

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.508.003>

Evaluation of Antimicrobial and Antioxidative Activities of the Aerial Plant Extracts of *Elytraria acaulis*

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ABSTRACT

Keywords

Aerial parts,
Antimicrobial activities,
Antioxidant assays,
Elytraria acaulis,
Organic solvents,
Pathogenic bacterial infections.

Article Info

Accepted:
06 July 2016
Available Online:
10 August 2016

Antimicrobial and Antioxidative activities of the aerial parts of the *Elytraria acaulis* a stem less perennial herb of *Acantheceae* family has been carried out in the present study. Extracts of the aerial parts of the plant (Stem & Leaves) were prepared in different organic solvents such as n-Hexane, ethanol, methanol and ethyl acetate. Initially total phenolic & total flavonoid content of the aerial parts in different solvent extracts was estimated. All the extracts were analyzed for antimicrobial properties against various pathogenic bacterial infections (*Escherichia coli*, *Klebsiella Species*, *Proteus vulgaris* and *Salmonella Typhi*). Amongst all the extracts, methanolic extract exhibited significant antimicrobial activity. The crude methanol extracts of leaves showed good inhibitory effects against pathogens. The free radical scavenging and antioxidant activity (AA) of the aerial extracts of *Elytraria acaulis* in different organic solvents was also assayed by DPPH assay, reducing power assay and FRAP assay. The aerial extracts of *Elytraria acaulis* has shown significant antimicrobial and anti oxidant activity. Hence further studies on this plant will enable elucidation of its therapeutic properties and medicinal applications.

Introduction

Anti-microbial substances are biological, semi-synthetic/synthetic origin produced by fungus/ bacterium or by medicinal herbs as secondary metabolites that stops or inhibits the growth of other micro-organisms *in-vitro* or *in-vivo* selectively. However, over the past few decades, the primary health care of humans are under threat due to emergence of drug-resistant bacteria, which makes it essential to investigate newer drugs with lesser resistance.

It has been known that drugs derived from natural sources play significant roles in the prevention and treatment of human diseases. Moreover, natural products of higher plants can be new source of antimicrobial agents with novel mechanisms of action. Therefore, the effects of plant extracts on bacteria have been studied by researchers worldwide and most of them on ethnomedicinal plants in India.

On the other hand, antioxidants are substances that protect cells from the damage caused by free radicals. Antioxidants interact with and stabilize free radicals and prevent the damage which may lead to cancer. Examples of antioxidants include beta-carotene, glutathione, lycopene, vitamins C, E, A well as enzymes such as catalase, superoxide dismutase and various peroxidases and other substances (Blot *et al.*, 1993). Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly for treatments of stroke and neurodegenerative diseases (Xianquan *et al.*, 2005).

Antioxidants are also widely present as ingredients in dietary supplements maintaining health and preventing diseases such as cancer and coronary heart disease. Although, initial studies suggested that antioxidant supplements might promote health, later, large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful (Steinmetz *et al.*, 1996).

Free-radicals are very unstable and react quickly with other compound by abstracting its electron to attain stability. When the attacked molecule loses its electron, it becomes a free-radical itself, resulting in the disruption of the substance especially in fatty foods. Environmental factors such as pollution, radiation, cigarette smoking and herbicides can also spawn free-radicals in the body (Borek, 1991).

Elytraria acaulis is a stem less perennial herb belonging to *acanthaceae* family of plant kingdom which is a small shrub that grows in shady dry places. Asian Scalystem is a stemless perennial herb with one to

several unbranched flowering stems; up to 30cm. Flowers are obrne in spikes held in tight, overlapping bracts. This plant is often found on often on rocky or sandy soils. It is a traditional herb, is the whole plant is used for medicinal purposes. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. The pharmacological industries have produced a number of new antibiotics; however, resistance to these drugs by microorganisms has increased due to their genetic ability to transmit and acquire resistance to synthetic drugs.

The microorganisms which are used to check the anti-microbial activity are *Escherichia coli*, *Salmonella typhi*, *proteus vulgaris*, and *klebsiella*. All these microbes are effective pathogens and cause numerous diseases in human beings. As mentioned earlier, due to prolong use of synthetic antibiotics, these microbes have gained resistance because of which infection/diseases caused by these have become difficult to cure.

