WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 1059-1066. Research Article ISSN 2277– 7105

INVITRO ANTIOXIDANT AND ANTIBACTERIAL STUDIES ON *ENICOSTEMMA LITTORALE* FLOWER EXTRACT

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Article Received on
26 Aug 2015,

Revised on 15 Sep 2015,
Accepted on 05 Oct 2015

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ABSTRACT

Enicostemma littorale is a glabrous perennial herb belongs to the family Gentianaceae. It is traditionally used as antidiabetic, urinary astringent, antiperiodic, anti-inflammatory, laxative and carminative agent. It possesses antioxidant, antimicrobial, antiedematologinic, antitumour activities. The objective of the present study was to evaluate antioxidant and antibacterial activity from the flower extract of *E. littorale*. In vitro antioxidant activity was evaluated from aqueous, methanol, ethanol, petroleum ether and chloroform flower extracts by studying 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity using the standard procedure. The antibacterial activity was evaluated against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Pseudomonas aeruginosa* of different concentrations (10mg/ml, 20 mg/ml & 30 mg/ml) of ethanolic flower extract of *Enicostemma littorale.* The ethanolic flower extract of *E.littorale* showed significant antioxidant activity and petroleum ether extract showed least radical scavenging activity. The bacterial strains showed maximum zone of inhibition at 30 mg/ml of ethanolic flower extract. It can be concluded that *E. littorale* flower extract can be used as a potent source of natural antioxidant and thus could prevent many free radical mediated diseases.

KEYWORDS: *Enicostemma littorale*, flower extract, antioxidant activity, antibacterial activity.

INTRODUCTION

Medicinal plants are the backbone of traditional medicine, covering more than 3.3 billion people in the less developed countries on a regular basis.[1] The World Health Organization

estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs.[2] Approximately 72,000 plant species were estimated for having medicinal properties, of which, India recognizes 3,000 plant species for having medicinal values. Plants are generally known for its acceptability by human and animal system and their therapeutic benefits are generally due to the active constituents present in it.[3]

Phytochemicals are the major constituents and their screening from various plants has been reported by many workers[4,5] revealing the presence of numerous chemicals, including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites[6] which are antioxidants and free radical scavengers.[7,8,9] Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body can cause oxidative damage of amino acids, lipids, proteins and DNA.[10,11] It has been established that oxidative stress is the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others.[12 ,13]

E. littorale (Family -Gentianaceae) is a perennial, tropical traditional medicinal herb with sessile lanceolate leaves, flowers arranged in clusters, fruit in a capsule. It is a good source of iron, potassium, sodium, calcium, magnesium, silica, chloride, sulphate, phosphate, vitamins B and C.[14] The plant has been used in the treatment of diabetes mellitus,[15] skin diseases, malaria, abdominal ulcers, arthritis, as anti–inflammatory,[16] antimalarial,[17] antimicrobial,[18] antipyretic,[19] antirheumatic,[20] antipsychotic, antihelmintic,[21] diuretic and hepatoprotective.[22] It has the property to increase the HDL levels and decreases the serum cholesterol, triglycerides, LDL, VLDL and LDL/HDL ratio.[23] Hence, the present study was performed to investigate the potential antioxidant and anti-bacterial activity, from the flower extract of *E. littorale*.

MATERIALS AND METHODS

Collection of *E. littorale*

Healthy plants of *E. littorale* were collected from Ambur, Tamil Nadu, India. The plant materials were washed under tap water and flowers were separated. The separated parts were cut into small pieces and then used for experimental studies.

Preparation of Flower extract

The *E. littorale* flowers were washed and dried in shade for 7 days. The air dried flowers were powdered using mortar and pestle which in turn was extracted using different solvent systems namely aqueous, methanol, ethanol, petroleum ether and chloroform. The extracts were then filtered through Whatmann No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10ºC and used for further studies.

Qualitative analysis of antioxidant activity of *E. littorale*

The antioxidant activity of flower extracts of *E. littorale* was determined by standard method.[24,25] 50 µl of flower extracts of *E. littorale* were taken in the microtiter plate. 100 µl of 0.1% methanolic 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered to be strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of *E. littorale*

The antioxidant activities were determined using DPPH, (Sigma- Aldrich) as a free radical. Flower extract of 100 µl were mixed with 2.7 ml of methanol and then 200 µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank containing the same amount of methanol and DPPH solution was prepared and measured as a control.[26] Subsequently, at every 5 minutes interval, the absorption maxima of the solution known synthetic standard of (0.16%) of butylated hydroxy toluene (BHT). The experiment was carried out in triplicates.

The capacity of scavenging free radicals was calculated as scavenging activity (%) = Absorbance in control - Absorbance in sample / Absorbance in control × 100.

Bacterial Strains

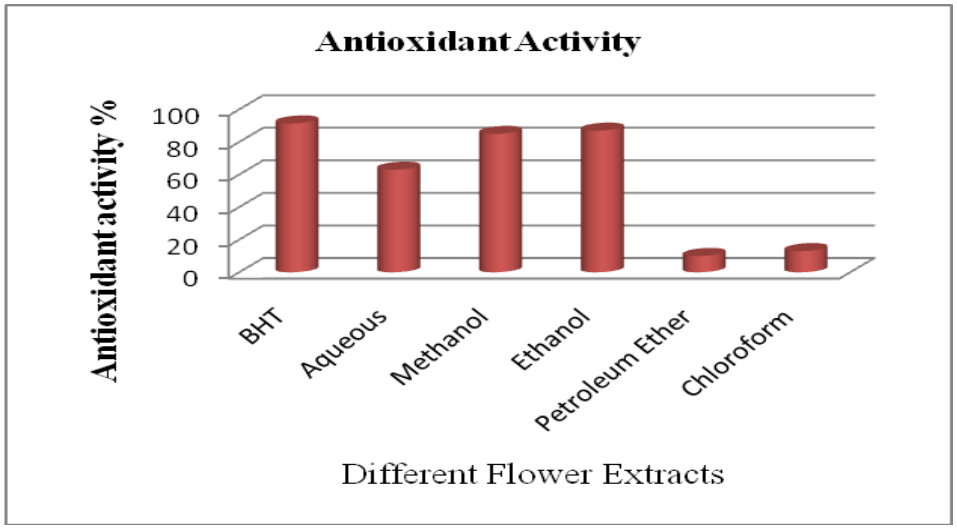
The four bacterial species used in this study were, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*. They were identified according to standard phenotype tests. The bacterial cultures were grown in Muller Hinton Agar and Muller Hinton Broth (Himedia).[27]

