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In vitro ANTIBACTERIAL ACTIVITY OF *Elytraria acaulis* LINDAU (ACANTHACEAE) AGAINST CLINICAL PATHOGENS

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Abstract

Currently the indiscriminate use of commercial antimicrobial agents has resulted in multiple drug resistance in human pathogenic microorganisms. To overcome the escalating problems associated with infectious diseases and drug resistance discovery of new antimicrobials is vital. Increased incidence of resistant in bacteria together with lack and high cost of new generation drugs has increased infection related morbidity and mortality particularly in developing countries. The present study was aimed to evaluate the *in vitro* antibacterial activity of *Elytraria acaulis* herb against the clinical pathogens. The *Elytraria acaulis* plant was collected; dried, powdered and serially extracted using the organic solvents in the Soxhlet apparatus and the antimicrobial activity was analyzed by Disc diffusion method and the Minimal Inhibitory Concentration (MIC) was analysed by Broth dilution technique against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus*. The methanolic extracts of *Elytraria acaulis* showed maximum antibacterial activity against all the tested pathogens and the MIC was ranged between 40 µg/ml to 5 µg/ml. The results obtained justified the antibacterial potential of the *Elytraria acaulis* against the clinically important pathogens. The bioactive compounds in the extracts will be helpful in herbal antimicrobial formulations.

Key words: *Elytraria acaulis*, Antibacterial activity, MIC and Clinical pathogens.

1. Introduction

Nature provides all of its constituents to the human and animal welfare as food, feed, medicinal, aromatic and pharmaceutical valuable products. In recent trends, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial agents commonly used in the treatment of infectious disease (Davis, 1994). In addition to this setback, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Ahmad *et al*., 1998). Most of the resistance developed from nosocomial infectious pathogens. Bacteria are the leading causes of nosocomial infections followed by viruses which are relatively second and occasionally fungi cause disease but rarely protozoa are involved. The exercise of healing and diagnostic equipment such as intravenous and urinary catheters, surgical procedure and transplantation has increased the risk of nosocomial diseases. Mostly nosocomial diseases are caused by Gram negative bacilli like *Escherichia coli* and *Pseudomonas aeruginosa*. Increased antibiotic therapy leads to the development of drug resistance against wide spectrum of antibiotics (Ravikumar *et al*., 2002).

Increased antibiotic resistance has turn out to be a global alarm, coupled with the problem of microbial perseverance, thus highlighting the need to widen novel microbial drugs that are not only

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dynamic against drug resistant microbes but more importantly, kill persistent microorganisms and shorten the time - span of treatment. Apart from toxicity, extensive therapy also creates poor patient fulfilment (Mariita *et al*., 2010). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Medicinal plants are a great source of economic values. Various plants and herbaceous plants used traditionally have potent antibacterial, antifungal and antiviral properties and such stories have called down the optimism about Phyto-antimicrobial agents (Das *et al.,* 1992). Since antiquity, medicinal plants have been mapping a deep source of antimicrobial and antiviral agent (Kane *et al.,* 1950).

Elytraria acaulis is a widely distributed perennial herb in South Africa, Ghana, Nigeria, Congo-Kinshasa, Somalia, Angola, Zambia, Malawi, Mozambique, Zimbabwe, Botswana and India. The Acanthaceae family consists of several important medicinal plants with extensive scope of biological actions and interesting phytochemical constituents [9]. Leaves of *Elytraria acaulis* were traditionally employed in the treatment of asthma, migraine, leucorrhoea, abscess of mammary glands, boils, burns, colic, diarrhoea, rickets, throat compliments tonsillitis, antihypoglycemic and snake bite (Jain *et al*., 2005). The present work was directed to study the antimicrobial activity of *Elytraria acaulis* extracts against the bacterial pathogens involved in urinary tract infections.

