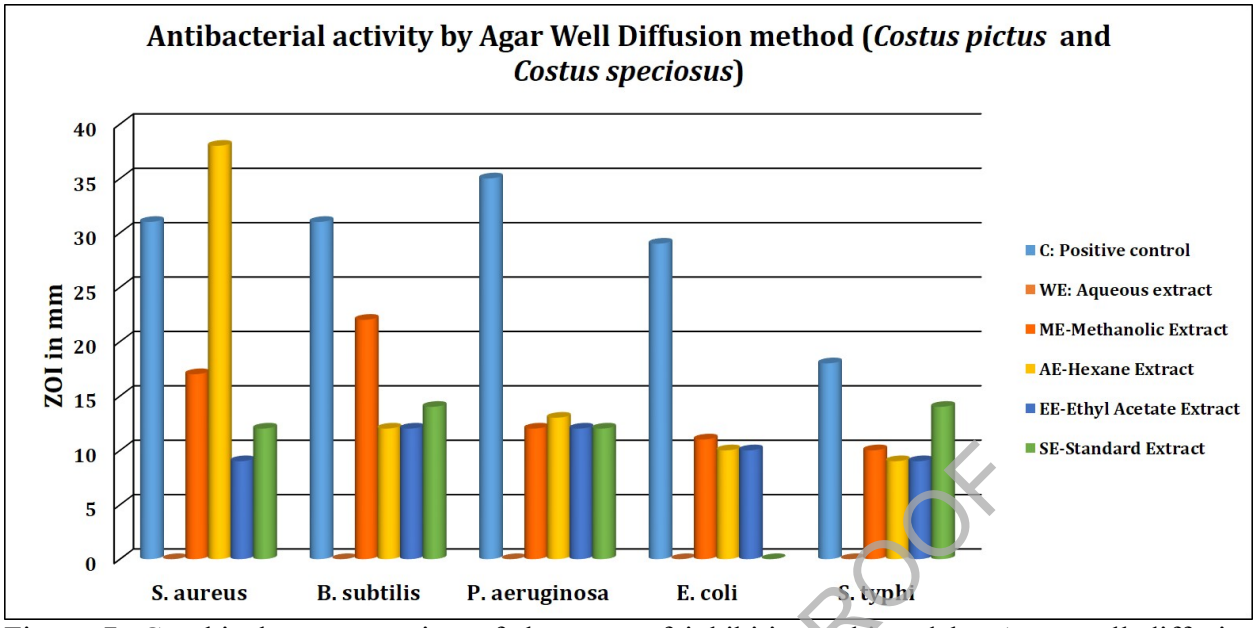


Figure 7: Graphical representation of the zone of inhibition achieved by Agar well diffusion method