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EVALUATION OF ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF *COSTUS PICTUS* AGAINST ESBL PRODUCING BACTERIA

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ABSTRACT

Due to misuse of antibiotics, bacteria are leading to an increase in multidrug resistance, thereby making the search for antibiotics from plants an effective and alternative approach imperative. In the present study, *Costus pictus* was selected for evaluation of antimicrobial activity against ESBL producing clinical isolates and additionally, biofilm inhibitory activity was determined with plant extracts. The results of phytochemical screening of *Costus pictus* leaf extract indicate the presence of Alkaloids, Flavonoids, Saponins Tannins and Phenols. The agar well diffusion method was utilized for the antimicrobial activity; the high polar extract of methanol showed greater activity then acetone extract. The methanol extract had a good battle against *E. coli* and *K. pneumoniae*. Furthermore, *Costus pictus* extracts had a good capacity to reduce biofilm formation. Further research is needed to understand which compounds found in the extract are responsible for the observed effects, including their respective modes of action.

KEYWORDS: *Costus pictus, ESBL, Antibiofilm, Antibacterial activity, E.coli.*

INTRODUCTION

The expansion of antimicrobial resistance (AMR) is a major health problem worldwide. This resistance to antibiotics is spread by the expanded use, selection of wrong antibiotics. The problem of AMR is particularly urgent in relation to antibiotic resistance in bacteria. Bacteria that cause common and serious infections are emerging with each new antibiotic that coming from the pharmaceutical market. It also influence the economical growth, use of antimicrobials, even in all the other fields like aquaculture, agriculture etc.[1] *Enterobacterales, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterococcus faecium, Escherichia coli* and *Staphylococcus aureus* are some of the clinical pathogens that has to be surveyed properly due to their extended resistance mechanisms which are named as "priority pathogen list" by WHO in 2007.[2]

Enterobacterales and few other gram negative bacteria are seemed to be resistant to carbapenems and cephalosporins which are achieved by the synthesis of extended spectrum beta lactamases (ESBLs) or carbapenemases enzymes.[3] The spread of pandemic diseases is also mediated by methicillin resistant *Staphylococcus aureus* (MRSA).[4] Pathogens like *S.aureus, Klebsiella pneumoniae, Enterococcus faecium, P.aeruginosa, Acinetobacter baumannii* and Enterobacter sp are exhibiting high level of resistance to a number of antibiotics and are commonly known as ESKAPE pathogens which are the major source of numerous infections that occurs in hospital environment.[5]

The infection with ESBL producing enterobacteriaceae is associated with a delay in initiating appropriate antibacterial therapy, which extends hospital stays and increase hospital costs. There seems to be a higher mortality rate in patients who fail to initiate antibacterial treatment at the beginning of their illness.[6] Due to this reason scientists are showing more interest in the efficiency of the antibiotics which are used now against many diseases. To overcome the resistant problem, scientists are searching for novel alternative substances to control such high resistant strains.

For the past many years, phytochemicals produced by plants are studied in this regard as they are many in number with minimal side effects.[7] These phytochemicals exhibit antimicrobial activity. Around 255 of the drugs that are used now are from plant derivatives.[8] Many traditional medicine systems like Indian Ayurveda and Chinese medicine are mainly based on plant sources.[9]

One among such plant is the *Costus pictus* commonly known as insulin plant, fiery costus, step ladder spiral ginger that is native to south and central America (with numerous medicinal properties like antidiabetic, antioxidant, antihelminthic and antitumor properties.[10] It also has hypoglycemic and anti inflammatory properties.[11] several study were determined the various parts of *Costus pictus* showed antibacterial, anthelmintic, and antitumor activities.[12,13] However, no one author showed efficacy of antimicrobial activity of leaves of *Costus pictus* against ESBL producing clinical isolates.

The present effort has been made to investigate the phytochemicals analysis and antimicrobial activity of the methanolic and acetone extract of leaves of *Costus pictus* against ESBL producing clinical isolates.

