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PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF *EVOLVULUS ALSINOIDES* EXTRACTS

Mukesh Kumar Yadav [*1], Santosh Kumar Singh [2], J. S. Tripathi [1] and Y. B. Tripathi [3]

Department of Kayachikitsa [1], Centre of Experimental Medicine and Surgery [2], Department of Medicinal Chemistry [3], Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221005, Uttar Pradesh, India.

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Correspondence to Author:
Mukesh Kumar Yadav

Ph D Scholar,
Department of Kayachikitsa,
Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221005,
Uttar Pradesh, India.

E-mail: kumar.mukesh097@gmail.com

ABSTRACT: Free radicals are atoms or groups of atoms with an unpaired number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. These free radicals become more lethal when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. Free radicals play a crucial role in a complex interplay of different mechanisms in both normal ageing and neurodegenerative diseases. Free radicals and other reactive oxygen species are generated continuously via normal physiological processes, more so in pathological conditions. The different solvent extracts of *Evolvulus alsinoides* at different concentrations *i.e.* 0.1mg/ml, 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1.0 mg/ml showed significant in-vitro antioxidant activity DPPH (2, 2-diphenyl -1- picrylhydrazyl) assay and hydrogen peroxide scavenging assay against standards L-ascorbic acid and Butylated hydroxytoluene (BHT) respectively. Therefore, the EC_{50} values for DPPH assay are EEA=196.08±2.16 µg/ml and HAEA=307.66±1.72 µg/ml and for hydrogen peroxide assay EC_{50} values are EEA= 460.20±0.632 µg/ml and HAEA=483.49±1.13 µg/ml.

INTRODUCTION: There is currently immense interest in natural antioxidants and their role in human health and nutrition. A considerable amount of data have been generated on the antioxidant properties of food plants around the globe. However, traditionally used medicinal plants await such screening. On the other hand, the medicinal properties of plants have also been investigated due to their potent pharmacological activities, low toxicity, and economic viability. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals.

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The use of plants as medicines predates written human history. Medicinal plants are in many ways having their own immense importance in the current era. All plants produce chemical compounds as part of their normal metabolic activities. *Evolvulus alsinoides* is often prostrate, slender and wiry with long hairs. It occurs throughout the region from India to west Cameroon and widely dispersed elsewhere in tropical Africa and worldwide. *E. alsinoides* has been proved to possess scientific potential in central nervous system depression, antioxidant [1], anticonvulsant activity [2], antibacterial [3], antidiabetic [4], anti-inflammatory activity [5].

MATERIALS AND METHODS:

Plant Material: Whole plant material of *Evolvulus alsinoides* (Linn.) was collected from village Ramnapur, Varanasi, Uttar Pradesh, India in October 2015 and identification was done by

Department of Botany, Banaras Hindu University, India and also herbarium of *Evolvulus alsinoides* (voucher specimen no. Convolvul./03/2015) of plant was deposited in the Department of Botany.

Chemicals and Reagents:

Plant Extraction: The extraction of plant was done with soxhlet method in different solvents (*i.e.* Petroleum ether, chloroform, ethyl acetate, ethanol, hydro-alcoholic) at 72 - 100 °C for 72 hours. The Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various natural sources. Generally, a small amount of dry sample is placed in a thimble. The thimble is then placed in distillation flask which contains the solvent of particular interest. After reaching to an overflow level, the solution of the thimble-holder is aspirated by a siphon. Siphon unloads the solution back into the distillation flask. This solution carries extracted solutes into the bulk liquid. The solute remains in the distillation flask and solvent passes back to the solid bed of the plant. The process runs repeatedly until the extraction is completed.

Phytochemical Screening: For preliminary phytochemical analysis, the freshly prepared crude ethanolic extracts of leaves were tested for the presence or absence of phytoconstituents such as alkaloids, tannins, Flavonoids, Saponins by using standard phytochemical procedures [6].

TABLE 1: PHYTOCHEMICAL EXAMINATION RESULTS OF EXTRACTS OF *E. ALSINOIDES*

S. no.	Phytochemical	Test	PEEA	CEA	EAEA	EEA	HAEA
1	Alkaloids	Wagner's reagent	+	+	+	+	+
2	Glycosides	Keller Kelliani's test	+	+	-	-	-
3	Flavonoids	Alkaline reagent Test	-	-	+	+	+
4	Phenols	Ferric chloride test	-	-	-	+	+
5	Saponins	Foam test	-	+	+	+	-
6	Tannins	Braymer's test	+	-	+	+	+
7	Terpenoids	Salkowki's test	+	+	-	+	-

PEEA- Petroleum ether extract of *E. alsinoides,* CEA- Chloroform extract of *E. alsinoides,* EAEA- Ethyl acetate extract of *E. alsinoides,* EEA- Ethanolic extract of *E. alsinoides,* HAEA- Hydro-alcoholic extract of *E. alsinoides*

DPPH Scavenging Activity: Free radicals are extremely reactive species and are known to damage proteins, cause breakdown of DNA strands, initiate the peroxidation of various compounds and thus leads to many health problems and degenerative diseases such as cancer, inflammation, atherosclerosis, accelerated ageing, *etc*. The free radical scavenging activity was measured by the DPPH method [7].