2. Materials and Methods

Collection of *Elytraria acaulis* plants

Elytraria acaulis from the Family of Acanthaceae was collected from the local areas of in and around Bhuvanagiri, Cuddalore district and identified morphologically and taxonomically, the specimens were presented to the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Bacteria tested

The antimicrobial activity of plant extract was tested against the urinary tract infection causing pathogens. The bacterial strains used in our present study were *Staphylococcus aureus Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Enterococcus faecali*s. Separate sterile Nutrient agar slants were prepared and the bacterial strains were individually inoculated into separate slants under aseptic conditions and incubated at 37 ºC for 24 hrs under aseptic condition the colonies were harvested separately from the slants and individually inoculated into sterile nutrient broths in separate test tubes and stored under refrigerated condition.

Preparation of plant extracts

The collected plant material was washed cleanly in tap water and then air dried under shadow condition at room temperature (25 °C) for 2 - 3 weeks until they are brittle. After complete drying, the plant material was ground to fine powder using electrical blender. Fifty gram of dried powder was packed in the Soxhlet apparatus with 300 ml of various solvents (Methanol, Acetone, Chloroform and Hexane) extracted until the extract was clear. The solvents from the extracts were evaporated using a rotary vacuum evaporator and the extract was stored in a refrigerator for further use.

Antimicrobial activity of the plant extract

The extracts were redissolved in 5 % DMSO and impregnated in sterile, empty discs of 6 mm in diameter. Mueller Hinton agar plates were prepared and the bacterial cultures isolated from POI wounds were inoculated, the discs with plant extracts along with positive controls were placed over the Mueller Hinton Agar medium and incubated for 24 hours at 37 °C. After 24 hours of incubation, the zone of inhibition of the bacterial species was measured using graduated zone measuring scale and expressed as mm in diameter. The data represent three replicates per microorganism.

Minimal inhibitory concentration

To determine the MIC for the extracts, fraction of *Elytraria acaulis,* extracts was serially diluted in Mueller Hinton Broth. Equal amount (2 ml) of bacterial suspension corresponding to 10^7 CFU mL^{-1} of the test organism was added to each of the test tubes and incubated at 37 °C for 24 hours. The turbidity in each tube was visualized. The lowest concentration of the respective extract that led to the inhibition of the growth of bacteria was considered as MIC. The data represent three replicates per microorganism.

3. Results and Discussion

Antibacterial activity of four solvents extracts of *Elytraria acaulis* were tested against the clinical pathogens and the results were presented in the Figure - 1. All four extracts showed maximum results at 100 µg concentrations. The methanol extract of *Elytraria acaulis* possessed maximum activity than other solvent extracts. The maximum zone of inhibition was observed against *Escherichia coli* of 18.0 ± 0.3 mm followed by *Klebsiella pneumoniae* of 14.0±0.5 mm of inhibition. The chloroform extracts showed moderates zone of inhibition against *Escherichia coli* of 11.0 ± 0.3 mm and the least activity was observed in the hexane extracts. The MIC values obtained from the present result were in the range of 20.0 – 80.0 µg/ml against the POI pathogens. The MIC values of methanolic extract against *Pseudomonas aeruginosa* and *E. coli* were 40.0 – 80.0 µg/ml. Shahbudin Saad *et al.* (2012) investigated the antimicrobial property of *S. alba* and methanol extract appeared to be the most effective extract while other extracts showed lower activity. *E. coli* appeared to be the most sensitive strain followed by *S. aureus* and *B. cereus.*

