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## Evaluation of antioxidant and antimicrobial potentials of *Eclipta prostrata* collected from the Nepal region

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**Abstract**

*Eclipta prostrata* is a perennial herb which is used to treat various ailments as a part of the traditional medicine in different parts of the world. Considering the ethnopharmacological importance of the plant, the antioxidant and antimicrobial activity of the leaf extract were evaluated. Antioxidant activity was determined using DPPH scavenging activity assay, ferric reducing power assay and total antioxidant assay. The phenol and flavonoid content were also quantitatively determined. The extract was able to scavenge DPPH free radical and reduce ferric ion. It showed a significant amount of phenol and flavonoid content. It inhibited the growth of pathogenic gram-positive and gram-negative bacteria effectively. Further study needs to be done for identifying the future applications of *E. prostrata* in drug and food industry.

**Keywords:** *Eclipta prostrata*, antioxidant, antimicrobial, free radicals.

**1. Introduction**

Plants and herbs with medicinal properties are an invaluable gift that nature has bestowed the mankind with. Classical Indian literature as well as various non-Indian literature have references of herbs being used by the saints and monks to alleviate the symptoms in sick and treat diseases. Plant preparations and herbs are used in Unani, Siddha medicine, Chinese traditional medicine, and Ayurveda. Various tribes and indigenous people across the globe still rely on herbal medicine. According to a report by World Health Organization, 80% of the population in Africa depends on traditional medicine for the primary health care [1]. The similar scenario can also be observed in many developing countries of Asia and Latin America where conventional medicine is the only available health service. In last few years, the practice of herbal medicine has burgeoned in developed nations of North America and Europe [2]. Due to side effects of synthetic drugs and their high cost, number of people are reverting to medicinal herbs for curing adverse health conditions.

The emergence of antibiotic-resistant bacteria has posed a serious threat to the humans and animals equally. Diseases caused by bacterial infections such as food poisoning, cholera, pneumonia, tuberculosis have caused havoc in the past, and if the bacteria keep evolving, they will create worse situation in future. Oxidative stress is another major health concern where the free radicals produced inside the body are not efficiently scavenged. The ineffective free radical scavenging is associated with cancer, Alzheimer's disease, cardiac diseases, atherosclerosis, arthritis and various other inflammatory diseases [3]. In this regard, plants with medicinal values can be very useful with the antibacterial and antioxidant property. In present study on *Eclipta prostrata*, we evaluated the total phenolic and total flavonoid content, antibacterial and antioxidant properties of the plant extract.

*E. prostrata* is a perennial weed growing in tropical areas of the Indian subcontinent, Malaysia, China and Australia [4-6]. It is the member of the Asteraceae family. The vernacular name of the plant is false daisy, and it is also called 'Bhringraj' in Sanskrit, mo cao in Wenchuan dialect of China and yerba de tago in Spanish [5, 7]. *E. prostrata* is used in ethnomedicine, Ayurveda and Chinese herbal medicine for the treatment of various ailments. AIDS patients use the plant as a self-medication for curing HIV infection and Giardial infection in Thailand [8-9]. In Thailand, *E. prostrata* is used to cure skin infections, bronchitis, hepatic diseases and as blood tonic [10, 11]. In traditional medicines of India, the plant is used as deobstruent and to cure jaundice, hypercholesterolemia, anticancer, obesity and spleen enlargement [10, 12]. Hoklos people in China use *E. prostrata* for the cure of bleeding nose and gastric bleeding, knee pain, cancer, hematuria, hypertension, cirrhosis of liver and nephritis [7, 13]. In Brazil, China and the Tamilnadu state of India, the plant is used for the treatment of

snake bites [14, 15]. Also, the plant has shown, Antivenom, antimalarial, and antidiabetic activity [14, 16, 17].

