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EVALUATION OF *IN VITRO* ANTIOXIDATIVE ACTIVITY OF *STEPHANIA WIGHTII* (ARN.) DUNN (MENISPERMACEAE) - AN ENDEMIC MEDICINAL PLANT

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ABSTRACT

In this study, antioxidant activity of *Stephania wightii* aerial parts and tuber extracts was evaluated by total antioxidant activity, property of metal chelating and reducing power assays. The total antioxidant capacity observed in the methanolic extract of aerial parts and tuber of *S. wightii* was 303.23 and 312.49 nM GAE/g extract. The reducing power of aerial parts and tuber extract of *S. wightii* was found to be 0.55, 0.57, 0.65, 0.73, 0.93nm and 0.59, 0.62, 0.68, 0.74, 1.05nm respectively. The aerial parts and tuber extracts have exhibited maximum chelating effects of 83.49 and 92.72 mg EDTA/g. Possibly, tuber extract showed potent antioxidant activity because it contains high amount of phytoconstituents, which could react with radicals to stabilize and terminate radical chain reactions.

Keywords: *Stephania wightii*, methanol extract, total antioxidant activity, metal chelating and reducing power assay.

INTRODUCTION

In recent years, phytochemicals of medicinal plants have received a great deal of attention mainly on their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen and various radicals as a damaging

side-effect of aerobic metabolism. These radicals are possibly involved in a number of disorders including cardiovascular malfunctions, tissue injury, DNA damage and tumor promotion [(1)]. Several studies suggest that antioxidants could prevent accumulation of these reactive oxygen species and be beneficial for treatment of these pathologies [(2)].

The genus *Stephania* belongs to family Menispermaceae, which includes about 60 species worldwide and occurs mainly in the tropical and semi-tropical zones of Asia and Africa [(3)]. The leaves are arranged spirally on the stem, and peltate, with the leaf petiole attached near the centre of the leaf. The name *Stephania* means "a crown". This refers to the anthers being arranged in a crown like manner. It is locally called as "Koloukone" by kanikar tribals. About 10,000 plant alkaloids have been identified in the genus *Stephania*. In traditional medicine, *S. wightii* has been used to treat a wide variety of ailments [(4)]. Presently an attempt has been made to study the antioxidant activity of methanolic extract of aerial parts and tuber of *Stephania wightii.*

MATERIALS AND METHODS

Plant material

Fresh aerial part (except Flower) and tuber of *Stephania wightii* (Menispermaceae) was collected from Waynad, Western Ghats, Kerala, India and authenticated by taxonomist, Botanical survey of India, Coimbatore, Tamilnadu.

Preparation of extracts

The dried aerial part and tuber plant material was subjected to size reduction to a coarse powder by using pulverizer and passed through sieve (40#). These powders were soaked into methanol solvent for 1 or 2 weeks. These extracts were filtered and concentrated under reduced pressure using rotary evaporator and dried in vacuum dryer till it become semisolid to solid mass and there stored in airtight containers in refrigerator below at 10°C.

Phosphomolybdenum assay

The total antioxidant activity of the sample was evaluated by the phosphomolybdenum method [(5)]. An aliquot of 0.1 ml of sample solution was mixed with 1 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped at room temperature and the absorbance of methanol solutions was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as nM gallic acid equivalents (GAE) per gram of dry extract.

Chelating effects on ferrous ions/ Metal chelating activity

The ability of the extract to chelate ferrous ions was estimated by the method of Dinis *et al.*, [6]. Briefly, 2 ml of various concentrations of the extracts in methanol were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). The mixture was then shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine - Fe^{2}+ complex formation was calculated as [(A0 - A1)/A0]×100, where A0 was the absorbance of the control, and A1 of the mixture containing the extract or the absorbance of a standard solution.

Determination of reducing power

The reducing power of extracts was determined according to the method of Oyaizu, [7]. 2.5 ml of various concentrations of the extract, 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml and 2.5 ml of 1% potassium ferricyanide were mixed and incubated at 50°C for 20 min and centrifuged for 10 min at 5000 g after addition of 2.5 ml of 10% trichloroacetic acid. 2.5 ml aliquot of supernatant was mixed with 2.5 ml of deionised water and 0.5 ml of 0.1% ferric chloride. After 10 min of incubation, the absorbance was measured at 700 nm against a blank.

RESULTS AND DISCUSSION

The phosphomolybdenum method is based on the reduction of molybdenum by the antioxidants and the formation of a green molybdenum (V) complex, which has absorption at 695 nm. The total antioxidant capacity observed in the methanolic extract of aerial parts and tuber of *S. wightii* was 303.23 and 312.49 nM GAE/g extract respectively (Table 1).

Table 1. Phosphomolybdenum assay of different concentrations of methanolic extracts of *Stephania wightii*

S.No	Plant parts used	Phosphomolybdenum assay
1	Aerial parts	252.49±0.26
2	Tuber	312.49±0.05

Values expressed as nM GAE/g extract

Figure 1 presents the reductive capabilities of the methanol extracts of aerial parts and tuber of *S. wightii*. At 10-50 µg/ml, reducing power of aerial parts methanolic extract of *S. wightii*

was found to be 0.55, 0.57, 0.65, 0.73 and 0.93nm respectively. In tuber extract, it was found to be 0.59, 0.62, 0.68, 0.74 and 1.05nm respectively. The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power of test solution. Greater absorbance at 700 nm indicated greater reducing power. The reducing power of the extract might be due to their hydrogen- donating ability. Since tuber contain several phytochemicals and it react with radicals to stabilize and terminate radical chain reactions.

