Free Radicals and Antioxidants, 2017; 7(1): 90-94
A multifaceted peer reviewed journal in the field of Free Radicals and Antioxidants
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Original Article

Total Phenolic Content, Total Flavonoid Content, *In vitro* Antioxidant Activity and Antimicrobial Activity against Human Pathogenic Bacteria of *Eremurus Himalaicus*–An Edible Herb of North Western Himalayas

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ABSTRACT

Introduction: A number of plants of Kashmir valley are unexplored and *Eremurus himalaicus* is one amongst them. *Eremurus himalaicus* is an edible herb of North Western Himalayas which is yet to be evaluated for possession of antioxidant and antimicrobial activities. **Method:** The antioxidant potential of the plant extracts (Ethyl acetate, EHE; Methanol, EHM and Aqueous, EHA) was assessed by determining the total phenolic content, total flavonoid content, DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity, ferrous metal ion chelating activity, hydrogen peroxide scavenging assay and total reduction capability assay. The plant was further investigated for antimicrobial activity using agar well diffusion method. **Results:** EHM showed highest total phenolic content followed by EHA and EHE with 270 mg GAE/g, 240 mg GAE/g and 110 mg GAE/g respectively. The flavonoid content was found to be highest in EHA with 85 µg QE/g followed by EHM with 65 µg QE/g and least in ethyl acetate extract with only 20 µg QE/g. Ferrous metal ion chelating potential was also highest for EHA with IC_{50} value of 200.336. For DPPH assay and H_2O_2 scavenging assay the activity was highest for EHM with IC_{50} value of 148.1788 and 182.3371 respectively. In total reduction capability assay again EHM showed highest reducing power. Besides, antibacterial activity was also screened on some human pathogenic bacterial strains where again the highest activity was shown by EHM. **Conclusion:** In conclusion, EHM and EHA showed highest antioxidant potential and EHE showed the least. Similarly the antimicrobial potential was highest for EHM showing *Eremurus himalaicus* as a promising new herb for various human diseases.

Key words: Antioxidant, Antimicrobial, MIC, IC_{50} value, Total phenolics content, Total Flavonoids content.

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DOI: 10.5530/fra.2017.1.14

INTRODUCTION

A number of human diseases are caused by oxidative stress due to imbalance between the formation and neutralization of pro-oxidants.[1] Oxidation reactions may produce free radicals, which play a vital role in damaging various cellular macromolecules. This damage results into various diseases like arthritis, diabetes, asthma, liver damage, atherosclerosis, inflammation and carcinogenesis.[2] Antioxidants terminate these chain reactions by removing free radical intermediates and getting themselves oxidized. Synthetic antioxidants like BHT and Ascorbic acid (Vc) possess good antioxidant potential; however these have some side effects and hence the need for natural antioxidants of plant origin as a potential source of natural antioxidants is the need of the hour. India has a rich biodiversity of plants particularly in the Himalayan belt and many of these plants have not been evaluated for their medicinal properties. One of such plants is *Eremurus himalaicus.* It is found on the rocky slopes of North-Western Himalayas and its leaves are consumed as food in case of anaemia. It is also used as galactagogue by practitioners of traditional systems of medicines.[3] Till date no scientific research work has been done on the medicinal values of this plant and a lot is needed to be explored. So the present work is aimed at assessing the antioxidant and antimicrobial activity of *Eremurus himalaicus.*

MATERIALS AND METHODS

Plant Material Collection and Identification

The first step was the collection of plant from Faqir Goojree area of Dhara, Srinagar, Kashmir at the proper time (in the month of May). The collected plant material was properly identified and the specimen voucher was deposited in The Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir under herbarium number: 1765 (KASH). It was ensured that the plant material was free from soil, pathogens, aflatoxins etc. The material was then dried under shade at room temperature and coarsely powdered in a grinding mill.

Extraction

The coarsely powdered plant material was extracted sequentially with different solvents of increasing polarity (petroleum ether, ethyl acetate, methanol and distilled water) by soxhlet extraction method. The recovered extracts were filtered and the filtrate was concentrated under reduced pressure at 35-45°C using rotary evaporator. The remaining moisture, if present, was removed by keeping the extracts in desiccators and finally stored at 4°C in well labeled storage vials for further experimental use.[4]

Determination of total phenolic content

The determination of total phenolic content was performed using Folin-Ciocalteu method as described by.[5] The positive standard used for the construction of calibration curve was Gallic acid and the range of concentrations taken was from 50 mg/mL to 150 mg/mL.

