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RESEARCH ARTICLE

# *In-vitro* evaluation of antimicrobial activity of *Costus pictus D. Don* aqueous leaf extract

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## ABSTRACT

**Background:** With the extensive use of antibiotics, antimicrobial resistance has become a serious threat to public health. Traditional plant medicine may serve as an alternative to this issue as they are relatively safe and has additional beneficial effects. **Aims and Objectives:** The aim of this study was to evaluate antimicrobial effect of *Costus pictus D. Don* aqueous leaf extract. **Materials and Methods:** Antimicrobial effect of *Costus pictus D. Don* aqueous leaf extract was tested by modified agar well diffusion method in triplets. 10 different types of bacterial isolates were studied. Zone of inhibition >8 mm was considered to be active. **Result:** Marked antimicrobial activity of *Costus pictus D. Don* aqueous leaf extract was observed against all the bacterial stains used, especially above 40 µl. **Conclusion:** Aqueous leaf extract of *Costus pictus D. Don* showed dose-related antimicrobial effect on the bacterial stains studied.

**KEY WORDS:** *Costus pictus D. Don;* Antimicrobial Activity; Modified Agar Well Diffusion Method; Bacterial Isolates

## INTRODUCTION

Antimicrobial resistance is one of the emerged global issues which create a very significant mortality and morbidity, especially in various developing countries. As all the available synthetic pharmacological agents not only produce its side effects and financial burden, it also leads to a very well-known global health issue that directly burdens the patients and attending physician. Bacterial isolates have already developed resistance against almost all the available antimicrobial agents. Due to inadequate and indiscriminate use of antibiotics, various resistance mechanisms such as Methicillin-Resistant Staphylococcus Aureus (MRSA), Extended Spectrum Beta-Lactamases (ESBL), Metallo-Beta-Lactamases (MBL), AmpC Beta-Lactamase (AmpC), Klebsiella Pneumoniae Carbapenemase (KPC), Vancomycin-Resistant Enterococcus (VRE) and Vancomycin-Resistant, Methicillin-Susceptible S. aureus (VR-MRSA) created a significant therapeutic challenge to all the physicians. This led to the search of new antimicrobial agents among various researchers. As each new antimicrobial compounds needs at least 2–3 decades to pass through all the phases, alternative medicines can be replaced to a maximum extent to overcome all these untoward effects. Among numerous medicinal plants, *Costus pictus D. Don* is one with diverse medicinal properties such as antidiabetic,[1] antioxidant,[2] anthelmintic,[3] and antitumor[4] effect.

Thus, this study was aimed to determine the antimicrobial effect of *Costus pictus D Don*, a medicinal plant.*n*. Considering that the use of antimicrobial compounds of medicinal plants has less side effects, better patient tolerance, relatively less expensive, and acceptance due to long history of use and being renewable in nature.

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## MATERIALS AND METHODS

### Plant Materials

*Costus pictus D. Don* leaves were collected at hot climate from the local garden in Pondicherry. The plant material was authenticated by the Department of Botany, Annamalai University, Chidambaram (No. 326). Specimen of plant is preserved in Sri Balaji Vidyapeeth, Pondicherry.

### Preparation of Plant Extract

The collected leaves were air dried in shade for a week. The dried leaves were powdered and subjected to soxhlation with distilled water. The final extract obtained was dried with rotary evaporator and refrigerated in brown airtight bottle.

### Drugs and Chemical

Bacterial isolates were procured from HiMedia Laboratories Pvt., Ltd.

### Evaluation of antimicrobial Activity

Antimicrobial activity of *Costus pictus D. Don* aqueous leaf extract was checked by *in vitro* modified agar well diffusion method at different volumes. After satisfactory sterility checks, sterile Mueller Hinton agar (MHA) was taken, and the list of test isolates was made lawn culture over it and kept for 10–15 min. Following that, multiple wells of 8 mm (0.8 cm) diameter were dug over it using sterile cork borer. Different volumes of extract at 10 µl, 20 µl, 30 µl, 40 µl, and 50 µl were poured in each MHA in various wells with different bacterial test organism. All the plates were incubated at 37°C for overnight incubation aerobically.[5] The experiment was carried out in triplicates and the average zone of inhibition surrounding each volume of wells with Costus pictus extract was recorded in millimeter (mm) and analyzed. An extract with zone of inhibition equal to or more than 8mm was considered as active.[6] Dimethyl sulfoxide which did not influence bacterial growth (50 µl) was added as control in each plate with test lawn cultures.