Materials and Methods

General

Chemicals: Nutrient Broth, Nutrient Agar, chloroform, methanol, ethyl acetate, n-hexane, etc., were obtained from Hi Media, Mumbai, India. The standard reference antibiotic, Streptomycin.

Microorganisms: The different strains of MRSA infected patients like that etc., *Klebsiella*, *Proteus vulgaris*, *salmonella typhii*, and *Escherichia coli* (ATCC 8739) at Global Hospital, Hyderabad. All the four

cultures were maintained in Muller Hinton broth as well as on agar slants at 4°C.

Preparation of solvent extracts from different parts of plant material

Fresh leaves and stem of *Elytraria acaulis* were procured from local gardens at Hyderabad, India. The collected material was washed thoroughly with distilled water and freeze dried. The dried samples were ground to powder and stored in tight container at -20°C until further use. Then the processed plant materials were subjected to fractionation using n-hexane, ethyl acetate, methanol and water in the order of increasing in polarity. The extraction procedure was carried out at room temperature for 72h using mass to volume ratio of 1:10. Plant samples were then subjected to sequential extraction using hexane (H), followed by Ethyl acetate (EA), Methanol (M), and finally with Ethanol (E). By using a Rotary Evaporator each solvent present in the extracted material was evaporated at its respective boiling point. The solvent free extracts were dissolved in DMSO and were screened for their activity against the selected multi drug resistant pathogens.

Standardized bacterial colony numbers

In order to ensure that the same number of bacteria was always used, a set of bacterial growth curves was established in the laboratory using the method described by Cappuccino & Sherman (2005) for each bacterial strain prior to the evaluation of antimicrobial activities. From these curves, we determined the optical density (OD) at 600 nm that corresponded to the desired number of colony forming units (CFU).

Preparation of test samples

One hundred milligrams (100mg) of plant extracts was dissolved in 1ml dimethyl

sulfoxide (DMSO) while 1mg of streptomycin was dissolved in 1ml sterile deionised water. Ten microliters of 100mg/ml plant extract (equivalent to 1mg dose), 10µl of 1mg/ml streptomycin (each equivalent to 10µg dose).

Total Phenolic Content Assay (Ali Ghasemzadeh *et al.*, 2010)

Chemicals: Galic acid (Standard solution-1mg/ml), Folin's reagent, Deionized water, and 20% NaCO₃.

Procedure: 1. Using Folin-Ciocalteu reagent with analytical grade gallic acid as standard 1 ml of extract or standard solutions (1mg/ml of range 100-1000 mg/ml) was added to 10 ml of deionized water to each sample. 2. To this add 0.1ml of F-C reagent to each sample. 3. After 5 minutes of incubation, 20% of sodium carbonate (2.0ml) was added to the each mixture. 4. After being kept in total darkness for 1hr, the absorbance was measured at 750nm using a spectrophotometer. 5. Amounts of total phenolic content were calculated using gallic acid calibration curve.

Total FlavinoidC ontent (Mccall *et al.*, 1999; Chen *et al.*, 2002)

Chemicals: Quercetin (Standard solution), 5% NaNO₂, 10% AlCl₃, 1M NaOH, and Distilled water.

Procedure: 1. Prepare standard solutions of concentration 1mg/ml (ranging from 100mg/ml – 1000mg/ml) and make up the volume to 5 ml using distilled water accordingly. 2. Add 0.3 ml of 5% NaNO₂ and leave it for 5min. Then add 0.3 ml of 10% AlCl₃. After 5 min add 2ml of 1M NaOH and make up the volume to 10ml with distilled water accordingly. 3. Read the absorbance at 510 nm using UV-

Spectrophotometer. 4. Plot a standard graph taking concentrations of the standard on x-axis against O.D on y-axis.

Antimicrobial Testing Methods

Well-in agar (wa): Well-in agar assay was carried out according to the method with some modifications. Bacteria colonies from plates were grown in NB until they reached their specific OD at 600 nm to give starting inoculums of 1×10^8 bacteria/ml. Nutrient agar plates were each divided into quadrants and labeled accordingly. One hundred micro liters of inoculums, equivalent to 10^7 CFU, was mixed with 6ml of molten soft NA (to ensure even distribution of bacteria) and poured immediately onto the base layer of NA. The plates were left to solidify for 10min. A sterilized 5mm borer was used to make holes in the centre of the divided areas. The bottom of the well was then sealed with molten soft agar. Ten micro liters of each of the test samples [plant extracts, streptomycin or penicillin (these were used as positive controls) & DMSO (used as negative control)] was then pipette into the holes.

