IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF *EQUISETUM DIFFUSUM* D. DON PLANT

Prabhat Soni*, Reearch scholar, Department of Biological Sciences, M.G.C.G.V.V, Chitrakoot, Satna (M.P) India
Sadhana Chaurasia, Associate Professor, Department of Energy and Environment, M.G.C.G.V.V, Chitrakoot, Satna (M.P) India
Ravindra Singh, Associate Professor, Department of Biological Sciences, M.G.C.G.V.V, Chitrakoot, Satna (M.P) India
Ravi Upadhyay, Professor, Department of Botany Govt. P.G. College Pipariya, Hoshangabad (M.P) India

ABSTRACT

Free radical is harmful to the living body it is unstable and donates to electron other molecules of living bodies. In everyone bodies, different types of free radicals are spread by exogenous and endogenous sources. Antioxidant molecules are inhibited to process of development free radical in bodies. At present two types of antioxidant uses as a drug, chemically synthesized in the lab and naturally plants develop as a secondary product. According to WHO (world health organization), plants are rich sources of natural products and secondary metabolites. *Equisetum diffusum* plants is a natural and virtuous source of antioxidant. The aim behind choosing this plant was that how much capacity this plant has to stop or eliminate free radicals. In all extracts of *E. diffusum* the best scavenging activity was carried out the inhibitory concentration of 50% (IC_{50}) in acetone extract (IC_{50} value = 25.940 µg/ml) and lowest in chloroform extract (IC_{50} value = 520.421 µg/ml) which were comparable with that of ascorbic acid (IC_{50} value = 21.387 µg/ml).

Keywords*: free radical, scavenging, 2, 2-diphenyl-1-picrylhydrazyl, and E. diffusum.*

INTRODUCTION

Free radicles are the byproduct of living cells. They originate from cells from causes of oxidative stress. It is a highly reactive, short-lived, unstable and unpaired electron [1]. They can contribute and take the electron forasmuch behaving as oxidants [2]. The ‘‘free radical theory of oxygen toxicity’’ was suggested by [3]. Hydroxyl (OH^-), Ozone (O_3), hydrogen peroxide (H_2O_2), nitric mono oxide (NO), nitric dioxide (NO_2), oxygen singlet (O^-_2), alkoxy radical (RO^-), chlorine monoxide (ClO^-) and peroxynitrite (HNO_3^-) are the most example of free radicals sources and they caused cancer disease, immune system disorder, lungs disease, kidney disease, neural syndrome, Alzheimer's

disease, arthritis, hypertension, organ transplantation, gastric ulcers, alcoholism, emphysema, muscular dystrophy and many diseases [4,5]. Many external and internal sources are formed free radicle such as internal sources: smoking, ozone, x-rays, gamma rays air pollution, radiation, pesticides, tobacco smoke, heavy metals, transition, drugs, chemical industries, and internal sources: phagocytosis, mitochondria, inflammation, xanthine oxidase, ischemia, peroxisomes, exercise, endoplasmic reticulum [6,7]. The terms of free radicle have frequently used in recent dialogues of disease contrivances [8]. In living cells and other molecules oxidized by free radicals: antioxidant molecules are discontinued the free radical chain reaction and preventing the oxidation of other molecules [9] [Figure 1.].

