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IN-VITRO ANTI-OXIDANT ACTIVITY OF *ELYTRARIA ACAULIS*

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ABSTRACT: The overproduction of oxidants in the body leading to different side effects and chronic diseases, including oxidative stress. The oxidative stress is the overproduction of the oxidant's condition in the body, which leads to metabolic imbalance and leading to diseases. It is one of the main diseases around the world, affecting the human lifestyle, either psychologically and physically. The natural products have been using as medicines and food supplements because of their nutritional values and less side effects. The current study was aimed to evaluate the free radical scavenging activity of *Elytraria acaulis* root extracts on different free radicals. The anti-oxidant activity was studied against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide, and hydroxyl free radicals using standard methods. The *Elytraria acaulis* root part extracts showed concentration-dependent antioxidant activity on free radicals. The extracts showed a moderate effect on free radicals compared to the standard drug ascorbic acid, and hydro-alcoholic extract possesses more activity than the other two extracts. The end results of the current study clearly state that *Elytraria acaulis* root possess free radical scavenging activity and also confirms its' traditional value in traditional medicine.

INTRODUCTION: The medicinal plants (MPs) are playing a vital role in maintenance of human health since ancient time^{1, 2, 3}. The main traditional medicines (TMs) using medicinal plants in the treatment of different ailments around the world are Ayurveda, Homeopathy, Tibetan medicine, Unani system, Chinese traditional medicine *etc.*³ The developments in science and technology enhanced the possibilities of treatment to different diseases within less time and affected the risks of infectious diseases^{4, 5}. But, at the same time, the use of modern medicine increased the risks of side effects and emergence of new diseases around the world⁶.

As above said, there were lots of MPs are available around the world and many of their chemical and biological activities are scientifically not explored. So, the current study was aimed to evaluate antioxidant activity of *Elytraria acaulis* root extracts. Oxidants are most unstable molecules and reactive. Oxidants are produced in body metabolisms and in the environment, because of their nature they can react with the other molecules and destabilize them⁷.

That destabilization of other molecules include proteins, lipids, deoxyribonucleic acids or ribonucleic acids, *etc.*, cause the cellular dysfunction. Antioxidants are molecules that are reacts with oxidants and stabilize them without reacting with bimolecular components⁸. The antioxidants are also produced in the body for like oxidants to stabilize them if the production of them is an imbalance, the body will be under oxidative stress⁹.

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The current day lifestyle and overconsumption of food are altering these levels and leading to different side effects and chronic diseases¹⁰. Thus, people are looking to take food supplements to equalize or reduce overproduced oxidants. Medicinal plant products are major nutritional supplements that have been using for healthier life¹¹. The knowledge about chemical constituents and biological activities of medicinal plants will help to enhance their usage around the world because of their less side effects and high nutritional values¹². *Elytraria acaulis* is one such medicinal plant that has been using in traditional medicine and less known about its' biological activities scientifically.

Elytraria acaulis is a perennial herb that belongs to the family Acanthaceae. *Elytraria acaulis* is growing widely in woodland, sandy land regions around the world. *E. acaulis* has been using in traditional medicine for different ailments^{13, 14, 15}. The root part has been using as paste for treatment of leucorrhoea, snake bites, abscess of mammary glands, throat compliments like tonsillitis. There were few reported biological activities but very were reported on root part¹⁶. So, the current work aimed to evaluate the antioxidant activity of *E. acaulis* root extracts.

MATERIALS AND METHODS:

Drugs and Chemicals: The chemicals and solvents used in the current study were analytical grades. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), linolenic acid, ammonium thiocyanate, ferrous chloride, butylated hydroxytoluene, riboflavin were purchased from sigma chemicals, USA. Nitroblue tetrazolium, was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai.

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, Government Degree College, Yeleswaram, E. Godavari, Andhra Pradesh, India. The roots were separated from the 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 the preparation of extracts successively with hexane, ethyl acetate, and hydro-alcoholic [70% Ethanol (hyd-alc)] using

maceration. The prepared extracts were stored in a desiccator for further use.

In-vitro Anti-oxidant Activity: Anti-oxidant activity of *Elytraria acaulis* root extracts was assessed using free radicals (superoxide, hydroxyl, and DPPH). Dimethyl sulphoxide (DMSO) used as vehicle¹⁷. The percentage of inhibition and IC₅₀ values were calculated.

Superoxide Radical Scavenging Activity: Superoxide scavenging activity of the selected plant extracts were evaluated as per standard methods. It is by absorption of light at 560 nm induction of superoxide free radical generation by riboflavin and corresponding reduction by nitroblue tetrazolium¹⁸.