0.5 ml different solvent extracts of *Evolvulus alsinoides* with different concentration *i.e.* 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml. Added 1.5 ml of 0.3 mM methanolic solution of DPPH. Incubated for 20 min in dark. Absorbance was measured at 517 nm against blank samples (DPPH solution only) and L-ascorbic acid (1.0 mg/ml) used as the standard. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH radical scavenging activity (%) =

$$\frac{A_b - A_s}{A_b} \times 100$$

Where,

A_b = Absorbance of Blank

A_s = Absorbance of Test sample

Hydrogen Peroxide Activity: 40 mM solution of H_2O_2 prepared in phosphate buffer (pH= 7.4). Plant extract (0.1 - 1.0 mg/ml) added to 0.6 ml of 40 mM solution of H_2O_2. (Butylated hydroxytoluene) BHT of 1.0 mg/ml concentration used as standard. Absorbance was taken at 230 nm against Blank solution (Phosphate buffer without H_2O_2).

H_2O_2 Scavenging activity [8] was measured by the following equation:

$$H_2O_2 \text{ Scavenging activity (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

Where, A_o = Absorbance of Control

A_1 = Absorbance of Sample

RESULT AND DISCUSSION:

DPPH Scavenging Activity: DPPH radical is a widely used method to evaluate the free radical scavenging ability of various plant samples. DPPH is a stable nitrogen- centred free radical the colour

of which changes from violet to yellow upon reduction by the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. It was found that the radical scavenging activities of extracts increased with increasing concentration. The EC_{50} for EEA=196.08±2.16 µg/ml and HAEA=307.66±1.72 µg/ml.

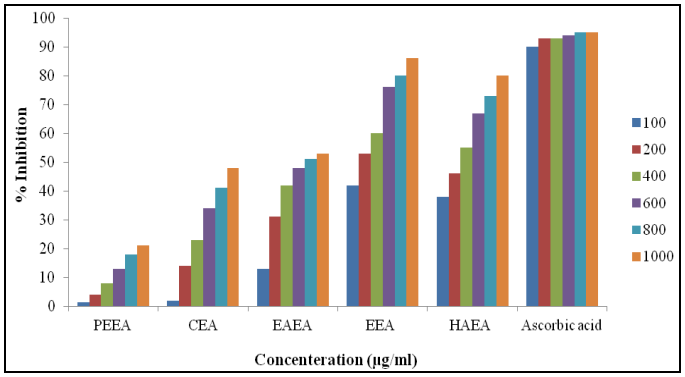

FIG. 1: DPPH SCAVENGING ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *E. ALSINOIDES*. L- ASCORBIC ACID WAS USED AS STANDARD

Hydrogen Peroxide Activity: Scavenging of H_2O_2 by ethanolic and the hydro-alcoholic extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The differences in H_2O_2 scavenging capacities between the two extracts may be attributed to the structural features of their active components, which determine their electron donating abilities. The ethanolic extract showed more percent inhibition than hydro-alcoholic extract of *E. alsinoides* respectively against the standard BHT (Butylated hydroxytoluene). EC_{50} for EEA= 460.20±0.632 µg/ml and HAEA=483.49±1.13 µg/ml Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems.

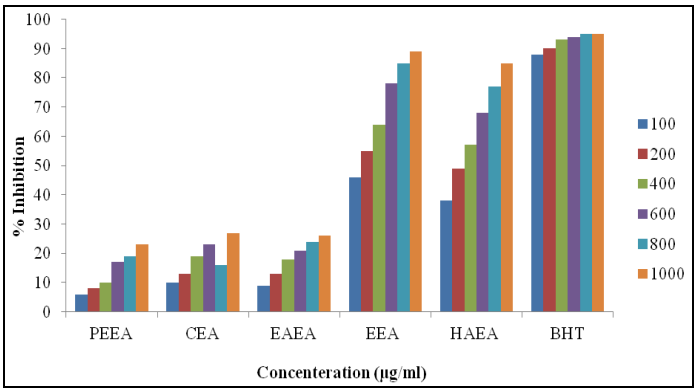

FIG. 2: HYDROGEN PEROXIDE SCAVENGING PERCENT INHIBITION OF DIFFERENT SOLVENT EXTRACTS OF *E. ALSINOIDES* BHT (BUTYLATED HYDROXYTOLUENE) WAS USED AS STANDARD

CONCLUSION: The study demonstrated the antioxidant potentials of different solvent extracts of *E. alsinoides*. The providing data can just enrich the existing comprehensive data of antioxidant activity of *E. alsinoides* extracts and insights the importance of antioxidant therapy in stress disorders and the consequent need to evaluate antistress agents in terms of their antioxidant ability.

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CONFLICT OF INTEREST: There is no conflict of interest.

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