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Evaluation of Anti-oxidant Activity of *Elytraria acaulis* Aerial Extracts

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ABSTRACT
Elytraria acaulis, a stem less perennial herb of *Acantheceae* family has many medicinal and therapeutic properties. Anti oxidative activity of the aerial parts of this *Elytraria acaulis* were assessed in the present study. The aerial parts of the plant (Stem & Leaves) were extracted in different organic solvents such as n-Hexane, Ethanol, Methanol, Ethyl Acetate and Chloroform. Initially, Total Phenolic & Total Flavonoids content in different solvent plant extracts were estimated. The free radical scavenging and antioxidant activity of the *Elytraria acaulis* aerial extracts in different organic solvents were also assayed by DPPH assay, FRAP assay. The aerial extracts of *Elytraria acaulis* have shown significant anti oxidant activity. Hence, further studies on this plant will enable elucidation of its therapeutic properties and medicinal applications.
Keywords: Aerial parts, phytochemical analysis, Antioxidant assays, *Elytraria acaulis.*

I. INTRODUCTION

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 *acantheceae* family of plant kingdom, which is a small shrub that grows in shady dry places. 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. Practitioners of traditional medicine have described therapeutic efficacy of many indigenous plants for several disorders. 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 present study was designed to analyze the phytochemicals present in the plant extract as well its free radical scavenging property (invitro anti-oxidant assay).

II. MATERIALS & METHODS

Collection and preparation of Plant Material: Fresh leaves and stem of *Elytraria acaulis* were procured from Seshachalam forest, Tirupathi, India. The dried samples were cut into small pieces and ground into fine powder using a dry grinder. The ground samples were sieved to get uniform particle size, then kept in an airtight container and stored in a freezer (-20°C)until further analysis.

Extraction of sample: Each ground sample was weighed and transferred into a beaker. Solvents like n-Hexane, Ethyl acetate, Ethanol, Methanol and Chloroform in the increasing order of polarity were added in the ratio of 1:10 and stirred with the aid of a magnetic stirrer. The extraction mixture was left to sediment for at least 72 h before the extract was separated from the residue by filtration through Whatman No. 1 filter paper. The solvent plant

extracts were lyophilized using a freeze dryer and the residual solvent of each extract was removed under reduced pressure using a rotary evaporator. Extracts were produced in duplicates and used to assay the antioxidant activity.

2.1 Total Phenolic content of *Elytraria acaulis* extracts

The total phenolic contents of *Elytraria acaulis* leaves & stem extracts were determined according to the method described by Malik and Singh. Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folinciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in FolinCiocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample (Malik et.al. 1980).

2.2 Total Flavonoid content of *Elytraria acaulis* extracts

The aluminum chloride method was used for the determination of the total flavonoid content of the sample extracts[5]. Aliquots of extract solutions were taken and made up the volume 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample (Mervat et. Al., 2009).

2.3. DPPH Assay of *Elytraria acaulis* extracts

Effect of *Elytraria acaulis* solvent extracts on DPPH radical was measured based on the method modified by **(Patel Rajesh *et al.*, 2011; ArunaPrakash, 2000)**. An aliquot of 200 µl of extract of various concentrations andascorbic acid as standard were mixed with 800 µl of 100 mMTris - HCl buffer (pH 7.4). The mixture was then added to 1 ml of 500 µM DPPH. This was made up to a DPPH final concentration of 250 µM. The mixture was shaken vigorously and left to stand at room temperature for 20 min in a dark room. Absorbance at 517 nm was measured using a UV-Vis spectrophotometer until the reading reached a plateau. The capability of seaweeds extracts to scavenge the DPPH radical was calculated by using the following equation:

% scavenging = [Absorbance of control-Absorbance of test sample/Absorbance of control] X 100.

IC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of seaweed extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged.

2.4. FRAP Assay of *Elytraria acaulis* extracts

Ferric ions reducing power was measured according to the method of Oyaizu with a slightest modification (Oyaizu 1986). Hydroalcoholic extract of *Kalanchoepinnata*in different concentrations ranging from 100 µl to 500 µl were mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 ml 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5 ml 0.1%, w/v ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard. All assays were run in triplicate way and averaged.