The concentration of phenolics in various extracts was expressed as GAE/g using standard gallic acid calibration curve equation.

$$Absorbance\ (\lambda 725) = 0.001\ x\ [GAE] + 0.017$$

Determination of total flavonoid content

For the determination of total flavonoid content aluminium chloride colorimetric method described by Chang *et al*., 2002 was employed. The standard used for construction of calibration curve was Quercetin and the range of concentrations that was employed was from 50 mg/mL to 150 mg/mL.

The concentration of flavonoids in various extracts was expressed as QE/g using standard Quercetin calibration curve equation.

$$Absorbance\ (\lambda 415) = 0.002\ x\ [QE] + 0.060$$

Determination of Antioxidant Potential

For the determination of antioxidant potential of *Eremurus himalaicus* various assays were performed on different extracts in triplicates and the mean values were taken. These assays included DPPH assay, H_2O_2 scavenging assay, Ferrous Metal Ion Chelating Assay and Total Reduction Capability Assay.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay: For the determination of DPPH assay the methodology discussed by.[6]

Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenging assay was performed according to the methodology of.[7]

Ferrous Metal Ion Chelating Activity

The ferrous metal ion chelating potential of various extracts of *Eremurus himalaicus* was assessed by the method of.[8]

Total Reduction Capability assay

The total reduction capability assay was performed using the methodology discussed by.[9]

Microbiological Assay

The test organisms that were employed for the microbiological assay are as listed in Table 1. The experiment was performed using Agar Well Diffusion Method according to National Committee for Clinical Laboratory Standards (NCCLS). The experiment was performed in triplicates and mean values were taken.

RESULTS

The total phenolics and total flavonoid content of various extracts of *Eremurus himalaicus* is shown in Table 3. The results reveal that the total phenolic content was highest in *EHM* with 270 mg GAE/g followed by *EHA* and *EHE* with 240 mg GAE/g and 110 mg GAE/g respectively. The results for the flavonoid content revealed that the highest content of flavonoids are present in *EHA* with 85 µg QE/g followed by *EHM* with 65 µg QE/g and least in *EHE* with only 20 µg QE/g.

The antioxidant assays, which were aimed at assessing the potential of extracts of *Eremurus himalaicus* in prevention of various diseases caused due to oxidative stress, gave variable results. The IC_{50} value for DPPH assay was lowest for *EHM* with a value of 148.18. The respective IC_{50} values for other two extracts i.e., *EHE* and *EHA* were 235.6 and 161.48 respectively. The results were compared with the reference sample butylated hydroxyanisole (BHA) and ascorbic acid (Vc) which showed an IC_{50} value of 81.84 and 48.25 respectively. The IC_{50} values in H_2O_2 scavenging assay were 182.33 (R2:0.995), 211.66 (R2:0.995) and 257.33 (R2:0.993) for EHM, EHA and EHE respectively. The IC_{50} value for the reference samples Vc and BHA were found to be 81.17 (R2:0.96) and 120.21(R2:0.992) respectively. Table 2 shows the ferrous metal ion chelating activity of *Eremurus himalaicus*. The results reveal that the IC_{50} values for *EHE, EHM and EHA* were 313.28 (R2:0.99), 216.87 (R2:0.989) and 200.34 (R2:0.99) respectively with an IC_{50} value of 56.35 (R2:0.974) for reference sample EDTA. Figure 1 shows the total reduction capability of *EHE, EHM and EHA*. As is evident from the figure, there is increase in absorbance with the increase in concentration of the extracts. The antioxidant potential was found to be highest in the reference samples Vc and BHA followed by *EHM, EHA and EHE.*

The results for the antimicrobial potential of *Eremurus himalaicus* against human pathogenic bacteria are shown in Table 4. The results reveal that the extract of *Eremurus himalaicus* with most promising antimicrobial potential is EHE followed by EHM. EHA showed the least antimicrobial potential. The positive reference used was streptomycin (25 mcg) and the negative reference used was DMSO which was also used as a solvent for dissolving the extracts.