Determination of antibacterial activity

Antibacterial activity was measured using the standard method of diffusion disc plates on agar.[28] 0.1ml of each culture of bacteria was spread on agar plate surfaces. For antimicrobial assay, all bacterial strains were grown in Muller Hinton Broth Medium (Himedia) for 24h at 37°C and plated on Muller Hinton Agar (Himedia) for agar diffusion experiments. Paper disc (6mm in diameter) were placed on the agar medium to load 20µl of different concentration (10 -30mg /ml) of flower extracts and were tested. Inhibition diameters were measured after incubation for 24hrs at 37°C. Blanks were also tested for antibacterial activity without the flower extract.

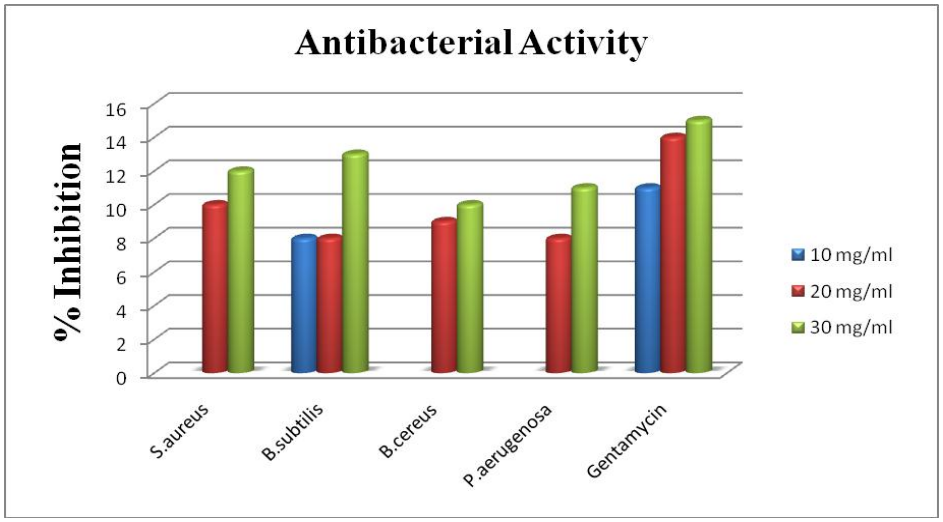
RESULTS

E. littorale flower extracts were analyzed for the presence of antioxidants."Graph 1" shows the qualitative antioxidant analysis in the flower extracts of *E. littorale*. The results revealed strong positive response from ethanolic flower extract.



"Graph 1" - Antioxidant activity of *E.littorale* flower extract.

Antibacterial activity of ethanolic *E. littorale* flower extract was tested against four bacterial strains and the results are depicted in "Graph 2". The results revealed that 30 mg/ml of flower extract showed maximum zone of inhibition against all the bacterial strains while only *B. subtilis* showed its activity when 10 mg/ml of flower extract was used.



"Graph 2"- Antibacterial activity of *E.littorale* flower extract.

DISCUSSION

As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they are considered as potential source of antioxidant compounds.[29] The antioxidative system protects the organisms from ROS induced oxidative damage. They are very good scavengers for the reactive oxygen species that prevents the damage in many cellular components such as; DNA, proteins and lipids.[30] The use of synthetic antioxidants has limitations and hence natural antioxidants have gained importance.

Many studies indicate a linear relationship between total phenolics and antioxidant activity.[31,32] The present study reveals that the flower extract of *E. littorale* possess potent antioxidant activity against ethanolic extract which is supported by the work of Abirami *et al.*, where the antioxidant activity from four parts of *Enicostemma littorale* (leaves, stems, roots and flowers) were evaluated and the flower extract showed maximum antioxidant activity.

The rapid increase in multidrug resistant pathogenic bacteria in human and animals, and the undesirable side effect of certain antibiotics has necessitated the search for safe antimicrobial drug of plant origin. Plants are being looked at as having great potential for therapeutic treatment of various bacterial diseases.

The antibacterial activity of ethanolic flower extract revealed that 30 mg/ml of flower extract showed maximum zone of inhibition against all the bacterial strains. Praveena *et al* (2011)[33] have studied the antimicrobial activity of *E. littorale* against many pathogenic

microorganisms by using different solvents like chloroform, ethyl acetate, methanol, petroleum ether. Among that, methanolic and ethyl acetate extract of *E. littorale* showed a prominent antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Shigella sonnei* and antifungal activity against *Aeromonas hydrophila, C. albicans.*

CONCLUSION

The present study concludes that the ethanolic flower extract of *Enicostemma littorale* possess rich antioxidant properties which may be attributed to its various phytochemicals such as flavanoids and phenolic compounds. The bioactive compound accountable for the antibacterial activity against the tested organisms should be elucidated to develop a new flower therapeutic material for various human ailments.

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