In-Vitro Evaluation of Antimicrobial and Antibiofilm Activity of Leaf Extract of Costus pictus D. Don against Multidrug Resistance Isolates

Deepa Kumari A and Chitra M

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 04

Res. Jr. of Agril. Sci. (2022) 13: 1206–1209

CARAS

Res. Jr. of Agril. Sci. (Jul-Aug 2022)
13(4): 1206–1209

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Full Length Research Article

In-Vitro Evaluation of Antimicrobial and Antibiofilm Activity of Leaf Extract of *Costus pictus D. Don* against Multidrug Resistance Isolates

Deepa Kumari A*[1] and Chitra M[2]

Received: 15 May 2022 | Revised accepted: 30 Jul 2022 | Published online: 04 Aug 2022

A B S T R A C T

Antimicrobial resistance has become a serious public health risk with the increased use of antibiotics. Modern plant medicinal products can be a solution to this issue, since they are relatively safe and have additional beneficial effects. The aim of this study was to evaluate antimicrobial effect of *Costus pictus* D. Don solvent leaf extract. Antimicrobial effect of ethanol and chloroform extracts was tested by modified agar well diffusion method. Four types of bacterial isolates and 2 of fungal isolates were studied. After 24 hours inhibition period, zone of inhibition was measured. Furthermore, antibiofilm activity also carried out with plant extract by using titter plant well method. Among the 2 solvent extracts, ethanol showed good inhibitory activity against all isolates especially while using 7.5mg of extract. The antibiofilm report was confirmed that significant amount of biofilm production inhibited by plant extract. The major components found in the fractions were Cyclohexanone, N-Acetylmannosamine, Ethanamine, and 2-Furanone as analyzed by GC–MS/MS technique. It can be concluded that a high polar active compound present in the *Costus pictus* extract may be responsible for the significant antimicrobial activity.

***Key words*:** *Costus pictus*, Antimicrobial activity, Agar well diffusion method, High polar compound

Antibiotic resistance is rising to dangerously excessive ranges in all components of the world. New resistance mechanisms are rising and spreading worldwide, threatening our capacity to treat frequent infectious illnesses. A growing list of infections, such as pneumonia, tuberculosis, blood poisoning, gonorrhea, and food borne infections, is arduous to treat and sometimes infeasible because antibiotics are less efficacious. In which antibiotics may be bought for human or animal use without a prescription, the emergence and spread of resistance is made worse. Similarly, in number of countries, without proper guidelines, antibiotics and treatment are often over prescribed by health workers and veterinarians and over used by the people. Many public health organizations have characterized the rapid emergence of resistant bacteria as a "crisis" or "nightmare scenario" that could have "disastrous consequences." In 2013, the CDC (Centers for Disease Control and Prevention) announced that the human race is now in the ' post-antibiotic age ' and in 2014, the World Health Organization (WHO) warned that the problem of antibiotic resistance is getting worse [1]. Amongst gram-positive pathogens, a worldwide pandemic of resistant *S. aureus* and enterococcus species presently poses the most important danger. The most serious gram-negative infections occur in health care settings and are most commonly caused by Enterobacteriaceae (mostly *Klebsiella pneumoniae*), *Pseudomonas aeruginosa*, and *Acinetobacter* [2]. Both gram-negative and positive pathogens are particularly worrying, as they become resistant to almost all the available antibiotic drug options, creating situations that remind of the pre-antibiotic era.

Alteration of target sites, active efflux of drugs and enzymatic degradations are the strategies employed through the pathogenic bacteria to develop intrinsic resistance to antibiotics. Numbers of mechanism are included for the antibiotic resistance, among them, betalactamase are one of the important mechanisms of resistance against beta-lactam antibiotics, and this is because betalactams are broad-spectrum antibiotics used to treat common infections [3]. Pathogens that produce extended-spectrum beta-lactamases (ESBLs) and carbapenemases (i.e., types of beta-lactamases) are reported to be spreading at an alarming rate, with more than 50% identifying drug-resistant pathogens are ESBL producers. This has led to an increased interest in medicinal plants since 25–50% of current prescribed are plant derived [4]. Crude extracts of medicinal plants could accommodate as an alternate source of resistance modifying agents owing to the wide variety of secondary metabolites [5]. In this point of view plant of *Costus pictus* possess many antimicrobial compounds and can be

