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ANTIOXIDANT ACTIVITY OF CISSUS QUADRANGULARIS L. STEM IN-VITRO

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ABSTRACT

The present study aimed to evaluate the *in-vitro* antioxidant activities of *Cissus quadrangularis L.* stem. The preliminary phytochemical assay using solvents of different polarity namely water, 50% hydroethanol, ethanol, acetone, chloroform and petroleum ether showed the presence of secondary metabolites like flavanoids, alkaloids, tannins, triterpenoids, carbohydrates, saponins, glycosides, ligins and inulins. Among all the extracts, hydroethanol extract showed the presence of more secondary metabolites. Hence it was utilized for antioxidant assays like superoxide radical scavenging activity, hydroxyl radical scavenging activity and metal chelating activity. These results revealed that the 50% hydroethanol extract of *Cissus quadrangularis L.* stem possessed potent antioxidant properties.

KEYWORDS: *Cissus quadrangularis L.*, phytochemistry, antioxidant activity.

INTRODUCTION

Medicinal plants exhibit various biological and pharmacological properties like anti-inflammatory, anti-bacterial, anti-cytotoxicity and so on.[1] Primary metabolites are required for the growth of plants. Some of them are proteins, lipids and starch. They act as a precursor for the pharmacologically active metabolites.[2] Secondary metabolites do not have any role in maintaining the life process of the plants, but they are essential for interaction with its environment for adaption and defence.[3] Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Superoxide, also called as hyperoxide is an important product of the one electron reduction of dioxygen, which normally occurs in the nature.[4] Hydroxyl radicals are highly reactive and have short life span. The destructive action of hydroxyl radicals are seen in neurological autoimmune

diseases like HIV-associated neurocognitive disorders.[5] An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidant vitamins (A, C, E) are found in vegetables, fruits, eggs, legumes and nuts. Plants with vitamins, flavanoids and polyphenols, have high antioxidants.[6] Nowadays, the search for medicinal plants with significant antioxidant potential is the intense focus of research.[7]

Many herbs have been reported to possess antioxidant properties.[8] *Cissus quadrangularis L.* (family Vitaceae) is a perennial plant scattered all over India, particularly in tropical regions. In Ayurvedic System of medicine, the stem of *Cissus quadrangularis L.* was used to treat piles, bone fracture, pain in joints, swelling, gout, asthma, scurvy, disease of ear and nose-bleeding.[9] In this study, we aimed to explore the antioxidant potential of hydroethanol extract of *Cissus quadrangularis L.* stem *in-vitro*.

MATERIALS AND METHODS

Collection and authentication of plant material

The stems of *Cissus quadrangularis L.* were collected from the local areas of Coimbatore and authenticated by Dr. N. Rajaram, Associate Professor in Botany, PSG College of Arts & Science. The collected plant parts were shade dried for 3 days, powdered using mechanical blender and was stored in an air tight container until further use.

Preparation of plant extract

10g of powdered aerial part of *Cissus quadrangularis L.* was dissolved in 100ml of solvents with different polarity like water, 50% hydroethanol, ethanol, acetone, chloroform and petroleum ether, subjected to cold maceration for 72 hours with intermittent shaking. It was then filtered using Whatman filter paper and the extracts obtained were used for preliminary phytochemical analysis using the standard procedures.[10]

Since the 50% hydroethanol extract showed positive result for many phytochemicals, it was used for further studies like quantitative analysis of phenols[11], tannins[12], carbohydrates[13] and free amino acids.[14] The extract was also subjected to free radical scavenging assays like superoxide radical scavenging assay[15], hydroxyl radical scavenging assay[16], and metal chelating activity.[17]

RESULTS AND DISCUSSION

Phytochemical analysis of *Cissus quadrangularis L.* stem

The results of the preliminary phytochemical analysis from table (1) showed that various phytochemicals like flavanoids, alkaloids, tannins, triterpenoids, carbohydrates, saponins, glycosides, lignins and inulins were present in *Cissus quadrangularis L.* stem. Among five extracts, the 50% hydroethanol extract showed the presence of maximum phytoconstituents.

Table 1: Preliminary phytochemical screening of *Cissus quadrangularis L.* stem extract.