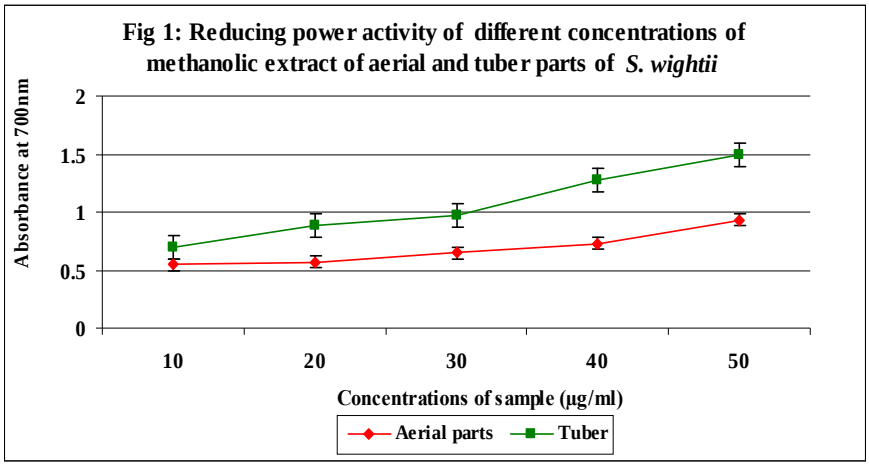

Fig 1: Reducing power activity of different concentrations of methanolic extract of aerial and tuber parts of *S. wightii*

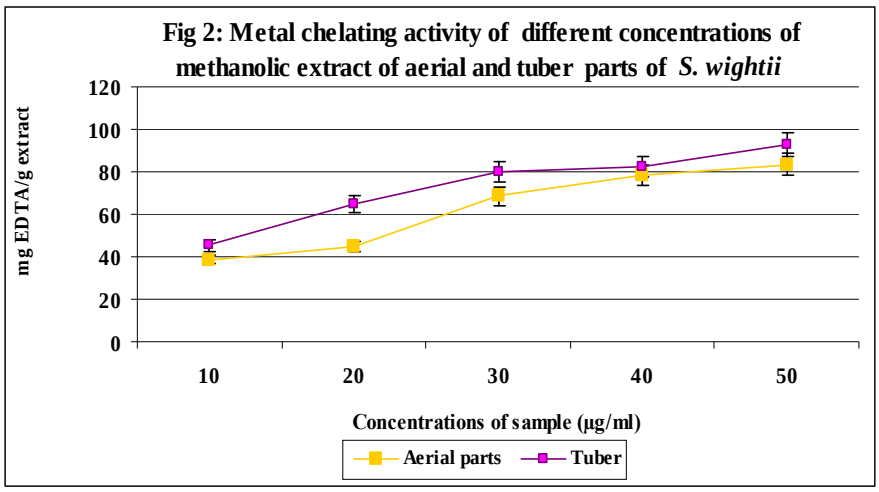

Fig 2: Metal chelating activity of different concentrations of methanolic extract of aerial and tuber parts of *S. wightii*

The chelating effects of methanolic extracts of *S. wightii* on ferrous ions increased with increasing concentrations (Fig. 2). At the concentration of 50µg/ml, the aerial parts and tuber extracts have exhibited maximum chelating effects of 83.49 and 92.72 mg EDTA/g extract, respectively. The results of the present study suggest that methanolic tuber extract of *S. wightii* exhibits good chelating activity on ferrous ions. Metal ion chelating activity of an antioxidant molecule prevents oxygen radical generation and the consequent oxidative

damage. Metal ion chelating capacity plays a significant role in antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in LPO [8] (Duh *et al.*, 1999).

The methanolic stem extracts of Menispermaceae members like *Anamirta cocculus, Coscinium blumeanum* and *Fibraurea tinctoria* showed appreciable antioxidant effects [9]. The alkaloidal fraction (AFCP) of roots of *Cissampelos pareira* possesses strong antioxidant activity which was revealed by its ability to scavenge the stable free radical DPPH and superoxide ions. AFCP showed a concentration dependent anti-radical activity by inhibiting DPPH radical with an IC_{50} value of 63.44µg/mL and it was also found to scavenge the superoxide radical generated in riboflavin-NBT-light system *in-vitro* and IC_{50} value was found to be 31.99µg/mL.

Antioxidant activity *Tinospora cordifolia* of was evaluated for total phenolic content (TPC), antioxidant (AOA) and their total flavanoid content (TFC) using the DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) screening assay. It showed high TFC (29.3µg/mg) and high AOA (87.86%) [10]. Mathew *et al.*, [11] have reported that the extract of *T. cordifolia* has been shown to inhibit the lipid peroxidation and superoxide and hydroxyl radicals *in vitro*. Moreover, administration of the extract partially reduced the elevated lipid peroxides in serum and liver as well as alkaline phosphatase and glutamine pyruvate transaminase. This indicates the use of *T. cordifolia* extract in reducing the chemotoxicity induced by free radical forming chemicals.

In vitro antioxidant potential of methanolic stems bark extract of *Sphenocentrum jollyanum* was evaluated by using superoxide ($O2^{\cdot-}$) and hydrogen peroxide (H_2O_2) radical scavenging assay. It inhibits superoxide anions and hydrogen peroxide radicals with IC_{50} values of 13.11 and 30.04µg/ml, respectively. The results indicated that methanolic extract of *S. jollyanum* possessed strong antioxidant property [12].

CONCLUSION

The previous reports revealed that the *Stephania wightii* used in the traditional medicine and it is a good source in the discovery of natural pharmaceutical drugs. The findings of the present study suggest that *Stephania wightii* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing oxidative stress related

degenerative diseases. Further work on isolation and identification of active compounds and its efficacy needs to be carried out.

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