* **Deepa Kumari A**
✉ jegadeeshkumar2@gmail.com

[1-2] P. G. and Research Department of Botany, Government Arts College, (Autonomous), Coimbatore - 64 1 018, Tamil Nadu, India

utilized as anti-infectious agents. Several studies have been carried out in the leaf extract of *Costus pictus* to evaluate the antidiabetic properties. The leaf and the rhizome are known for the antidiuretic, antihelmintic, antibacterial and antitumor activities [6]. However, antibiofilm activity of *Costus pictus* was has not yet been evaluated. The present study was undertaken to study the preliminary phytochemicals analysis and antibacterial and antibiofilm activity of the leaf extract of *Costus pictus*.

MATERIALS AND METHODS

Collection of plant

The leaf of *Costus pictus* was collected from Coimbatore district, Tamilnadu and was shade dried, powdered and extracted in Soxhlet apparatus successively with ethanol and chloroform solvents. The extracts were stored at 4°C for phytochemicals screening and antimicrobial analysis.

Phytochemical screening of bark extract

The presence of various phytochemicals compounds in the leaf of *Costus pictus* was confirmed by using the methods of Solomon *et al.* [7] procedure.

Microorganisms

The Multidrug resistance isolates of *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumonia* and *Pseudomonas aeruginosa,* in case of fungal, *Candida albicans* and *Aspergillus niger* were procured from Microtech, Microbiology Laboratory, Coimbatore and used for the study.

Determination antibacterial activity of plant extract

This test was carried out according to the method of Khan and Tewari [8]. The plates were inoculated with freshly prepared overnight each inoculum which were swabbed over the entire surface of each MHA medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10^8 CFU/ml of bacteria. The 6mm diameter of well was made with borer on the agar plates. Different concentration of leaf extracts were filled in well with the help of micropipette. Ampicillin (10µg/ml) was used as positive control and 100µl of DMSO was added in to well, which was negative control. In case of antifungal activity, ketoconazole was (10µg/ml) was used as positive control. The plates were incubated at 37°C for 24 hours for bacterial isolates and 48hrs for fungal isolates. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

Anti-biofilm activity of plant extract

The microtiter plate well method was used to determine the anti-biofilm activity of the plant extract according to Gurunathan *et al.* [9] procedure. Individual wells of the plates were filled with 180 µl of Muller Hinton Broth, and 10 µl of the test pathogens (OD = 1.0, 600 nm) were added. Subsequently, 10 µl containing of plant extract was added, and the preparation was thoroughly mixed. After completing the preparation of the test plates, they were incubated for 24 h at 37°C and mixtures without bacteria were used as control. After incubation, the contents were discarded and gently washed with phosphate buffered saline (PBS, pH 7.2) to remove free-floating non-adherent bacterial cells from the walls and bottom of the wells. The wells of microtiter plates were then air dried for 45 min. After drying, adherent "sessile" bacteria in the wells were fixed with 2% w/v sodium acetate and the wells were then stained with crystal violet stain (0.1%, w/v) and incubated for 30 min. After that, wells were thoroughly washed with sterile deionized water to get rid of all excess dye. After the plates were air dried, 200 µl of 95 percent, v/v, and ethanol were added to each well, and the absorbance at 620 nm was measured. The percentage of inhibition of biofilm formation was calculated using following equation:

% biofilm inhibition = [1 − (OD620 of cells treated with Ag NPs or plant extracts / OD620 of the non-treated control) × 100].

GCMS analysis

Chromatograph interfaced to a mass spectrometer (GC-MS – Perkin-Elmer) equipped with an Elite-1, fused silica capillary column (30 m ´ 0.25 mm ID ´ 1 m df, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min and an injection volume of 2 ml was employed (Split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 2000°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 35 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

RESULTS AND DISCUSSION

Plants are a very vital supply of potential precious bioactive compounds for the development of new therapeutic agents. Hence, these observations have assisted in developing new drugs for the therapeutic use in human beings. However, not numerous studies are accessible on the pharmacological properties of *Costus pictus.* The results of phytochemicals screening of leaf extract of *Costus pictus* showed the presence of various phytochemicals (Table 1). Two solvents such as chloroform and ethanol showed positive results for the presence of Alkaloids, Flavonoids, Tannins and Phenols. In addition, the ethanol extracts showed the presence of Saponins, Sterols and Quinones. The Carbohydrates, Terpenoids, and proteins are absent in both of the extracts and ethanol extract scored the highest number of phytochemicals. In 2013, Shiny *et al.* [10] observed the number of phytochemicals from various solvents extract of *Costus pictus*. The other hand of found that number of phytochemicals from ethanol extract of *Costus pictus* [11].