Firstly in 1825, D. Don was described *Equisetum difjusum* in prodromus flora Nepalensis [10]. In “Handbook of fern allies" *E. diffusum* plant mention the book by [11]. *E. diffusum* plant are belonging to the family of Equisetaceae (Systematic classification display in Table 1). They widely distributed in Madhya Pradesh (Sehore, Satpura Hills Hoshangabad, Amarkantak Anuppur, Tamia Chhindwara), Sikkim Jammu and Kashmir, Shimla, Meghalaya, Assam, in India and other countries namely Nepal, Bhutan, Myanmar, Japan, and Vietnam [12-16]. It is commonly known as ‘Choti hadjod’ or 'Horsetail' or 'Scouring rushes'. The monomorphic stem is an annual, horizontal and hollow branched aerial part of the stem is the dark green and underground part of creeping; the perennial rhizome is dark brown. Stems are differentiated into nodes and internodes. Biangulate ridges and two keeled sheaths noticeably in the internode of the branch (having a narrow longitudinal groove along the apex of each ridge) according to [17]. At each internode, a circle of scale-like leaves is found which fused to the base and some height to form a leaf sheath according to [18]. Fertile and sterile branches in the plant are almost identical or monomorphic and each branch can be produced cones. Studies of previous literature and documents have presented that, there was no study of In-vitro free radical scavenging activity in the whole plant (Wh. P.) of *E. diffusum* plant species. So, the current study was performed on the *In-vitro* free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH): of *E. diffusum* D. Don plant which was helpful in the progress of effective drug development and prevent the oxidization of living cells molecule, will motivate researchers to study in this field in future.

Table-1: Systematic classification of *E. diffusum* plant

Kingdom	Plantae
Class	Polypodiopsida
Order	Equisetales
Family	Equisetaceae
Genus	Equisetum
Species	*E. diffusum*

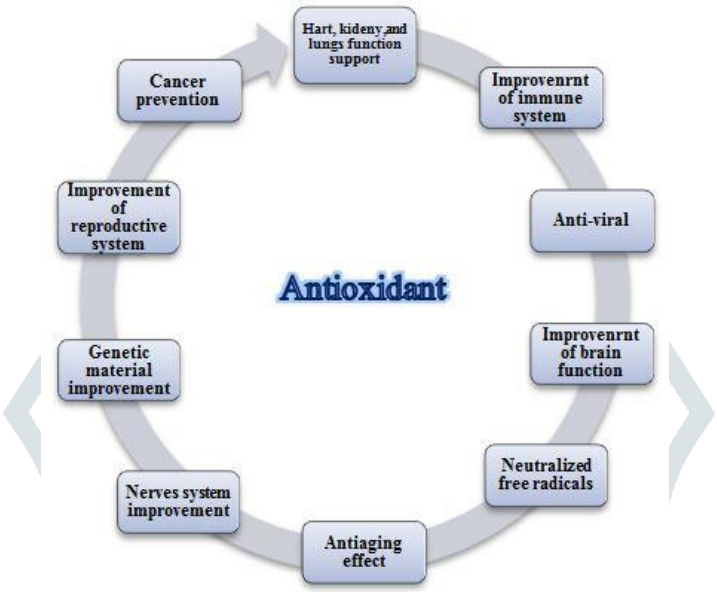


Figure- 1: Role of antioxidant in the body [9]

MATERIAL AND METHOD

Collection and validation of plant material

Equisetum diffusum whole plant (Wh. p.) was collected from the Budani in forest range of 'Khanda bad' (latitude 22.78, longitude 77.59) of District, Sihor, Madhya Pradesh, India. The famous taxonomist Prof. Ravi Upadhyay Head of Department in Botany, Government Post Graduate College Pipariya District Hoshangabad Madhya Pradesh, India was to validate plant samples. A sample prepared by standards of Ayurvedic pharmacopeia of India (API).

