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Case Study

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In-Vitro Antibacterial Activity of *Elytraria acaulis*

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ABSTRACT

The current study was aimed to evaluate antibacterial activity of *Elytraria acaulis* root extracts on different gram positive and negative bacteria. The hexane, ethyl acetate and hydro-alcoholic extracts of *Elytraria acaulis* were tested using cup plate method for their activity with their zones of inhibition. The tested extracts of *E. acaulis* roots showed dose dependent antibacterial activity. As the concentration increased their zone of inhibition on tested bacterial strains was increased. The extracts showed better activity on gram negative strains compared to gram positive. Among three extracts hydro alcoholic extract showed more activity. The outcome of the current study concludes that *E. acaulis* root extracts have biological active compounds which inhibit microbial growth and confirm the traditional use of *E. acaulis* root in different ailments.

Keywords: *Elytraria acaulis;* Roots; Gram positive; Gram negative; Zone of inhibition.

1. INTRODUCTION

The microorganisms became resistant to current day using drugs since last decade because of several reasons around the world (Aslam *et al.,* 2018). The main reason behind the microbial resistance was adequate use of antibiotics and the emergence of new diseases (Sweileh, 2017). As the new diseases emergence and ample use of antibiotics causing side effects and their resistance insisting the current researchers to identify new bioactive molecules from natural resources (Ramsay *et al.,* 2018; Ganga Rao *et al.,* 2011; Fair and Tor, 2014). Medicinal have been using as medicines in traditional medicines around the world such as Ayurveda, Unani, Chinese Traditional medicine and are one of the major resource for identification, isolation and development of new bioactive molecules (Pan *et al.,* 2013; Wells, 2011; Patwardhan *et al.,* 2005). But, there were ample of medicinal plants are not reported about their biological activities scientifically (Zhu *et al.,* 2018; Maheswara Rao and Aniel Kumar, 2018). So, the current research was aimed to evaluate the anti bacterial activity of *Elytraria acaulis* root part.

Elytraria acaulis is an perennial herb belongs to the family Acanthaceae. *Elytraria acaulis* is grow widely in woodland, sandy land regions around the world. *E. acaulis* has been using in traditional medicine for different ailments (Kaido *et al.,* 1997; Shikarwar *et al.,* 2008; Kumudhavalli and Jayakar, 2011). The root part have been using as paste for treatment of leucorrhoea, snake bites, abscess of mammary glands, throat compliments like tonsillitis.

2. MATERIALS AND METHODS

Chemicals

The solvents and chemicals utilized in current research were analytical grade and used standard drug ciprofloxacin was procured from local market (Dr. Reddy's Laboratories).

Plant material collection and extracts preparation

The plant material *Elytraria acaulis* was at pulnadu region, Andhra Pradesh, India and authenticated by Dr. Prayaga Murthy. Pragada, Govt. Degree College, Yeleswaram, E. Godavari, A.P. India. The roots were separated from freshly collected plant material and wash under running tap water to remove unwanted material. The cleaned roots were shade dried and granulated into fine powder for further use. The powder was used for preparation of extracts successively with hexane, ethyl acetate and hydro-alcoholic [70%Ethanol (hyd-alc)] using maceration. The prepared extracts were stored in desiccator for further use.

Selected bacterial strains

Gram positive and gram negative bacterial strains were tested in the current *In-vitro* antibacterial activity of *E. acaulis* study (Table 1). The bacterial strains were taken from National Chemical Laboratory (NCL), Pune.