DISCUSSION

Total phenolic and flavonoid content, and *in vitro* antioxidant activity of ethyl acetate, methanol and aqueous extracts of *Eremurus himalaicus* was determined. Various solvents were used for extraction with increase in their polarity which has been proven to be effective in earlier studies.[10]

The phytocompounds like flavonoids and phenolics exhibit various biological activities, the most important being the antioxidant activity.[11]

Table 1: Test organisms used for microbiological assay

Test organism	Strain
Staphylococcus aureus	MTCC 11949
Escherichia coli	MTCC 10312
Proteus vulgaris	MTCC 7299
Klebsiella pneumonia	MTCC 3384
Bacillus subtilis	MTCC 11554
Pseudomonas aeruginosa	MTCC 10636

Table 2: Total phenolics and flavonoids content of various extracts of *Eremurus himalaicus*

Sample	Total Phenolics Content	Total Flavonoid Content
	mg GAE/g	mg QE/g
EHE	110.00 ± 18.00	20.00 ± 8.00
EHM	270.00 ± 12.00	65.00 ± 5.00
EHA	240.00 ± 19.00	85.00 ± 7.00

Table 3: Antioxidant potential of various extracts of *Eremurus himalaicus*

Sample	DPPH assay		H_2O_2 assay		Fe^{2+} Ion Chelating assay	
	IC_{50}	R^2	IC_{50}	R2	IC_{50}	R^2
Vc	48.25	0.981	81.17647	0.96	----	----
BHT	81.84685	0.978	120.2076	0.992	----	----
EDTA	----	----	----	----	56.35135	0.974
EHE	235.5951	0.962	257.3298	0.993	313.2848	0.99
EHM	148.1788	0.998	182.3371	0.995	216.8733	0.989
EHA	161.4843	0.96	211.6653	0.995	200.336	0.99

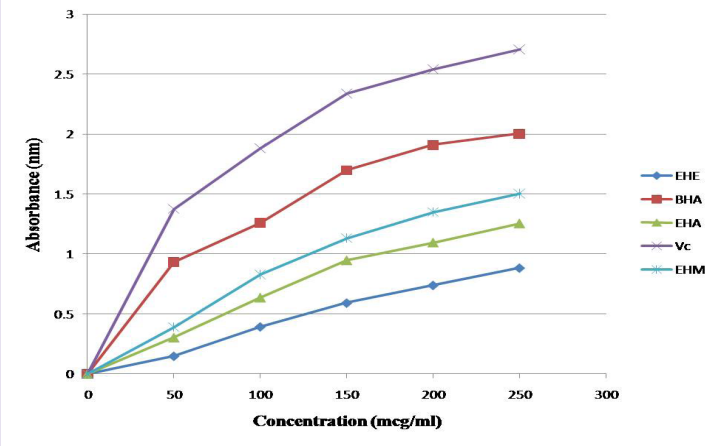

Figure 1: Total reduction capability assay of various extracts of *Eremurus himalaicus*

Table 4: Antibacterial activity of various extracts of *Eremurus himalaicus* using agar disc diffusion method

Sample	Zone of inhibition diameter (mm)					
	Bacterial strains					
	S. aureus	*E. coli*	*P. vulgaris*	*K. pneumonia*	*B. subtilis*	*P. aeruginosa*
EHE						
6 mg	15.39	13.54	15.56	12.89	11.34	10.54
10 mg	17.28	15.54	23.84	17.90	15.13	14.67
14 mg	23.28	20.36	24.82	19.87	21.32	19.23
Streptomycin, 25 mcg	25.36	30.83	25.65	25.18	30.25	25.38
EHM						
6 mg	10.41	10.11	10.53	-	10.67	7.37
10 mg	13.74	13.49	15.83	8.33	13.92	15.50
14 mg	15.32	17.32	16.43	15.19	15.54	20.35
Streptomycin, 25 mcg	20.63	25.54	20.67	30.32	30.87	30.52
EHA						
6 mg	-	5.65	-	-	8.22	-
10 mg	9.43	7.77	-	-	9.43	-
14 mg	15.59	9.38	10.48	-	10.58	-
Streptomycin, 25 mcg	25.67	30.40	25.34	30.80	30.31	30.16

They directly or indirectly scavenge the free radicals through a series of coupled reactions.[12] In our study the extracts of *Eremurus himalaicus* showed the highest phenolic content in *EHM* followed by *EHA* and *EHE*. However the flavonoid content was highest in *EHA* followed by *EHM*. The flavonoid content in *EHE* was very less. From this we can conclude that the methanolic and aqueous extracts possess good amount of phenolics and flavonoids which confers antioxidant potential to these extracts.