## Materials and Method

### Collection of leaves and extract preparation

Fresh leaves of *Eclipta prostrata* were collected from Simara, Bara, Nepal (Latitude: 27.1602, Longitude: 84.9796) in the first week of January 2017. The leaves were subsequently washed with water to remove dust particles and soil. The washed leaves were sundried for a day and then dried in hot air oven. The mechanical grinder was used to powder the leaves. The extract was prepared using Soxhlet extraction method in 90% methanol as solvent and after plant extract was obtained, it was dried in a water bath at 65°C to evaporate methanol. The extract was kept in the refrigerator for future studies.

### Determination of total phenolic content

Total phenolic content was measured by Folin Coilcateau method as mentioned in Akhtar *et al.* [18, 19]. Gallic acid was used to construct a standard curve for determining the total phenol content.

### Determination of total flavonoid content

The flavonoid content of methanolic extract of *E. prostrata* was determined by following aluminium chloride reducing method as mentioned in Kaur *et al.* [19, 20]. Quercetin was used to construct a standard curve for determining the total flavonoid content.

### DPPH Scavenging Activity

The antioxidant property of the *E. prostrata* leaves extract was measured in vitro by 2, 2 diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging activity as mentioned in Kaur *et al.* [21, 22]. Dilutions of plant extract in methanol ranging from the concentration of 10-140 µg/ml were used. Ascorbic acid was used as a standard. The DPPH scavenging activity was calculated as:

$$\% \text{ DPPH scavenging activity} = \left\{ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \right\} \times 100$$

Where,

$\text{Abs}_{\text{control}}$  = Absorbance of methanol + DPPH

$\text{Abs}_{\text{sample}}$  = Absorbance of sample (plant extract/ Ascorbic acid) +DPPH

### Ferric Reducing Power Assay

The ferric reducing power of leaves extract of *E. prostrata* was determined by using the method of Oyaizu as mentioned in Akhtar *et al.* [23, 19]. *E. prostrata* extract with a concentration range between 100-200 µg/ml was prepared in distilled water and used for the estimation.

### Total Antioxidant Activity

The total antioxidant activity of *E. prostrata* was evaluated by following the method of Prieto *et al.* as mentioned in Akhtar *et al.* [23, 19]. Plant extract of different concentrations ranging from 25-100 µg/ml in ethanol was used for the estimation.

### Antibacterial Activity Determination

The culture of *Escherichia coli*, *Pseudomonas putida*, *Salmonella enterica*, *Staphylococcus aureus*, and *Streptococcus pyogenes* was obtained from Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. Antibacterial

activity of the *E. prostrata* extract was evaluated by agar well diffusion method as discussed in Akhtar *et al.* [25, 19].

## Results and Discussion

The generation of the Antibiotic resistance towards microbes is a serious health issue worldwide. Microbes are becoming less responsive to antibiotics because of which diseases which were easily cured by the antibiotic administration are becoming difficult to treat. Pollution, stress and modern lifestyle are also affecting the human health insidiously. These factors are causing oxidative stress which oxidizes lipid, proteins and nucleic acids in the body. Oxidative stress is associated with several diseases such as rheumatoid arthritis, cancer, atherosclerosis, Parkinson's disease, cardiac diseases, Alzheimer's disease [26]. Scientists across the globe are exploring new therapeutic compounds with antioxidant and antibacterial activities. Plants are rich in phytochemicals showing myriad of pharmacological properties [10, 12, 27, 28]. With the onset of the human civilization, people have trusted on plants for their healthcare needs. Plant-based products are still the most reasonable and readily available source of treatment in the primary healthcare system [29]. In Ayurveda, Unani medicine, and Chinese herbal medicine, plants are used for the treatment of skin infections, gastrointestinal infections, wounds, cancer, arthritis and many more diseases. Considering the importance of plants, the extract of *Eclipta prostrata* was studied for its antioxidant and antibacterial activity.