Statistical analysis

Experimental results are expressed as means ± standard deviation (SD). All measurements were replicated three times. IC50 values were also calculated by linear regression analysis. Experiments results were further analyzed for Pearson correlation coefficient(r) between total phenolic, flavonoid and DPPH radical scavenging assay using the Microsoft Excel 2007 software and two-way analysis of variance (ANOVA) was applied to investigate the differences among means by using the Microsoft Excel 2007. The values were considered to be significantly different at $P < 0.05$.

III. 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 containfree 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.

3.1 Total Phenolic content of *Elytraria acaulis*

Phenolic compounds are a class of antioxidant agents, which act as free radical terminators. Total phenols were measured by FolinCiocalteu reagent in terms of Gallic acid equivalent. According to our study, the high contents of this Phytochemical in aqueous extract of *Elytraria acaulis* can explain its high radical scavenging activity (Table 1). Figure 1 shows the activity for *Elytraria acaulis* plant extract in all solvents was found to be lower than the standard (0.078) at 200 µg/ml concentration. It was found that the activity of *Elytraria acaulis* plant extract in ethanol was found to be higher (0.075) followed by methanol, ethyl acetate, chloroform and hexane solvents respectively at 1000 µg/ml concentration.

3.2 Total Flavonoid content of *Elytraria acaulis*

According to our study, the high contents of this Phytochemical in aqueous extract of *Elytraria acaulis* can explain its high radical scavenging activity (Table 2). Figure 2 shows the activity for *Elytraria acaulis* plant extract in all solvents was found to be lower than the standard (0.866) at 200 µg/ml concentration. It was found that the TFC of *Elytraria acaulis* plant extract in methanol was found to be higher (0.506) followed by ethanol, ethyl acetate, hexane and chloroform solvents respectively at 1000 µg/ml concentration but less when compares to that of standard Quercetin.

3.3 DPPH Assay of *Elytraria acaulis* extracts

DPPH is a stable free radical at normal temperature. It shows specific absorbance at 517nm due to color of methanolic solution of DPPH. Body also contains man free radicals, which assumed same as DPPH. Decrease in absorbance of mixture indicates the radical scavenging activity (Table no: 3). Inhibition activity for *Elytraria acaulis* plant extract in Methanol solvent was found to be higher (82.0) when compared with the other solvents at 1000 µg/ml concentration and it is lower than standard Ascorbic acid (86.0). Inhibitory activity was found to be higher for Methanol followed by Hexane, Ethanol and Chloroform extracts (Fig. 3).

3.4 FRAP Assay of *Elytraria acaulis* extracts

FRAP assay is based on a redox-linked reaction, whereby antioxidants present in plant extracts act as reductants while ferric ions in reagents serve as oxidants. Reduction of ferric-tripyridyltriazine to ferrous complex forms an intense blue color with maximum absorption at 593 nm. The ferric reducing ability of extracts of *Elytraria acaulis*is shown in Table 6. Figure 6 showed that Methanol, Ethanol, Ethyl acetate, Chloroform and Hexane extract reduced ferric ions efficiently and had reducing activity in the range of 0.127+0.02– 0.153+0.01. Hexane and Chloroform extracts showed least reducing activity with 0.119+0.01 and 0.127+0.02 respectively. Methanol extract of *Elytraria acaulis* displayed significant reducing ability (0.153+0.01). Reducing activity of all the extracts was lesser than the standard quercetin (0.236+0.02).

IV. CONCLUSION

In conclusion, the present study was undertaken to analyze Antioxidant activity of the aerial extracts of *Elytraria acaulis* and the findings clearly indicated that *Elytraria acaulis* has profound antioxidant activity. These results are supportive to the traditional use of *Elytraria acaulis* as antioxidative agent. The selected plant has varieties of phytochemicals such as phenolics, flavonoids and tannins and possesses various activities such as radical scavenging, antioxidant and reducing activities. Therefore, this plant species may be attempted to derive the drugs of antioxidant properties proving the efficacy of this research work whichis highly valuable for identification of newer antioxidant principles. The scientific community has begun to interest in the identification, recovery and enhanced performance of natural antioxidant principles from plant sources. The purpose of this present study is also guide to the invention of new antioxidant sources from *Elytraria acaulis*.