MATERIALS AND METHODS

Collection of bacterial isolates

This study was conducted from Jan 2021 to Dec 2021. Totally 6 bacterial species of *Escherichia coli*, *Salmonella sp*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* were obtained from the Microtech Diagnostic Centre, Coimbatore, Tamilnadu. All isolates of *E.coli* were isolated from UTI patient's urine samples. The following information was provided by the submitting laboratories: sex, age, date of collection and patient location. One isolate per patient was included.

Confirmation of the isolates

All the bacterial cultures were tested for viability and purity once again by sub-culturing on nutrient broth. The identification of the isolates was re-confirmed by performing selective media (EMB, MacConkey and chromogenic). The confirmed isolates were cultured in nutrient agar slant and store in 4^0C condition.

Isolation of ESBL producing isolates

According to Balan (2013) ESBL producers were identified.[14] Using a sterile cotton swab, the test inoculums (0.5 McFarland tube) were applied on Mueller-Hinton agar (MHA) plates as a lawn. On the surface of MHA, a disc of CAZ (30 g)+clavulanic acid (10 g) was placed, and the disc of CAZ (30 g) was then placed 15 mm away from the ceftazidime+clavulanic acid disc via the edge to edge. The inoculated plates were incubated at 35°C in the incubator for 18–24 h. The zone of inhibition between the CAZ and ceftazidime+clavulanic acid was compared. The difference in the zone diameter of ≥5 mm was interpreted as positive for ESBL production.

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 methanol and acetone solvents. The extracts were stored at 4ºC for phytochemicals screening and antimicrobial analysis.

Phytochemical Screening of *Costus pictus* extract

The presence of various phytochemicals compounds in the leaf of *Costus pictus* was confirmed by using the methods of Solomon *et al* procedure (2013).[15]

Determination of antibacterial activity of *Costus pictus* extract

The Mueller - Hinton agar plates were inoculated with freshly prepared overnight inoculums (10^8 CFU/ml), which were swabbed over the entire surface of the medium. The 6mm diameter of the well was made with sterile stainless steel borer on the agar plates. A 100 µl of different concentrations of extracts were filled in well with the help of micropipette and one well filled with solvent control and another well filled with antibiotic control (ampicillin -10mcg) and plates were kept for incubation at 37°C for 24 hours. After incubation, observed the zone of inhibition around each well and recorded in mm.

Antibiofilm activity of *Costus pictus* extract

In vitro experiments evaluated the ability of the extract of *Costus pictus* to inhibit biofilm formation on 96-well polystyrene plate. The bacterial culture was diluted to 10^6 CFU/mL and transferred to wells of the plate and 100 µl of extract at different concentrations were added into wells and incubated the plates at 37 °C for 24 hrs. After incubation, the wells were washed with PBS, dried, and stained with 200 µL of 0.1% crystalline violet. Then, wells were thoroughly rinsed with sterile deionized water until all excess dye is removed. The plates were air dried and added 200 µl of ethanol (95%, v/v) to each well and 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].

RESULTS AND DISCUSSION

Totally 5 bacterial genera of 22 species were procured from clinical laboratory and subjected to determination of ESBL producers. The confirmation of ESBL production is provided by demonstration of synergy between CAZ and clavulanate. Among the 22 isolates, 12 (54.5%) of were ESBL producers, the *E.coli* and *K.pneumoniae* (50%) were predominant and followed by *E.faecalis* (25%).

The ESBL producers are key members from the group of antibiotic resistant pathogens lead to nosocomial infection. ESBL-producing bacteria are a major concern because of their association with MDR microorganisms, resulting in a limited range of antibiotics for treatment Infections leading to increased morbidity, mortality and hospitalization costs. In this context, understanding ESBL formation and the antimicrobial spectrum of bacterial isolates is critical to provide patients with reliable and empirical antibiotic therapy. Researchers are currently conducting extensive research on alternative therapy solutions, including most plants and phytonutrients.