Bacterial growth condition: Acquisition Plates with bacteria and test samples were incubated at 37 °C for 16 to 18 h after which the inhibition diameter (ID) was measured using a caliper. Each experiment was carried out on at least three separate occasions.

MIC and MBC: The minimum inhibitory concentration and maximum bactericidal concentration were determined by the broth dilution method with modification done. 4ml nutrient broth was dispensed in 6 test tubes. Into the first tube add 1ml of plant extract and then serially dilute. The stock concentration of plant extract was 5mg/ml and it was diluted up to 0.004 mg/ml. Then add 0.1ml of 24 hrs old bacterial culture in

all the tubes and incubate it for 24 hrs at 37° C. After incubation MIC & MBC were calculated by comparing it with blanks (positive control). The lowest concentration that did not show any visible growth after macroscopic evaluation was considered as MIC. After the determination of MIC, the tubes which did not show any visible growth was diluted 100 fold with drug free NB and incubated at 37°C for 48 hrs. The lowest concentration of the tube that did not show any visible growth was considered as the maximum bactericidal concentration (MBC). The assays were performed in triplicates.

Anti-Oxidant Activity

DPPH Assay (Patel Rajesh *et al.*, 2011; Aruna Prakash, 2000)

Chemicals: DPPH – 0.1mM, Ethanol, and Ascorbic acid – 98% (standard solution).

Procedure: 1. Prepare 0.1mM of DPPH solution dissolved in ethanol. 2. Simultaneously prepare standard solution of concentration 1mg/ml (100mg/ml–1000mg/ml). 3. Make up the volume to 1ml using ethanol for each standard solution. 4. Add 3ml of DPPH to each test tube and incubate for 15 min. 5. Read the OD at 517 nm. 6. Calculate the antioxidant activity using the following formula:

% scavenging = $\frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \times 100$.

Frap Assay (Selvakumar; Gohari *et al.*, 2011; Iris *et al.*, 1996)

Chemicals: FeSO₄.7H₂O (standard solution of 100–1000µg/ml), Frap Reagent – a) acetate buffer – 30 mM, Ph-3.6 [200 ml], TPTZ –10mM in HCl (40 mM) [20 ml],

FeCl₃–20mM [20ml], and distilled water [24 ml].

Procedure: 1. 200 MI of extracted sample was mixed with 3ml of FRAP reagent in a test tube. The same is repeated taking standard solution as ferrous sulphatehepta hydrated (FeSO₄.7H₂O). 2. Incubate at 37 degree celcius for 30 min in water bath. 3. Read the absorbance at 593 nm. 4. Plot a standard graph taking concentrations of the standard on x-axis against OD on y-axis.

Results and Discussion

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain

free radical scavengers like polyphenols, flavonoids and phenolic compounds.

Several reports have conclusively shown close relationship between total phenolic contents and antioxidative activity of the different plants. Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also needs their characterization. These results from various free radical-scavenging systems revealed that the *Elytraria acualis* had significant antioxidant activity and free radical-scavenging activity.

Table.1 Determination of total phenolic content for methanol, ethanol and ethyl acetate extracts of *Elytraria acualis*

Concentration (100-1000µg/ml)	Methanol	Ethanol	Ethyl acetate	Gallic Acid
200	0.021	0.023	0.021	0.78
400	0.031	0.048	0.027	.113
600	0.039	0.057	0.033	0.120
800	0.046	0.061	0.041	0.147
1000	0.058	0.075	0.049	0.198

Table.2 Determination of total flavonoid content for methanol, ethanol and ethyl acetate exacts.