Conflict of Interest

We declare that we have no conflict of interest.

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S.No.	Concentration (□g/ml)	Methanol	Ethanol	Ethyl Acetate	Hexane	Chloroform
1.	200	271+0.35	227+0.32	258+0.48	174.0+0.24	194.0+0.24
2.	400	421+0.35	376+0.43	351+0.25	242+0.35	242+0.35
3.	600	532+0.24	489+0.35	482+0.56	324+0.25	314+0.25
4.	800	642+0.45	557+0.21	576+0.48	452+0.57	478+0.57
5.	1000	752+0.63	632+1.32	672+0.56	526+0.26	575+0.26

*Each Value represents mean value+ standard deviation of three replicates

Table 1: Total Phenolic Content (Expressed as mg Gallic Acid/g dry weight) of *Elytraria acaulis*

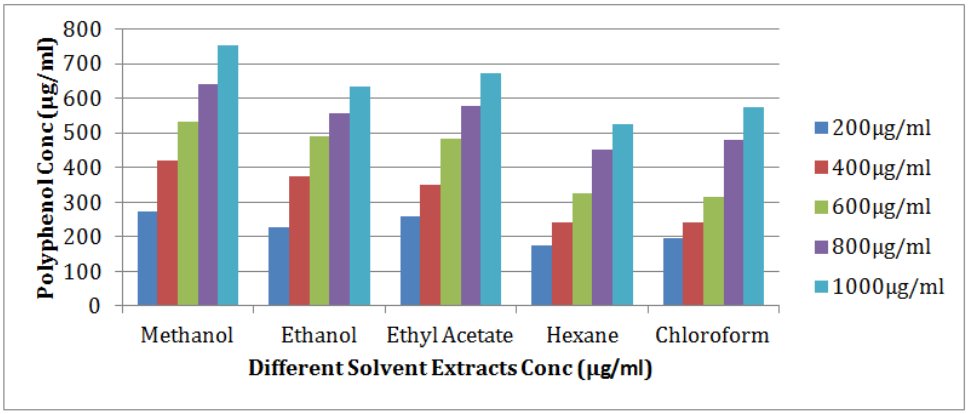

Fig 1: Total Phenolic Content of *Elytraria acaulis* extracts

Concentration (□g/ml)	Methanol	Ethanol	Ethyl Acetate	Hexane	Chloroform
200	32.02+1.25	54.5+0.32	83.17+1.35	74.0+0.24	94.0+0.24
400	74.45+1.35	108.3+1.23	173.4+1.56	142.2+0.35	162.3+0.35
600	103.67+1.3	284.4+1.35	216.82+1.3	224.3+0.25	214.4+0.25
800	142.34+0.45	357.1+1.21	376.32+1.48	352.2+0.57	378.5+0.57
1000	178.34+0.63	432.2+1.32	421.23+1.56	426.4+0.26	475.6+0.26

Table 2: Total Flavanoid content (Expressed as mg quercetine/g dry weight) of *Elytraria acaulis*