Table 1 Phytochemical Constituents of *Costus pictus*

Constituents	Ethanol extract	Chloroform extract
Alkaloids	+	+
Carbohydrates	-	-
Flavonoids	+	+
Tannins	+	+
Saponins	+	-
Phenols	+	+
Sterols	+	-
Terpenoids	-	-
Quinones	+	-
Proteins	-	-

Costus pictus is one such medically important plant, which is commonly known as 'Insulin plant' as its leaves are

proved to produce the antidiabetic effects [12]. This plant containing phytochemicals compounds are secondary metabolites that serve as a defense mechanism against many microbes. Results of antibacterial activities of the ethanol and chloroform extracts of *Costus pictus* were shown in (Fig 1-2) respectively. The results showed that both non-polar (chloroform) and polar (ethanol) solvent extracts were active against 4 bacterial genera and 2 fungi genera. In case of ethanol extract, the highest zone of inhibitory was observed against *Staphylococcus aureus* and *Klebsiella pneumoniae*; the zones of inhibition were ranging from 12 to 21 mm in diameter. Among the 4 types of genera, *Proteus spp* was slightly sensitive to ethanol extract and while using 7.5mg of extract, all the isolates were suppressed.

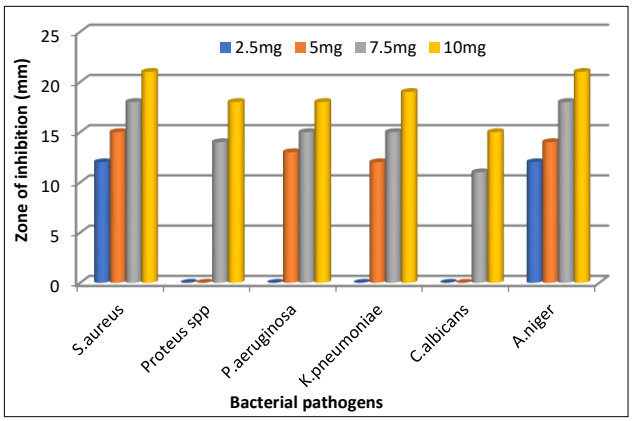

Fig 1 Antimicrobial activities of ethanol extract of *Costus pictus*

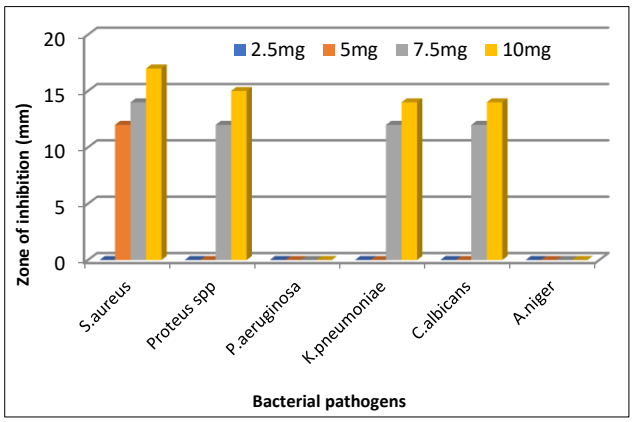

Fig 2 Antimicrobial activities of chloroform extract of *C. pictus*

Results found in this study were supported and/or opposed in the data reported in literature. Rai and Kalaivani [13] conducted a study which supports the finding of the present study in which the water extract was able to have inhibitory effects against Staphylococcus and Pseudomonas, whereas Ramya and Dhamotharan [6] oppose the findings concerning the Gram-positive bacteria. In case of chloroform extract, this was highly suppressed to *Staphylococcus aureus* and second most to *Klebsiella pneumoniae*. Presently, *Pseudomonas aeruginosa* was resistance to chloroform extract, while using 10mg of extract, 3 bacterial isolates were suppressed. In case of fungi genera, *C. albicans* was slightly suppressed and *Aspergillus niger* was not suppressed. A review study was indicated that *Costus pictus* was not active against to *Aspergillus niger* [14].