Concentration (100-1000µg/ml)	Methanol	Ethanol	Ethyl acetate	quercitin
200	1.539	0.437	0.306	0.066
400	1.724	0.640	0.591	0.170
600	1.906	0.725	0.623	0.193
800	2.406	0.870	0.714	0.262
1000	2.661	0.906	0.808	0.324

Table.3 Antibacterial effect of *Elytraria acaulis* plant aerial extract against pathogenic bacterial strains

Name of the organisms	Diameter of zone inhibition (mm) (10mg/ml Concentration)				
	Ethyl acetate	Methanol	Ethanol	Hexane	Streptomycin
<i>Escherichia coli</i>	13 ±10	NI	12 ± 10	20 ±7	39
<i>Klebsiella pneumonia</i>	15 ± 9	11 ± 9	13 ± 10	16 ± 10	27
<i>Proteus vulgaris</i>	15 ± 10	15± 8	20 ± 9	14 ±11	35
<i>Salmonella typhi</i>	16 ±12	15 ±10	14 ± 4	14 ±12	16

Table.4 Determination of Minimum inhibitory and bactericidal concentrations of *Elytraria acaulis* plant aerial extract and Streptomycin effect on pathogenic bacterial strains

Plant extract 10mg/ml		<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Proteus vulgaris</i>	<i>Salmonella typhi</i>
Ethyl Acetate	MIC	0.312	0.312	0.312	0.312
	MBC	1.25	1.25	1.25	1.25
Methanol	MIC	-	0.312	0.312	0.078
	MBC	-	1.25	1.25	0.312
Ethanol	MIC	0.312	0.312	0.312	0.312
	MBC	1.25	1.25	1.25	1.25
Hexane	MIC	0.312	0.312	0.078	0.312
	MBC	1.25	1.25	0.312	1.25
Streptomycin	MIC	0.04	0.008	0.25	0.004
	MBC	0.08	0.032	0.05	0.032

Table.5 Determination of FRAP activity for methanol, ethanol and ethyl acetate extracts

Concentration (100-1000µg/ml)	Methanol	Ethanol	Ethyl acetate	FeSO ₄ .7H ₂ O
200	1.890	0.989	0.417	0.076
400	2.236	1.208	0.520	0.154
600	2.792	1.509	0.696	0.250
800	3.106	2.364	0.730	0.280
1000	3.341	2.967	0.837	0.352

Table.6 Standard table of DPPH scavenging assay for ascorbic acid

Concentration (100-1000µg/ml)	Methanol	Ethanol	Ethyl acetate	Ascorbic acid	Inhibition (%)
200	8.41	17.75	17.75	0.192	10.28
400	28.50	57.47	38.31	0.173	19.15
600	57.00	64.48	50.93	0.159	25.17
800	86.44	75.23	55.00	0.132	38.31
1000	95.00	81.77	78.50	0.116	45.79

Fig.1 Total phenolic content for methanol, ethanol and ethyl acetate extracts of *Elytraria acaulis*

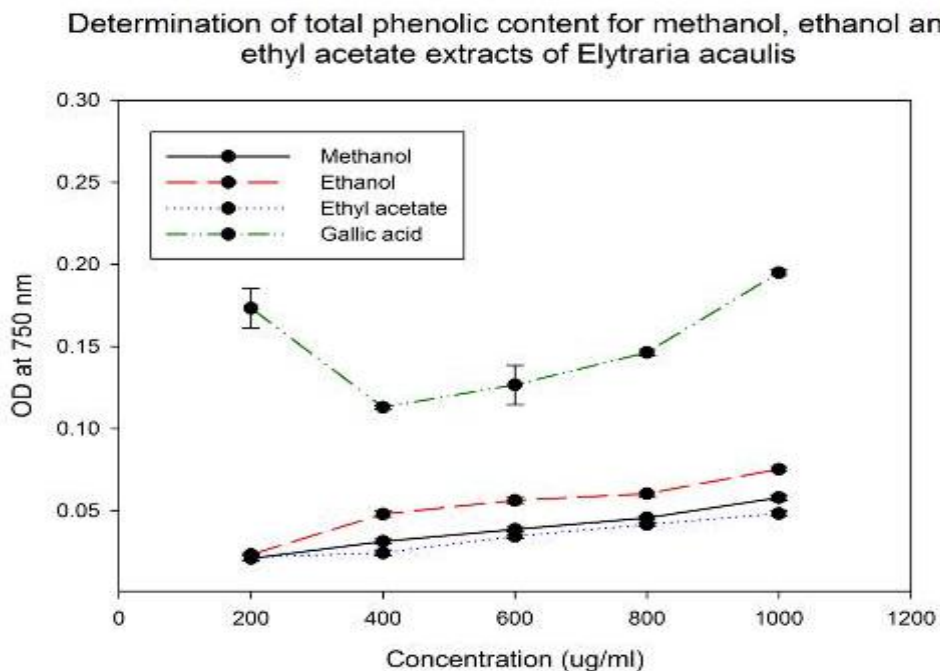


Fig.2 Total flavonoid content for methanol, ethanol and ethyl acetate extracts.