According to the above statement the present research has used *Costus pictus* to know the medicinal properties of the plant. Results of phytochemical screening of *Costus pictus* leaf extract indicate the presence of various phytochemicals. Alkaloids, Flavonoids, Saponins Tannins and Phenols were observed from both methanol and acetone solvents extracts. In addition, the methanol extracts showed the presence of Quinones and Sterols were observed.

Iqbal *et al*., (2015) reported that the flavonoids have several functions as an antioxidant[16], antibacterial, antiinflammation, anti-allergy and anti-mutagenic.[17,18] Alkaloid had been reported as cytotoxicity, antimalarial, and anti-inflammatory. Steroids and triterpenoid could be effective as an analgesic and also possess antibacterial and insecticidal properties.[19] In light of the aforementioned claims, present investigation reveals that *Costus pictus* has a range of beneficial phytochemicals.

The literature reveals that the presence of these phytochemicals is responsible for their antimicrobial activity against many microorganisms. Therefore, *Costus pictus* extracts were evaluated for antimicrobial activity and confirmed promising results as antimicrobial agents. The highest antimicrobial activity was observed while using methanol extract compared than acetone extract. The zone of inhibition was ranged between 10mm to 18mm, among the bacterial pathogens, *E.coli and K.pneumoniae* were highly suppressed and positive control of antibiotic used in the study does not show any markable antibacterial activity against the selected pathogenic isolates. In 2012 Majumdar and Parihar analyzed the leaf extracts of *C. pictus* against *Klebsiella pneumoniae*, and *Escherichia coli*.[20]

Research on the inhibition of ESBL-producing bacteria with this plant has not been extensive; however, Raj and Kalaivani reported that leaf extract of *C. pictus* was active against to ESBL producing *E.coli* isolates[21]; but research on killing more bacterial species has not been found. In the present study, 6 wide range of ESBL producing bacterial isolates were suppressed with 5mg of concentration of methanol extract, but same time, 7.5mg of acetone extract was inhibited the all isolates (Fig.1). The presence of several phytochemicals with antibacterial activity in the extracts may have contributed to the synergistic destruction of the bacteria.[22]

Antibiofilm activity was another aspect evaluated in a selection of plant extracts. Biofilm plays a major role in reducing the effectiveness of antibiotics, increasing the rate of virulence, and increasing the ability of bacteria to attach to a substance.[23] Therefore, this complex problem must be properly and carefully managed to kill the bacteria that form the biofilm. Thus, some researchers have been done to prevent biofilm formation and kill bacteria using plant and its products.[24,25,26] In the present study, 6 bacterial genera were utilized for the inhibition of biofilm activity, among them *E.coli* and *S.aureus* were highly suppressed, methanol extract highly reduce the formation of biofilm, specifically, better biofilm reduction was observed at higher concentrations of extract. The similar line of results were observed by Abraham *et al*., and Ravichandiran *et al*.,[27,28] The 3mg methanol extract reduced all bacteria forming biofilms more than the acetone extract.

The phytocompounds present in the plant extracts may be responsible for the antibiofilm activities observed in this study. In a recent study, isolated phytocompounds from plants had antibiofilm activity against different bacterial pathogens. Some studies indicate that this activity may be due to saponins, terpenes, tannins, and alkaloids. These phytochemicals were observed in current study also. Plant extracts may disrupt bacterial cell-to-cell communication strategies (quorum sensing), thereby reducing biofilm formation.

The present study indicate that leaf of *C. pictus* are rich in phytocompounds, particularly saponins, terpenes, tannins, and alkaloids, and possess in vitro antibacterial activity against ESBL producing isolates activity, moreover, antibiofilm activity also observed against ESBL producing isolates. The *C. pictus* extracts can be an alternative to control the formation of bacteria and its producing biofilms formation. However, future study attempts will be made to isolate the compounds which may responsible for the antibiofilm effects of the active plant extracts.

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