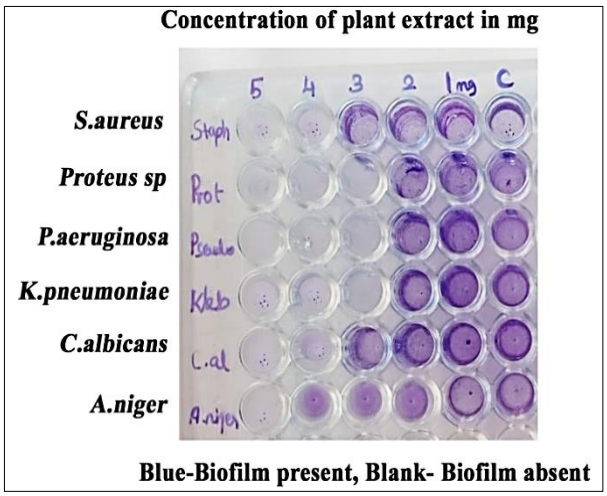

Fig 3 Antibiofilm activity of *Costus pictus*

antibiofilm activity was observed against to *Klebsiella pneumoniae*, (56.8%) and followed by *Proteus sp* (54.1), and *Pseudomonas aeruginosa* (51.2%), which was suppressed on 3mg of concentration. In case of fungus isolates, *Candida albicans* producing biofilm was highly suppressed (Fig 3). This observed to be the first study to determine the antibiofilm activity of the *Costus pictus* extract. However, previous study of Jdco *et al.* [15], were observed the antibiofilm activity with leaf extracts of *E. leitonii*, *Epiperipatus brasiliensis*, *Eugenia myrcianthes* and *Eugenia involucrate* against *Candida albicans*.

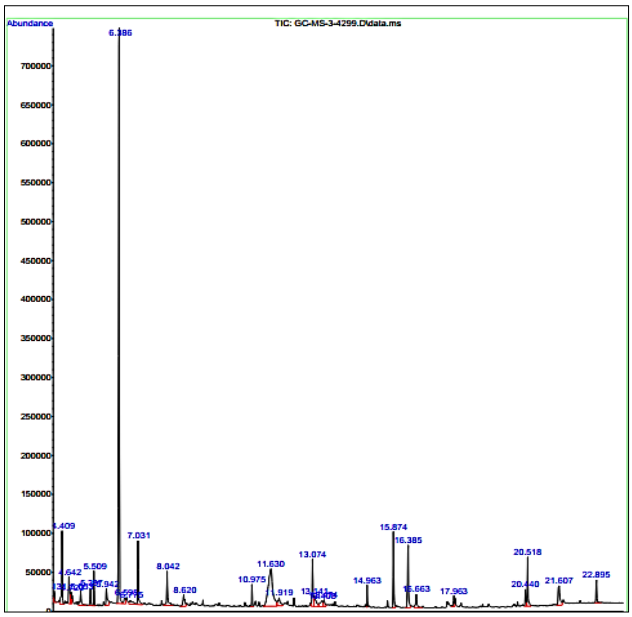

Fig 4 GCMS analyses of ethanol extract of *Costus pictus*

The antibiofilm activity also carried out with ethanol extract of *Costus pictus.* Bacterial biofilms remain a global health threat due to their high therapeutic resistance and ability to exacerbate nosocomial infections. Therefore, searching for novel effective molecules to overcome this problem is a priority. In this study, the activities of the ethanol extract of *Costus pictus* was tested against the biofilms of the bacteria species. Among the bacterial isolates, better result of

Furthermore, GCMS analysis was carried out with ethanol extract of *Costus pictus.* Numerous antimicrobial compounds of phytochemicals were observed (Fig 4). The Cyclohexanone, N-Acetylmannosamine, Ethanamine, and 2-Furanone were observed. Recently found the antifungal compound of 2-Furanone from Kei Apple extract [16]. Previous study was observed the antimicrobial activity of Cyclohexanone from *Murraya paniculata*. The cyclohexane

and derivates possess exceptional nature of mode of action as antibacterial agents [17].