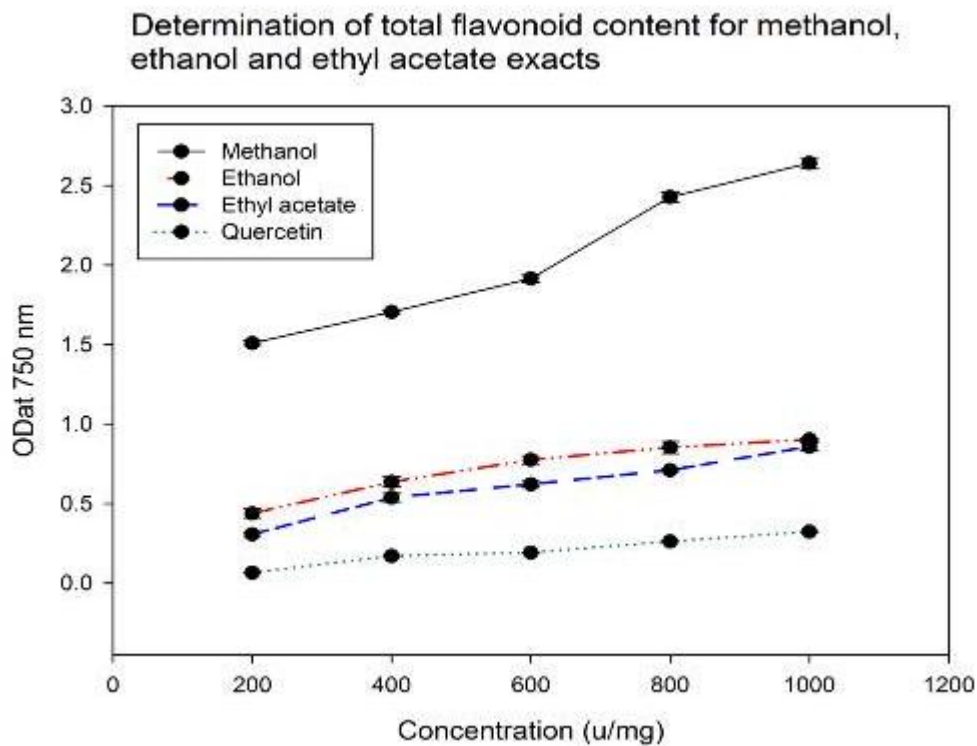


Fig.3 Antibacterial effect of *Elytraria acaulis* extract with different solvents in comparison with streptomycin (a positive control antibiotic).

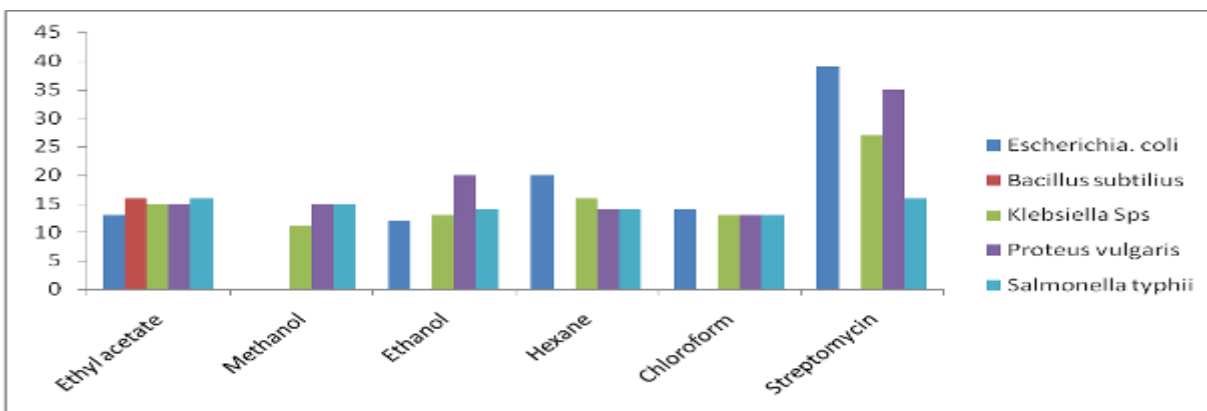


Fig.4. Antibacterial effect of different solvents and *Elytraria acaulis* extract of arial parts in comparison with streptomycin (a positive control antibiotic)