Hydroxyl Radical Scavenging Activity: The scavenging activity of selected plant extracts on hydroxyl radical was measured as per the established method.

It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe⁺²/EDTA/H₂O₂ system¹⁹.

DPPH Radical Scavenging Activity: The DPPH radical scavenging activity was measured as per methods. This method is based on the measure of color absorbance of alcoholic DPPH solution (Blue color) after the addition of the antioxidant solution (Extract / Compound). If antioxidants present in the test compound blue color yellow color due to DPPH^{20, 21}.

Calculation of Percentage Inhibition: The percentage inhibition of superoxide production by the extract was calculated using the formula:

$$\text{Inhibitory ratio} = (A_0 - A_1) \times 100 / A_0$$

A₀: Absorbance of control; A₁: Absorbance of plant extract or/and Ascorbic acid.

IC₅₀ Calculation form Percentage Inhibition: The optical density obtained with each concentration of the extract/ascorbic acid was plotted, taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract / ascorbic acid.

RESULTS: The tested extracts of *Elytraria acaulis* root part showed concentration-dependent antioxidant activity on tested free radicals. The extracts showed a moderate effect on free radicals compared to the standard drug ascorbic acid **Table 1** and **Fig. 4**. The hexane extract showed low antioxidant activity compared to other two extracts. The IC₅₀ values of hexane extract on different free radicals at tested concentration was also not detectable. It has showed more activity (46.67 ± 2.60) on DPPH free radical than others **Fig. 1**.

The ethyl acetate extract showed moderate activity on tested free radicals. It has almost same percentage of inhibition on superoxide and hydroxyl free radicals **Fig. 2** and **Fig. 3**, but showed more on DPPH free radical (74.67 ± 2.19) **Fig. 1**. The hydro-alcoholic extract showed more percentage of free radicals inhibition compared to other two extracts and it has almost equal inhibition of tested free radicals but little more on DPPH free radical **Table 1**, **Fig. 1** and **Fig. 4**.

TABLE 1: IC₅₀ VALUES OF ELYTRARIA ACAULIS EXTRACTS ON DIFFERENT FREE RADICALS

Name of the plant/ compound	Name of the extract	IC ₅₀ value in μg on different free radicals		
		DPPH	Hydroxyl	Superoxide
<i>Elytraria acaulis</i>	Hexane	ND	ND	ND
	Ethyl Acetate	155	251	257
	Hydro-Alcoholic	139	140	144
Ascorbic acid		95	103	72