Table 1: Tested bacterial strains list

S. No	Gram Positive	Gram Negative
1.	*Streptococus pneumoniae*	*Pseudomonas aeruginosa*
2.	*Staphylococcus aureus*	*Yersinia enterocolitica*
3.	*Clostridium sporogenes*	*Escherichia coli*
4.	*Listeria monocytogenes*	*Salmonella typhimurium*

***In-vitro* antibacterial activity**

The antibacterial activity of selected plant extracts were evaluated using agar well diffusion method (Ganga Rao *et al.,* 2011; Ganga Rao *et al.,* 2012; The Wealth of India, 2006). The extracts' solutions were prepared in dimethyl sulfoxide at different concentrations i.e. 40, 80, 150 and 250 mg/mL. The agar plates were prepared with nutrient agar and were autoclaved, then cooled to room temperature. The cooled agar was equally poured in Petri dishes and refrigerated for solidification, then each plate was separately inoculated with testing bacterial strains as spread plate technique with sterilized spreader and with sterile steel borer (6mm) made wells on petri dish to equal distribution (100µl) as 4, 8, 15 and 25 mg/well and standard drug at 100µg/well was placed. Then, prepared plates were placed with no disturbance for transmission of placed samples in wells. Then Petri plates were incubated for 24hrs at incubator (37±2˚C). Finally, after 24hrs plates were examined for extracts' antibacterial activity by measuring their activity as zones of inhibition. The experiment was repeated for three time and results were showed as mean±SD.

3. RESULTS AND DISCUSSION

The present research was carried to evaluate the anti-bacterial activity of *E. acaulis* root extracts on selected gram positive and negative strains and found the extracts are found to have moderate inhibition on bacterial growth compared to standard drug. The results were showed in Table 2 to 4. All the extracts were showed dose dependent zone of inhibitions on tested bacterial strains. Among three extracts hydro alcoholic extract showed better activity, hexane extract showed less and ethyl acetate showed moderate activity. The extracts showed low activity at low concentrations and no inhibition on bacterial growth.

The hexane extract have more activity on gram positive compared to negative strains and did not show any zones on inhibition at 4mg on *Pseudomonas aeruginosa* and *Yersinia enterocolitica.* The extract showed more activity on *Escheichia coli* and *Clostridium sporogenes* (Table 2).

The ethyl acetate extract showed more on gram negative compared to positive strains and did not show any inhibition at 4mg concentration on *Listeria monocytogenes* and the extract showed maximum on *Escheichia coli* at 25mg (Table 3).

Table 2: Zone of inhibitions of *Elyraria acaulis* hexane extract

Name of the microorganism	Concentration of the extract (mg/100µL)					
	4	8	15	25	#	##
	Zone of inhibition (in mm)					
Streptococus pneumoniae	1.33± 0.33	3.67± 0.58	5.67± 0.88	8.67± 0.33	20.67± 0.3	-
Staphylococcus aureus	1.67± 0.33	3.33± 0.67	6.33± 0.33	9.33± 0.67	19.67± 0.33	-
Clostridium sporogenes	1.67± 0.33	3.67± 0.67	5.33± 0.67	7.67± 0.33	23.33± 0.33	-
Listeria monocytogenes	1.67± 0.33	2.33± 0.88	4.33± 0.67	6.67± 0.33	18.67± 0.58	-
Pseudomonas aeruginosa	-	2.67± 0.58	4.33± 0.88	6.67± 0.33	21.33± 0.33	-
Yersinia enterocolotica	-	1.33± 0.67	2.67± 0.33	5.33± 0.67	20.67± 0.33	-
Escheichia coli	2.33± 0.67	3.67± 0.33	5.67± 0.33	8.33± 0.67	24.33± 0.67	-
Salmonella typhimurium	2.67± 0.33	3.33± 0.67	6.33± 0.67	10.3± 0.67	21.33± 0.67	-

Ciprofloxacin(100µg/200µL); ## DMSO(100µL)