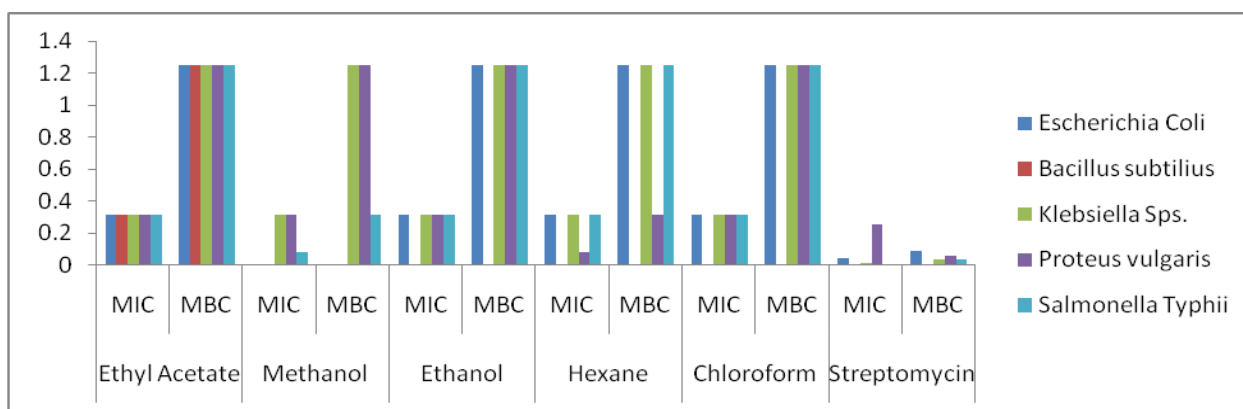


Fig.5 FRAP activity for methanol, ethanol and ethyl acetate extracts

Determination of FRAP activity for methanol, ethanol and ethyl acetate extracts

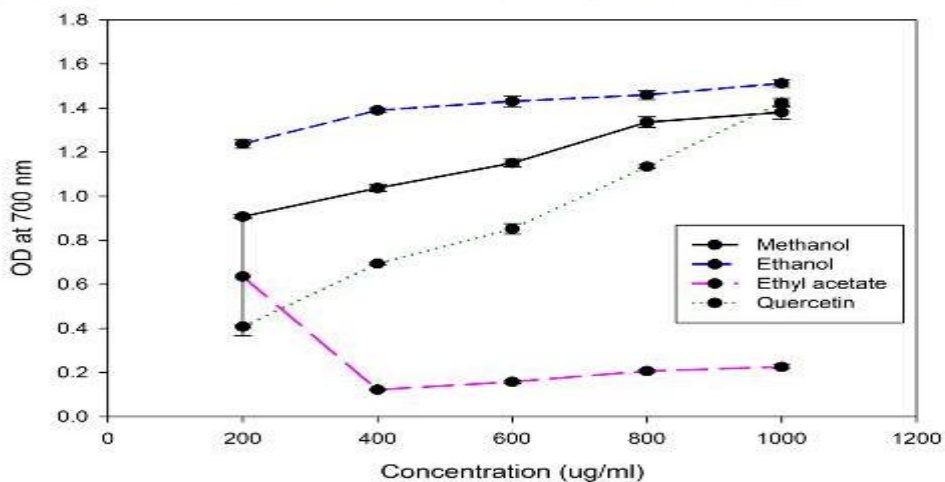
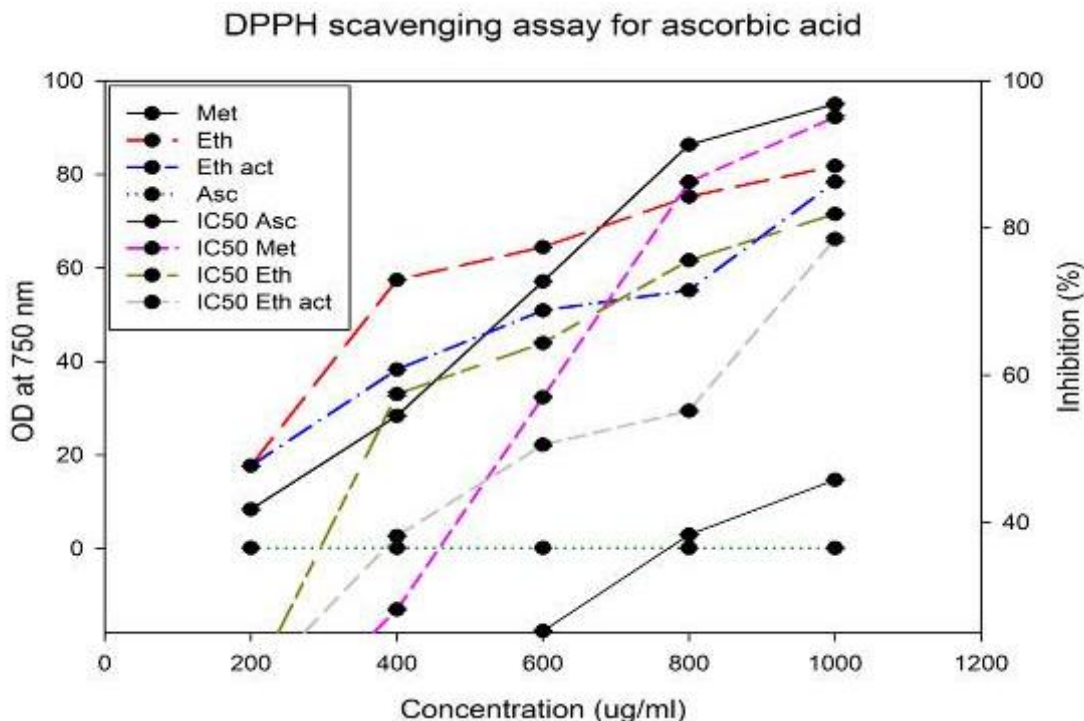