Antibiotic resistance results in reduced efficacy of antibacterial drugs, making the treatment of patients difficult, costly or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality. Extraction of bioactive compounds from plant material is principally dependent on the type of solvent used for the extraction procedure. The traditional healers use primarily water as the solvent, but in our study we used methanol for extraction and it showed better results against the tested pathogens (Allero and Afolayan, 2006; Parekh and Chanda, 2007). Xixu and Lee (2001) reported that the plant flavonoids highly inhibit the growth of multiple drug resistant organisms. *E. acaulis* contains alkaloids, flavonoids, glycosides, sugar compounds and terpenes (Praveen Kumar *et al*., 2014). It was demonstrated that 80 % of aqueous ethanolic extracts of *Lophostemon suaveolens* and *Syncarpia glomulifera* showed microbicidal activity against the sensitive and resistant strains of *Staphylococcus aureus*. Ahoua *et al*. (2015) investigated antimicrobial activity of 27 medicinal plants against *Escherichia coli, Pdeidomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Candida glabrata* in which 24 methanol extracts (18 %) showed activity against bacteria and 6 extracts (5 %) were active against yeasts. Jaya Prakash Priya *et al*. (2012) investigated the methanol extracts of *Vigna radiata* L. against bacterial pathogens involved in food spoilage and food borne diseases which showed significant concentration dependent antibacterial activity against almost all the test pathogens. Activity index clearly showed that the various solvent extracts of this plant, antibacterial activity found to be more than gentamicin a well known synthetic antibiotics indicating that this plant have significant antibacterial activity against the tested pathogens.

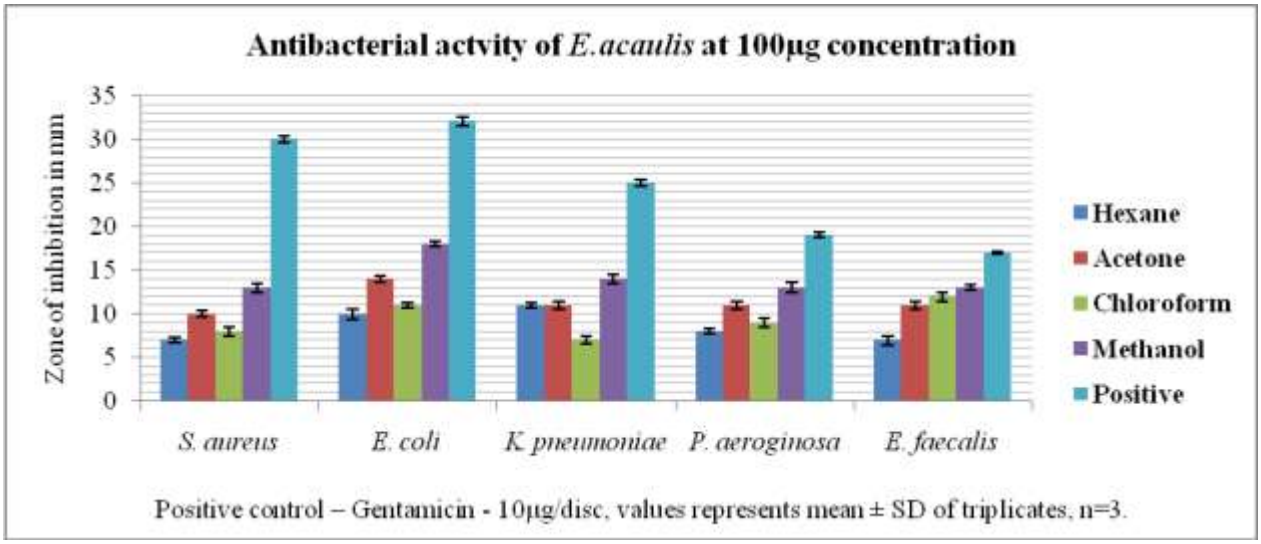

Figure - 1: Antibacterial activity of *E. acaulis* crude extracts

Table - 1: Minimal inhibitory concentration of *E. acaulis* extracts

Bacteria	Minimum Inhibitory Concentration of *Elytraria acaulis* (in µg/ml)				
	Hexane	Chloroform	Acetone	Methanol	Positive control
S. aureus	80	80	40	20	2.5
E. coli	80	40	20	10	1.25
K. pneumoniae	80	80	40	20	2.5
P. aeroginosa	80	80	40	40	5
E. faecalis	80	40	40	20	5

4. Conclusion

The findings of this study indicate that the solvent extracts of *Elytraria acaulis* have a high potential for the production of pilot compounds with antimicrobial properties against the multi drug resistant pathogens. Further study with purification of the compound present in the plant helps in identifying new antimicrobial agents.

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