Table 3: Zone of inhibitions of *Elyraria acaulis* ethyl acetate extract

Name of the microorganism	Concentration of the extract (mg/100µL)					
	4	8	15	25	#	##
	Zone of inhibition (in mm)					
Streptococus pneumoniae	-	1.33± 0.67	2.33± 0.67	4.67± 0.33	20.67± 0.3	-
Staphylococcus aureus	1.33± 0.33	2.67± 0.33	4.33± 0.67	6.67± 0.33	19.67± 0.33	-
Clostridium sporogenes	1.67± 0.33	3.33± 0.33	5.33± 0.67	7.67± 0.33	23.33± 0.33	-
Listeria monocytogenes	-	0.67± 0.33	2.33± 0.88	4.67± 0.33	18.67± 0.58	-
Pseudomonas aeruginosa	0.67± 0.33	2.33± 0.67	4.33± 0.33	6.67± 0.33	21.33± 0.33	-
Yersinia enterocolotica	1.33± 0.67	2.67± 0.33	4.33± 0.67	6.33± 0.58	20.67± 0.33	-
Escheichia coli	1.67± 0.33	3.33± 0.67	5.33± 0.88	8.33± 0.88	24.33± 0.67	-
Salmonella typhimurium	0.67± 0.58	2.33± 0.58	4.33± 0.67	7.33± 0.67	21.33± 0.67	-

Ciprofloxacin(100µg/200µL); ## DMSO (100µL)

The hyd-alc extract has showed almost equal activity on tested bacterial strains compared to other two extracts. The hyd-alc extract showed zone of inhibitions even at 4mg not like other extracts and showed more activity on *S. typhimurium* and *E. coli* (Table 4).

The present day drugs in current world facing microbial resistance and their adequate usage causing different side effects and chronic diseases (Sweileh, 2017; Levy, 2002). The current scenario demanding the researchers to search for new antibiotics with broad spectrum which are posses no or less side effects (Wells, 2013). In this point of view, the current work carried to assess antibacterial activity of *E. acaulis* root extracts on infectious bacteria and found that *E. acaulis* have moderate antibacterial activity.

Table 4: Zone of inhibitions of *Elyraria acaulis* hydro-alcoholic extract

Name of the microorganism	Concentration of the extract (mg/100µL)					
	4	8	15	25	#	##
	Zone of inhibition (in mm)					
Streptococus pneumoniae	1.67± 0.33	3.33± 0.67	5.67± 0.33	10.67± 0.33	20.67± 0.3	-
Staphylococcus aureus	2.33± 0.33	4.33± 0.67	6.33± 0.67	9.33± 0.67	19.67± 0.33	-
Clostridium sporogenes	1.67± 0.58	3.33± 0.58	5.33± 0.67	8.33± 0.67	23.33± 0.33	-
Listeria monocytogenes	1.33± 0.67	3.33± 0.67	5.33± 0.58	8.33± 0.67	18.67± 0.58	-
Pseudomonas aeruginosa	2.33± 0.67	4.33± 0.67	7.33± 0.88	10.67± 0.33	21.33± 0.33	-
Yersinia enterocolotica	1.67± 0.33	3.67± 0.33	6.33± 0.58	8.67± 0.33	20.67± 0.33	-
Escheichia coli	2.67± 0.33	5.33± 0.67	8.67± 0.33	12.33± 0.67	24.33± 0.67	-
Salmonella typhimurium	1.67± 0.33	4.33± 0.67	7.67± 0.33	11.67± 0.33	21.33± 0.67	-

Ciprofloxacin(100µg/200µL); ## DMSO(100µL)

The extracts showed more activity on gram positive compared to gram negative organisms. The previous studies on different natural products saying that, the use of herbal medicines have fewer side effects (Pan *et al.,* 2013). There were many pure biological active compounds were isolated from medicinal plants and have been using for treatment of different ailments (Kumudhavalli and Jayakar, 2011). The extracts

of *E. acaulis* showed antibacterial activity may be the presence of individual effective compounds or synergetic compounds interaction, the isolation of pure compounds from these extracts will be more worthful research and the further research is going on.

Conclusion

The present research confirms the traditional medicinal use of *E. acaulis* root and its anti bacterial activity. Further research is worth full on isolation of bioactive compounds from different extracts of *E. acaulis.*

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Conflicting of Interests

We have none to declare.

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