When the plant extract was used to evaluate the total phenolic content, it was found that 50 µl (1mg/ml) of it was equivalent to 10.18 µg/ml of gallic acid. In case of total flavonoid content, 100 µg/ml of *E. prostrata* extract was found to be equivalent to 3.36 µg/ml of quercetin. The presence of phytochemicals especially phenolic compounds plays an important role in free radical scavenging activity and antibacterial activity of the plants [30]. As the extract was in the crude form, the extract displayed less phenolic and flavonoid content. Flavonoids are a group of secondary metabolites with powerful antioxidant activity. Phenolics and flavonoids are important metabolites which could be used in treating a number of ailments.

Phenolics and flavonoids are the compounds which can show free radical scavenging activity. By keeping this in view, the DPPH free radical scavenging activity of the extract of *E. prostrata* was evaluated, and it was found to be increasing with the increase in plant extract concentration. As the concentration of plant extract increased, the absorbance decreased which indicated the reduction in the free radicals numbers. The IC<sub>50</sub> value of *E. prostrata* and reference compound ascorbic acid was 42.1µg/ml and 6.62µg/ml, respectively. The IC<sub>50</sub> value of plant extract is significant as compared to ascorbic acid as plant extract is in a crude form which is having a number of other compounds also which may or may not have free radicals scavenging activity.

To further establish the antioxidative strength of the *E. prostrate*, we evaluated total reducing power and antioxidant activity of the plant extract. In the determination of the reducing power of methanolic extracts of *E. prostrata*, gallic acid was used as a control. With the increase in the concentration of the leaves extract of *E. prostrata*, there was an increase in absorbance. The ferric reducing power of the extract having a concentration of 200µg/ml was found to be equivalent to 12.18 µg/ml of gallic acid. Flavonoid and phenols have the ability to reduce ferric ion. For the total

antioxidant activity of *E. prostrata*, 25 µg of methanolic extract was found to be equivalent to 11.75 µg of ascorbic acid. These tests showed that *E. prostrata* is having significant total reducing and antioxidant power. It also established that total antioxidant power was more predominant as compared to the total reducing power in the plant extract.

*E. prostrata* plant extract was further used to find the antibacterial activity against pathogenic as well as non-pathogenic bacteria. It showed the ability to inhibit the growth of all the bacteria under study. *E. prostrata* extract (100mg/ml) formed a zone of inhibition with diameter 14mm, 13mm, 9mm, 12mm and 15 mm for *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas putida*, *Salmonella enterica* and *Streptococcus pyogenes* respectively (Table 1). *E. prostrata* had highest antibacterial activity against *S. pyogenes*. The extract inhibited the growth of bacteria in a dose-dependent manner. The present study showed that the plant extract was showing the ability to inhibit the growth of both gram-positive (*S. pyogenes*, *S. aureus*) as well as gram-negative bacteria (*E. coli*, *S. enterica*, and *P. putida*).

### Conclusion

Medicinal plants are rich in phytochemicals with a plethora of pharmacological activities. By virtue of these compounds, plants have been successfully used from antiquity to cure human diseases. The *Eclipta prostrata* leaves extract showed antioxidant and antibacterial activity. The extract was able to scavenge DPPH free radical and reduce ferric ion. Although the extract was crude in form, it showed a significant amount of phenol and flavonoid content. It showed the inhibitory effects on the growth of pathogenic gram-positive and gram-negative bacteria. Further study needs to be done for the isolation of these compounds from the extract and their future applications in drug and food industry.

**Table 1:** Antibacterial activity of *Eclipta prostrata*

	Diameter in mm (20 mg/ml)	Diameter in mm (50 mg/ml)	Diameter in mm (100 mg/ml)
Gram-positive			
<i>S. pyogenes</i>	8	10	15
<i>S. aureus</i>	8	11	13
Gram-negative			
<i>E. coli</i>	11	12	14
<i>S. enterica</i>	10	11	12
<i>P. putida</i>	0	7	9

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