Test	Aqueous	50% hydro Ethanol	Ethanol	Acetone	Petroleum ether
Flavanoids	+	+	-	-	-
Alkaloids	+	+	-	-	-
Tannins	-	+	+	+	-
Triterpenoids	+	+	-	-	-
Carbohydrate	+	+	+	-	-
Saponins	+	+	+	+	+
Glycosides	+	+	+	-	-
Lignins	+	+	+	+	-
Inulins	+	+	+	+	+

+ indicates presence, - indicates absence

Quantitative analysis of *Cissus quadrangularis L.* stem

In the quantitative analysis of the hydroethanol extract of *Cissus quadrangularis L.*, the total phenolics, tannins, carbohydrates and free amino acids were found to be 18mg/g, 10.5mg/g, 12.8mg/g and 10.5mg/g respectively as shown in the fig (1). Phenolics are believed to be cancer chemopreventives, rich in antioxidant effects and promote healthy aging. Tannins protects from heart disease, prevent cellular damage. Carbohydrates promote weight loss and keep the memory sharp. Free amino acids are mainly used to build the proteins.[18]

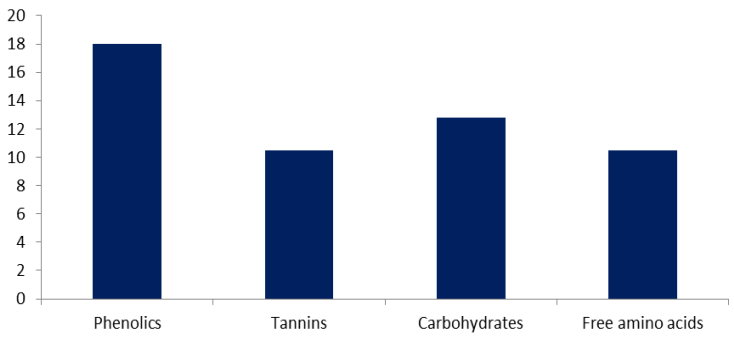

Fig. 1: Quantitative analysis of hydroethanol extract of *Cissus quadrangularis L.* stem.

Free radical scavenging assays

Superoxide radical scavenging assay

Superoxide is biologically toxic and is produced in immune actions. The phagocytes produce a large amount of superoxide by the enzyme NADPH oxidases. Superoxide is deleterious, produced during mitochondrial respiration and by the enzyme xanthine oxidase.[19] The superoxide radical scavenging activity of the *Cissus quadrangularis L.* extract was assessed by using ascorbic acid as standard. From the fig (2), it is evident that the superoxide radical scavenging efficacy of both the standard and the plant extract increased in a dose dependent manner. Maximum radical scavenging activity was found at maximum concentration (100µg/ml) in which ascorbic acid showed 93.1% inhibition whereas the *Cissus quadrangularis L.* stem extract showed 65.9% inhibition. The IC_{50} value for standard and the plant extract were found to be 40.80±1.02µg/mg and 48.86±1.23µg/g respectively. Since *Cissus quadrangularis L.* stem extract showed an inhibition on superoxide radicals, it can be used as a component of antioxidant source from medicinal plants.

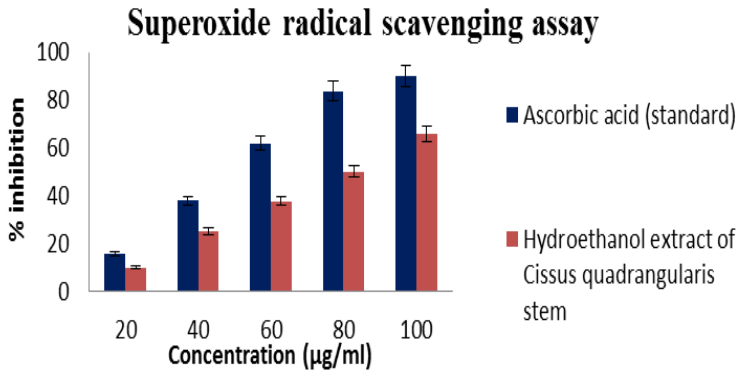

Fig 2: Superoxide radical scavenging potential of *Cissus quadrangularis L.* stem.