Fig.6 DPPH scavenging assay for ascorbic acid



In this case, for FRAP assay the maximum absorbance was shown at 200µg/ml itself i.e., 1.890, 0.989 and 0.417 OD at 593 nm for methanol, ethanol and ethyl acetate extracts respectively when compared to that of the ferrous sulphate hepta hydrate taken as a standard of concentration 1000 µg/ml showing 0.352 OD units.

Among the five extracts and standard tested for the in vitro antioxidant activity using the DPPH method and the crude methanolic, ethanolic and ethyl acetate extracts of *Elytraria acualis* showed antioxidant activity, with maximum of 57% (at 600 µg/ml); 57.47% (400 µg/ml) and 50.93% (600 µg/ml) respectively when compared to that of the standard (ascorbic acid) that gave 45.79% (at 1000 µg/ml) activity. The results indicate that the antioxidant activity of the crude extract of *Elytraria acualis* is higher than that of ascorbic acid. The results and observations are presented in tables 1, 2, 3, 4, 5, and 6 and figs.1, 2, 3, 4, 5 and 6.

In conclusion, in view of the continuous rise of antibiotic resistant bacterial strains, the present study was undertaken to analyze antibacterial activity and Anti oxidants of the aerial extracts of *Elytraria acaulis* and the findings clearly indicate that *Elytraria acaulis* has profound antimicrobial activity against the pathogenic strains. These results are supportive to the traditional use of *Elytraria acaulis* against multidrug resistant parasites of pathogenic strains. Many substances consumed by man either through foods, drinks and inhalation, even effect of exogenous material (ultraviolet radiation) on the skin may be destructive to the health and thus, shortening the life span of man. Free radicals when generated in the body system of man causes damage to which eventually leads to death at shorter time. Continuous usage of the same vegetable oil which is not even properly stored and re-using the already fried oil (rancid) lead to generation of free radicals through lipid peroxidation. Further research is being carried out in our

laboratory for the determination of mechanism of action, toxicological studies and formulation of the compound.

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How to cite this article:

Suresh Babu, B., M. Lakshmi Narasu, B. Venkanna, Kodaparthi Ashwitha, CH. Kalyani and Narmada, CH. 2016. Evaluation of Antimicrobial and Antioxidative Activities of the Aerial Plant Extracts of *Elytraria acaulis*. *Int.J.Curr.Microbiol.App.Sci.* 5(8): 20-29.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.508.003>