DPPH assay is one of the most widely used methods for screening antioxidant activity. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). The colour changes to yellow due to the scavenging of DPPH radicals by antioxidant compounds via donation of hydrogen atom.[13] The absorption decreases and coincides with the number of electrons taken up. The maximum antioxidant potential in this study was observed for *EHM* in a concentration–dependent manner which was followed by the other two extracts i.e., *EHE* and *EHA* respectively. The results were compared with the reference sample BHA and Vc.

The scavenging of DPPH radicals can be correlated with the number of available hydroxyl groups. Thus from the results we can infer that the antioxidant activity of EHM may be due to the presence of molecules with an available hydroxyl group.

H_2O_2 is a non-radical molecule that causes oxidative stress to cells. It is produced by dismutation of superoxide anion by univalent reduction. It

is highly important because of its ability to penetrate biological membranes. It causes breaking up of DNA, resulting in single strand breaks and formation of DNA protein cross-links.[14] Hence, the elimination of H_2O_2 is very essential for protecting the *in-vivo* systems. In Hydrogen peroxide scavenging assay the activity is assessed on the basis of decrease in absorbance caused by consumption of H_2O_2 by antioxidant moieties. Thus we can say that EHM has highest H_2O_2 scavenging potential followed by EHA and EHE respectively.

In living systems, iron stimulates and accelerates the process of lipid peroxidation.[15] However, in presence of chelating agents, there is disruption in the complex formation with the result there is decrease in the color intensity. Thus, the measurement of colour reduction leads to the estimation of the chelating potential or antioxidant potential of the chelating agent. The results for this assay revealed that the extracts of *Eremurus himalaicus* interfered with the formation of ferrozine–Fe^{2+} complex. The ferrous metal ion chelating potential of *Eremurus himalaicus* followed the order *EHE, EHM and EHA*.

For the measurement of reductive ability the transformation of Fe3+ to Fe2+ in presence of various extracts of *Eremurus himalaicus* was examined and it was found to possess considerable reducing ability which serves as an indicator of electron donating potential which represents the antioxidant potential.[15] The activity was found to be concentration dependent and the results ascertained that the extracts of *Eremurus himalaicus* possess significant reducing capacity with *EHM* having the highest potential.

The second part of the study was antimicrobial activity which was performed by agar well diffusion method. It has been reported that the antimicrobial activity reflects the constituents of the plant extracts.[16] Flavonoids and phenolics are mostly found to be responsible for the antimicrobial activity of plants.[17,18] However, many plants have shown similar antimicrobial activity with different phytocompound combinations. The antimicrobial activity of the *Eremurus himalaicus* extracts against test organisms revealed that the most promising antimicrobial potential was present in EHE followed by EHM and EHA. The extracts of *Eremurus himalaicus* showed more inhibitory effect on *S. aureus,* and *P. vulgaris* strains of bacteria. However, lesser inhibitory activity was seen for *K. pneumonia* and *P. aeruginosa strains*.

CONCLUSION

Eremurus himalaicus, an unexplored plant of Kashmir Himalayas, possesses significant amounts of flavonoids and phenolics, which are important phytocmpounds having various biological activities. Besides, *Eremurus himalaicus* has good potential of preventing oxidative damage *in vitro*, establishing the antioxidant activity of this plant. Moreover, the plant also has a capability of preventing the growth of bacteria and hence can be considered a good source for antimicrobial drugs. Overall, *Eremurus himalaicus* can be considered as a promising new herb that has antioxidant as well as antimicrobial activity and can be a good source for the discovery of new potential drugs.

ACKNOWLEDGEMENT

The authors are thankful to the University of Kashmir, Srinagar, for proving necessary facilities for the study.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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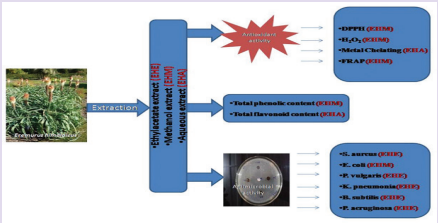
PICTORIAL ABSTRACT



SUMMARY

- *Eremurus himalaicus* is a scientifically unexplored plant of North-Western Himalayas.
- Possesses good content of phenolics and flavonoids.
- It has good potential of preventing oxidative damage *in vitro*.
- It also has capability of preventing the growth of bacteria.
- Can be considered as a promising new herb that has antioxidant and antimicrobial activity.

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