Hydroxyl radical scavenging assay

Hydroxyl radicals are highly reactive and degrade organic contaminants.[20] If this OH^- damages the DNA, it causes cancer.[21] The scavenging of hydroxyl radicals by the standard (ascorbic acid) and the plant extract (100-500µg/ml) were concentration dependent. Maximum radical scavenging activity was found at 500µg/ml in which ascorbic acid showed 97% inhibition with an IC_{50} value of 25.35±1.31µg/g, whereas the plant extract showed 86.2% inhibition with an IC_{50} value of 20.36±1.12µg/g as shown in fig (3). Since the hydroethanol extract of *Cissus quadrangularis L.* stem showed an inhibition for hydroxyl radicals, it could be used to destroy the hydroxyl radicals produced in the body during various metabolic reactions.

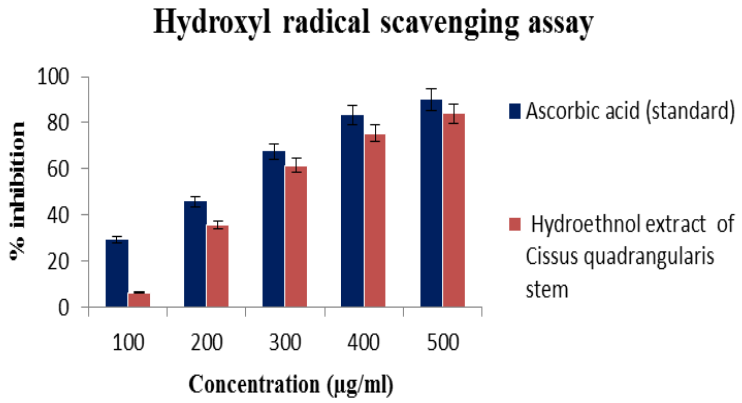

Fig 3: Hydroxyl radical scavenging potential of *Cissus quadrangularis L.* stem.

Metal chelating activity

Iron chelation reduces the formation of free metal ions. Metal ion toxicity is due to accumulation of metals like sodium, potassium, calcium, zinc, Copper, iron and magnesium. These can be treated with metal ion chelators.[22] The chelating activity increased with increase in concentration (100-500µg/ml) of the plant extract as shown in fig (4). The maximum metal ion chelation was obtained at 500µl with 58.2% inhibition for the plant extract and the IC_{50} found to be 71.76±1.089µg/g whereas the standard EDTA showed 72.7% inhibition and the IC_{50} value was 82.50±1.278µg/g. Iron toxicity can lead to vascular congestion of heart, liver, kidney, GI tract, spleen, adrenal, thymus and central nervous system.[23] Since *Cissus quadrangularis L.* stem extract showed an inhibition against the iron toxicity, it can be used as a potential source for managing disease conditions that arise from iron toxicity.

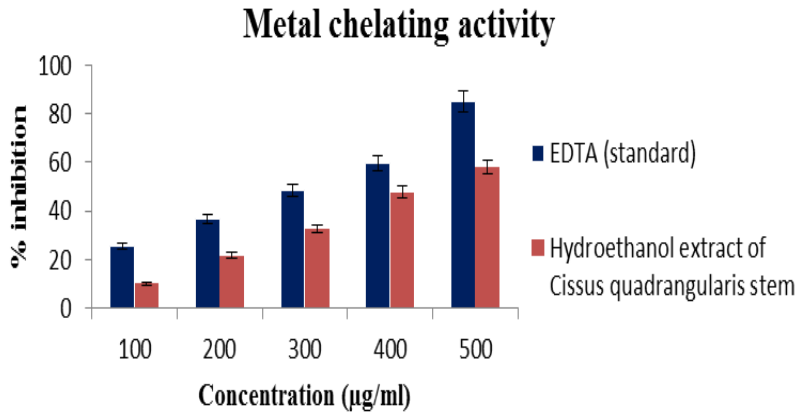

Fig 4: Metal chelating activity of *Cissus quadrangularis L.* stem.

Statistical analysis

All the experiments were performed in triplicates (n=3)**.** Results are represented as mean ± standard deviation.

CONCLUSION

From the present study, it may be concluded that *Cissus quadrangularis L.* stem is an important source of natural antioxidants with good free radical scavenging and anion radical scavenging capacities.

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