DPPH assay

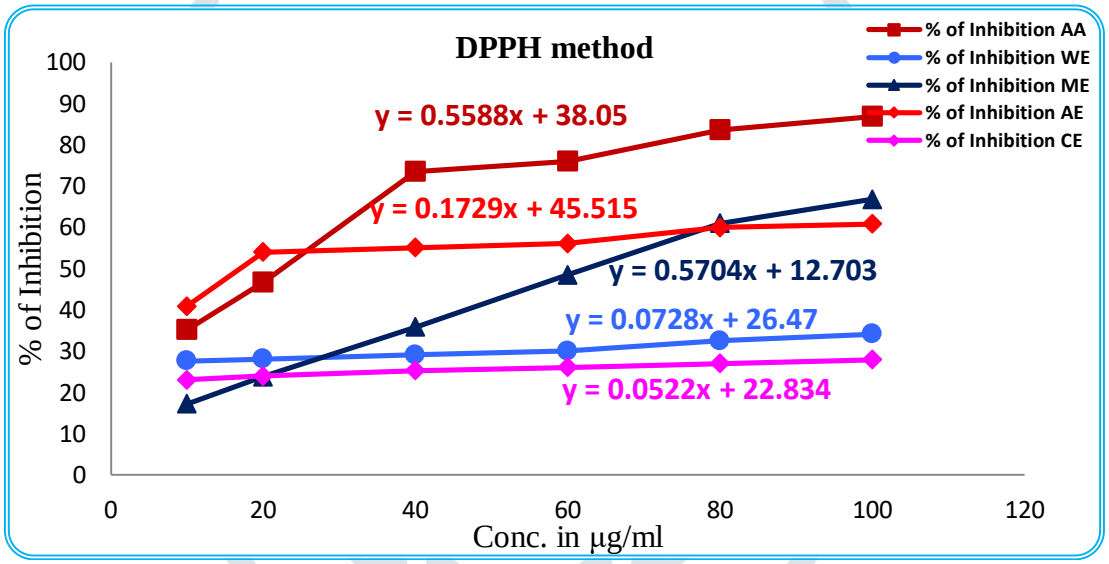
DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extracts at different concentrations (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5

ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm in Labindia 3000 + UV/VIS Spectrophotometer [19].

$$\text{Calculation of \% Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Statistical examination- All data analyses found out in triplicate and data are expressed as mean ± standard deviation (SD). The mean, standard deviations, correlation and IC_{50} values analysis were calculated using MS-Excel software.

RESULT AND DISCUSSION



AA- Ascorbic acid, WE- Water extract, ME- Methanolic extract, AE- Acetone extract, CE-Chloroform Extract

Figure-2: IC_{50} values (50% inhibitory concentration) of DPPH scavenging activity of different plant extracts

The assessment of antioxidant activity in various water, methanol, acetone & chloroform extracts of the whole plant of *E. diffusum* was determine. The highest activity found in acetone extract (IC_{50} value = 25.940 µg/ml) followed by methanol extract (IC_{50} value = 65.387 µg/ml), water extract (IC_{50} value = 323.201 µg/ml) and chloroform extract (IC_{50} value = 520.421 µg/ml) sequentially. (IC_{50} value was 21.387 µg/ml) of ascorbic acid used as a standard (Figure-2).

Among all four extracts highest antioxidant activity was recoded in acetone extract (IC_{50} value = 25.940 µg/ml) and lowest in chloroform extract (IC_{50} value = 520.421 µg/ml) extract which were comparable with that of ascorbic acid. The acetone extracts of whole plant showed best free radical

scavenging antioxidant activity in comparison to all other extracts. The IC_{50} values of all tested extracts of plants indicated that ascorbic acid was more active than tested extracts, the sample extracts.

Several research scholars and scientists are found free radicle activity in genus Equisetum and other Pteridophytes plants. Free radicle scavenging activity of aerial stems of *Equisetum debile* Roxb. Synonyms of *Equisetum diffusum* methanolic plant extract in four pure compound, results (B-3 7.0 µg/mL and B-4 9.48 µg/mL compound) were significant [20].

CONCLUSION

E. diffusum is showing *In-vitro* free radical antioxidant activity in different plant extracts. Property of the antioxidant molecule is inhibited and reduces the chain of free radicle in living cells and they prevent many diseases. The power of this plant is being used by tribal and general people of different places in the country to various diseases, especially bone fractures. *E. diffusum* plant has been classified as traditional and popular uses of drugs. Till now no such study has been done in this plant, so there is a so possibility of studying in this plant. This can be used in the welfare of mankind and also it can be used to make drug/medicine in pharmaceutical industries and other commercial uses.

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