ND: Not detected

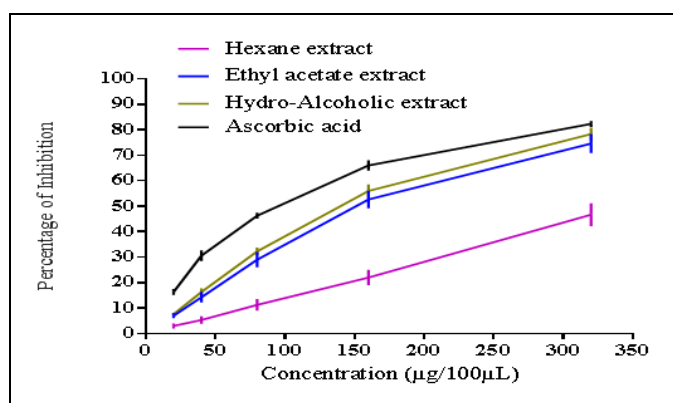


FIG. 1: DPPH FREE RADICAL SCAVENGING ACTIVITY OF ELYTRARIA ACAULIS EXTRACTS

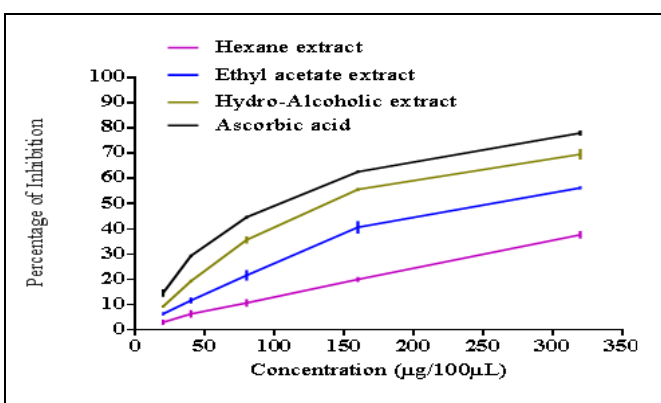


FIG. 2: HYDROXYL FREE RADICAL SCAVENGING ACTIVITY OF ELYTRARIA ACAULIS EXTRACTS

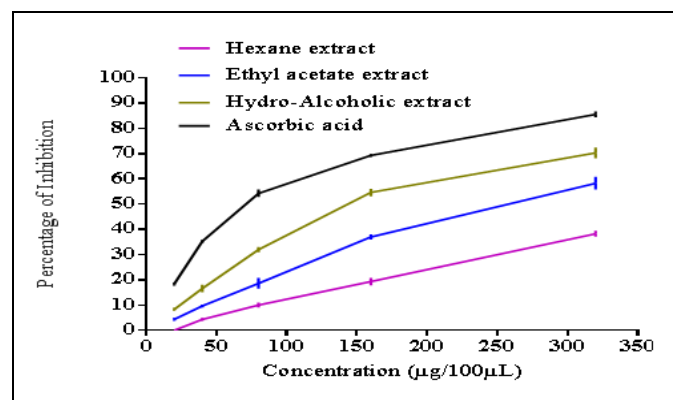


FIG. 3: SUPEROXIDE FREE RADICAL SCAVENGING ACTIVITY OF ELYTRARIA ACAULIS EXTRACTS

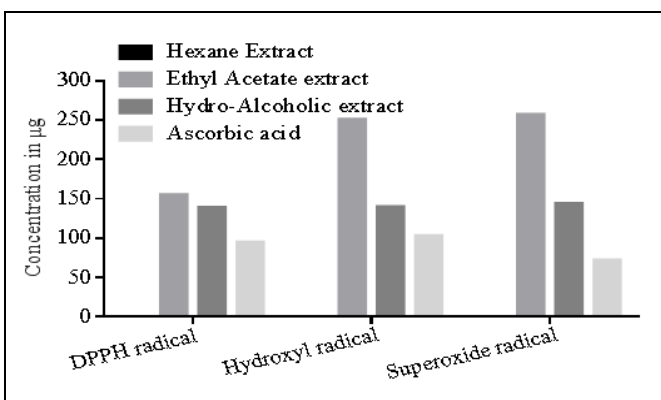


FIG. 4: IC₅₀ VALUES OF ELYTRARIA ACAULIS EXTRACTS ON DIFFERENT FREE RADICALS

DISCUSSION AND CONCLUSION: Nowadays, oxidative stress (OS) became one of the major chronic diseases around the world. The main cause OS is an overproduction of reactive oxygen species (ROS) against antioxidants in the body^{22, 23}. These

ROS play an important role in body metabolisms and signal transduction at moderate levels²⁴. As above said, the over oxidants playing a vital role in the chronic disease development and their side effects, and they cause the oxidative damage

macromolecules (lipids, proteins, nucleotides, nucleosides, etc.,)^{19, 22, 25}. In the last two decades, several research studies reporting that there is a direct connection between them and acute stress (psychological/physiological) can lead to OS^{26, 27, 28}. The main ROS is superoxide free radical and is a precursor to others such as hydroxyl, hydrogen peroxide, etc. The produced ROSs enhances the endogenous reactions and damages the cellular functions and finally leads to cell death^{29, 30}. So, there is a need to prevent the OS from different ROS and their side effects. Natural products have been using in traditional medicines for a long time in the treatment of different diseases and are using nutritional supplements^{31, 32}. The researchers from the last two decades are working on unexplored natural products in the identification of new biologically active compounds to treat different diseases, including OS³³. The natural products contain different chemical constituents like polyphenols, alkaloids, tannins, steroids, glycosides etc.. All these compounds work either individually or synergistically on diseases^{34, 35, 36}. There were different reports on these compounds against OS. In this point of view, the current study was carried to assess the anti-oxidant activity of unexplored traditional medicinal plants, i.e., *Elytraria acaulis*.

The root parts of *E. acaulis* have been using in the treatment of leucorrhoea, snake bites, abscess of mammary glands, throat compliments. In our previous studies, we found, the extracts of *E. acaulis* possess antibacterial activity on different pathogenic bacteria. The present study also confirms the anti-oxidant activity of *E. acaulis*. In both studies, hydroalcoholic extract posses more activity compared to the other two extracts. Further studies are under progress, such as the identification of chemical components, isolation of pure compounds, and evaluation of different biological activities like hepatoprotective, anti-inflammatory activities.

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CONFLICTS OF INTEREST: None to declare.

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