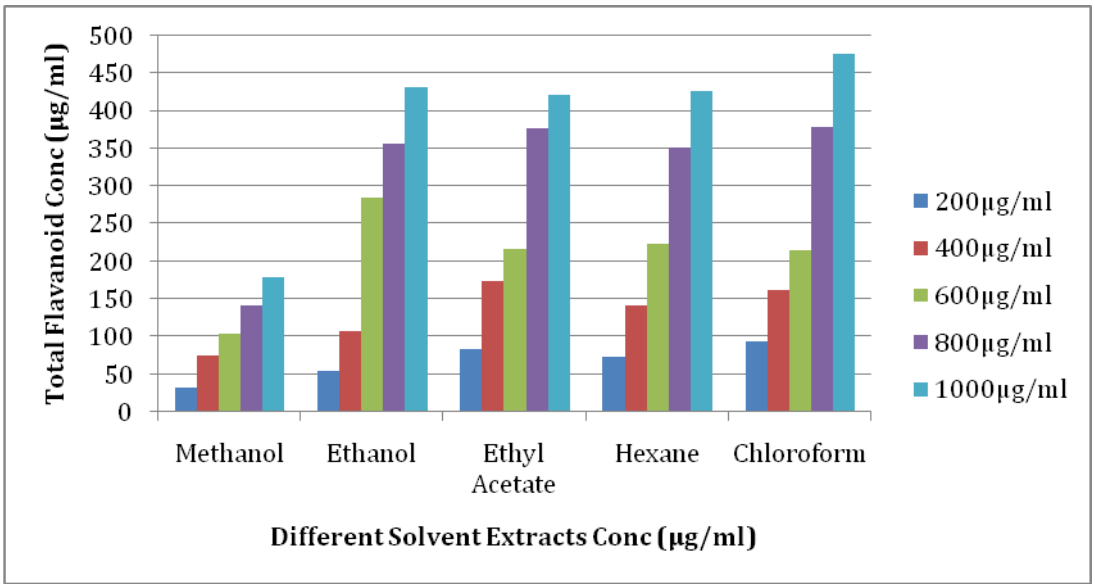

Fig 2: Total Flavonoid Content of *Elytraria acaulis* extracts

S.No.	Concentration	Ascorbic Acid	Methanol	Ethanol	Chloroform	Hexane
1.	50	50.42+0.50	34+0.60	32+0.59	33+0.58	30+0.62
2.	100	56.64+0.52	46+0.58	45+0.50	43+0.53	42+0.54
3.	150	63.52+0.43	62+0.40	59+0.43	60+0.48	58+0.42
4.	200	70.42+0.35	68+0.48	65+0.39	66+0.42	67+0.39
5.	250	78.62+0.28	73+0.34	70+0.30	72+0.35	69+0.30
6.	300	86.0+0.22	82.0+0.20	79+0.24	76+0.23	81+0.22
IC50		52.3	118.58	129.27	127.67	135.38

*All data were average (+SD) of three replicates

Table 3: DPPH Assay of *Elytraria acaulis* extracts

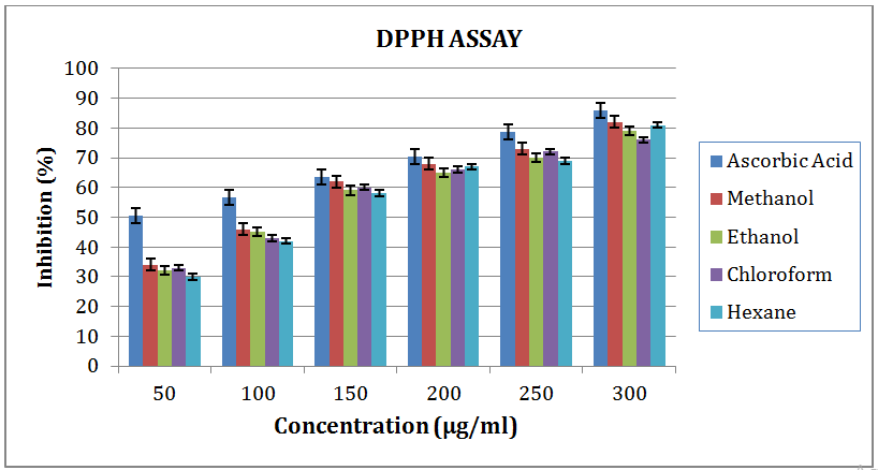

Fig 3: DPPH Assay of *Elytraria acaulis* extracts

S.No.	Elytraria Acaulis	
1.	Quercetin	0.236+0.02
2.	Methanol	0.153+0.01
3.	Ethanol	0.142+0.02
4.	Ethyl Acetate	0.139+ 0.01
5.	Hexane	0.119+0.01
6.	Chloroform	0.127+0.02

*Each value represents mean values+standard deviation of three replicates.

Table 6: Ferric Reducing Ability – FRAP (Expressed as mM $FeSO_4$/g dry weight) of Elytraria Acaulis