CONCLUSION

The results obtained from this study provided evidence that the ethanol and chloroform extracts of the *Costus pictus* was exhibited beneficial antimicrobial and antibiofilm activity against bacterial and fungal isolates. The highest antimicrobial activity was observed for ethanol extract, which showed a MIC was 6.5mg and further characterization revealed the presence of cyclohexane and 2-Furanone derivatives that might be responsible for the observed antimicrobial activity. It can be concluded that a high polar active compound present in the *Costus pictus* extract may be responsible for the significant antimicrobial activity, whereas *Aspergillus niger* was not suppressed. Therefore, further investigations are necessary to evaluate antimicrobial activity with various extracts. Moreover, other parts of the plants need to be studied to evaluate the studied plant extracts as a potential antimicrobial agent.

LITERATURE CITED

1. Michael CA, Dominey-Howes D, Labbate M. 2014. The antibiotic resistance crisis: causes, consequences, and management. *Front Public Health* 2:145.
2. Lee Ventola MS. 2015. The antibiotic resistance crisis. *Pharmacy and therapeutics* 40(4): 277-283.
3. Saeidi S, Amini Boroujeni N, Ahmadi H, Hassanshahian M. 2015. antibacterial activity of some plant extracts against extended-spectrum beta-lactamase producing *Escherichia coli* isolates. *Jundishapur Jr. Microbiology* 8(2): e15434.
4. Gupta PD, Birdi TJ. 2017. Development of botanicals to combat antibiotic resistance. *Journal of Ayurveda and Integrated Medicine* 8(4): 266-275.
5. Brijesh, Daswani P, Tetali P, Antia N, Birdi T. 2009. Studies on the antidiarrheal activity of *Aegle marmelos* unripe fruit. *BMC Complement Alternate Medicine* 9(47): 1-12.
6. Ramya R, Dhamotharan R. 2016. Antibacterial activity and screening of antibacterial compounds of *Costus pictus* D. Don using GC-MS. *International Journal of Science and Research* 5(6): 810-814.
7. Solomon SU. 2013. Preliminary phytochemical screening of different solvent extracts of stems bark and roots of *Dennetia tripetala*. *Asian Journal of Plant Science and Research* 3(3): 10-13.
8. Khan JA, Tewari S. 2011. A study on antibacterial properties of *Rosa indica* against various pathogens. *Asian Journal of Plant Science and Research* 10: 2838-2846.
9. Gurunathan S, Han JW, Kwon DN, Kim JH. 2014. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale Res. Letters* 9(1): 373.
10. Shiny CT, Saxena A, Gupta SP. 2013. Phytochemical and hypoglycaemic activity investigation of *Costus pictus* plants from Kerala and Tamilnadu. *International Journal of Pharmaceutical Science Invention* 2(5): 11-18.
11. Prejeena V, Suresh SN, Varsha V. 2017. Phytochemical screening, antioxidant analysis and antiproliferative effect of *Costus pictus* d. don leaf extracts. *International Journal of Recent Advances in Multidisciplinary Research* 4 (3): 2373-2378.
12. Jose B, Reddy LJ. 2010. Analysis of the essential oils of the stems, leaves and rhizomes of the medicinal plant *Costus pictus* from southern India. *Int. Jr. Pharmacy Pharm. Science* 2(2): 100-101.
13. Rai JB, Kalaivani R. 2018. In-vitro evaluation of antimicrobial activity of *Costus pictus* D. Don aqueous leaf extract. *Natl. Jr. Physiol. Pharm. Pharmacology* 8(8):1107-1109.
14. Devi GMR. 2019. A comprehensive review on *Costus pictus* D. DON. *International Journal of Pharm. Science and Research* 10(7): 3187-3195.
15. Jdco S, Ia F, Jg L, Infante J, Sm DA. 2017. Unexplored endemic fruit species from Brazil: Antibiofilm properties, insights into mode of action, and systemic toxicity of four *Eugenia Spp*. *Microb. Pathog*. 105: 280-287.
16. Qanash H, Yahya R, Bakri MM, Bazaid AS, Qanash S, Shater AF, Abdelghany TM. 2022. Anticancer, antioxidant, antiviral and antimicrobial activities of Kei apple (*Dovyalis cafra*) fruit. *Scientific Reports* 12: 5914-5918.
17. Sonter S, Mishra S, Dwivedi MK, Singh PK. 2021. Chemical profiling, in vitro antioxidant, membrane stabilizing and antimicrobial properties of wild growing *Murraya paniculata* from Amarkantak (M.P.). *Scientific Reports* 11: 9691-9693.