Bacterial isolates used to check *in vitro* susceptibility testing were as follows 1. *Staphylococcus aureus* ATCC (25923), 2. *Pseudomonas aeruginosa* ATCC (27853), 3. *Escherichia coli* ATCC (35218), 4. *K. pneumoniae* ATCC (700603), 5. *Enterococcus faecalis* ATCC (29212), 6. *E. faecalis*, 7. *MDR P. aeruginosa*, 8. MDR *K. pneumoniae*, 9. Methicillin-resistant *Staphylococcus aureus* (MRSA), and 10. ESBL-producing *E.coli*.

## RESULTS

The findings of the present study are recorded in Table 1.

## DISCUSSION

Due to extensive use of various antimicrobial agents, there is a current need to find an alternative to tackle this issue to protect future humanity, which created brainstorming to find a biologically active compound from plants extracts against the potential pathogens and its resistance mechanisms.[7,8] Nature as an ally and resource in finding new strategies to combat infectious diseases, *Costus pictus D. Don,* commonly referred as insulin plant or spiral ginger was studied. Considering all the facts, we have studied this plant extract with various ATCC control bacterial isolates and some clinical isolate, to prove its antibiotic effects. Following agar well diffusion method with aqueous leaf extract of Costus pictus D. Don, *ATCC Staphylococcus aureus* and *ATCC K. pneumoniae* isolates showed active zone of inhibition with all the test

**Table 1:** List of bacterial isolates used for ***in vitro*** susceptibility testing

| Bacterial isolates | Aqueous leaf extract used in various concentration (µl) | | | | | DMSO (50µl) |
|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | |
| | Diameter of zone of inhibition in mm | | | | | |
| *S. aureus* ATCC (25923) | 9* | 12 | 15 | 18 | 21 | - |
| *P. aeruginosa* ATCC (27853) | 6 | 6 | 9* | 11 | 14 | - |
| *E. coli* ATCC (35218) | 6 | 7 | 8* | 10 | 12 | - |
| *K. pneumoniae* ATCC (700603) | 8* | 9 | 10 | 12 | 18 | - |
| *E. faecalis* ATCC (29212) | 6 | 6 | 8* | 11 | 15 | - |
| *E. faecalis* | 6 | 6 | 8* | 9 | 13 | - |
| MDR *P. aeruginosa* | 6 | 6 | 6 | 10* | 13 | - |
| MDR *K. pneumoniae* | 6 | 6 | 10* | 14 | 16 | - |
| MRSA | 6 | 10* | 12 | 14 | 16 | - |
| ESBL producing *E. coli* | 6 | 6 | 7 | 8* | 10 | - |

*Active zone of inhibition. *S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, K. pneumonia: Klebsiella pneumonia,* MRSA: *Methicillin-Resistant Staphylococcus aureus*, ESBL: *Extended Spectrum Beta-Lactamases*

volumes used. *ATCC P. aeruginosa, ATCC E. coli,* and *ATCC E. faecalis* showed active zone with extract volumes from 30 µl to 50 µl. Among clinical isolates from various samples, *E. faecalis* and *MDR K. pneumoniae* also showed active zone only with 30 µl, 40 µl, and 50 µl. In case of MRSA clinical isolate, significant active zone of inhibition was documented from 20 µl to 50 µl, respectively. With 40 µl and 50 µl, *MDR P. aeruginosa* and *ESBL producing E.coli* showed active significant zone of inhibition.

In comparison with our results, Reddy *et al*. found very significant zone of inhibition using *Costus pictus* leaf oil against various bacterial isolates when compared with various antibiotic.[9] Similarly, Majumdar and Parihar also witnessed very marked antimicrobial effects against various bacterial strains.[10] We, in this study witnessed satisfactory zone of inhibition surrounding each isolates in various concentrations similar to others. Although it is not a new idea to advocate plant extracts to cure infectious diseases, this ancient holistic approach was attempted to prove its antimicrobial affects an evidence-based approach.

The limitations of our study could be less number of isolates tested, lack of MIC values, and *in vivo* effects.

## CONCLUSION

Aqueous leaf extract of *Costus pictus D. Don* has definite but dose-related antibacterial effect on the stains tested. Remembering the economic and health burden caused by synthetic antibiotics, plants and plant-derived medicine will be a better and safe alternative.

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