Research Article

Free Radical Scavenging Activity of *Stephania wightii* (Arn.) Dunn (Menispermaceae) - An Endemic Medicinal Plant

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ABSTRACT

A number of Indian medicinal plants have been used for thousands of years in traditional system of medicine. *Stephania wightii* is an important member of Menispermaceae family. It is an endemic to the southern Western Ghats, India. The aim of this study was to investigate the free radical scavenging activity of *S. wightii*. The methanolic extract of aerial parts and tuber of *S. wightii* were used for this study. The extracts were assayed for radical scavenging activity, using the stable free radical diphenylpicrylhydrazyl, OH radical and nitric oxide radicals scavenging activity. The IC50 values of methanol extract of aerial parts and tuber on DPPH radical, hydroxyl radical and nitric oxide radicals scavenging were found to be 10-30µg/ml, respectively. The free radical scavenging activity of the plant extracts may be due to the presence of phytoconstituents. In all the methods, the methanolic tuber extract has exhibited the good scavenging activity compared to the aerial parts extract. It may have promising antioxidant agents and may also helpful in the treatment of the diseases caused by free radicals.

Keywords: DPPH free radicals, hydroxyl free radicals, nitric oxide free radicals, *stephania wightii.*

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INTRODUCTION

Medicinal plants form an important part of Ayurveda, practiced in India and other traditional systems of medicine used by two thirds of the world population. Realizing their importance, extracts of plant parts are extensively explored for different bioactivities including antioxidants [1]. Recent interest has increased considerably in finding naturally occurring antioxidants for use in food or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity [2]. Many medicinal plants contain large amounts of phytochemicals, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [3]. The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Vitamin C, vitamin E, selenium, b-carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants. Apart from these, in plants we get secondary metabolites such as alkaloid, flavonoids and terpenoids play an important role in the defense against free radicals [4].

The family Menispermaceae is well known as an important source of isoquinoline alkaloids, one of the largest groups of natural products which display interesting pharmacological activity [5]. The genus *Stephania* belongs to this family, a large family of about 65 genera and 350 species, distributed in warmer parts of the world. The *Stephania* plants have been proven to be a rich source of substances of

phytochemical interest. Many interesting compounds have been isolated, including aporphine alkaloids, hasubanan alkaloids, isoquinoline alkaloids, morphine alkaloids, protoberberine alkaloids, miscellaneous alkaloids and miscellaneous compounds [6]. *Stephania wightii* is a slender climber with peltate and membranous leaves. The flowers are umbelliform cymes while inflorescence is axillary and arising from old leafless stem. In traditional medicine, most of the plants of the genus *Stephania* have been used to treat a wide variety of ailments such as dysentery, pyrexia, tuberculosis, diarrhea, dyspepsia, urinary diseases, abdominal ills, asthma, ascariasis, dysmenorrhea, indigestion, wounds, head-ache, sore-breasts and leprosy [7].

Habit of *Stephania wightii*

In this study, DPPH radical scavenging activity, Hydroxyl radical scavenging activity and Nitric oxide radical scavenging activity have been used to measure the antioxidant property of the plant sample *S. wightii*.

MATERIALS AND METHODS

DPPH radical scavenging activity

The scavenging effect of extracts on DPPH radicals was determined according to the method of Shimada *et al.* [8]. Various concentrations of sample (4 ml) were mixed with 1 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. The percentage inhibition was calculated according to the formula: (A0-A1)/A0]×100, where A0 was the absorbance of the control and A1 was the absorbance of the sample.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of extracts was assayed by the method of Smirnoff and Cumbes, [9]. The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM $FeSO_4$, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and varied concentrations of the extracts. After incubation for 1 hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The scavenging activity of hydroxyl radical effect was calculated as follows: [1-(A1-A2) / A0] x 100, where A0 is absorbance of the control (without extract) and A1 is the absorbance in the presence of the extract, A2 is the absorbance without sodium salicylate.

Scavenging of Nitric oxide radical activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction [10]. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration (10-50 µg/ml) of the methanol extracts of plant sample dissolved in phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25ºC for 5 hr. Control experiments without the test compounds, but with equivalent amounts of buffer were conducted in an identical manner. After 5hr, 0.5ml of incubation solution was removed and diluted with 0.5ml of Griess reagent (1% sulphanilamide, 2% 0-phosphoric acid and 0.1% napthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was read at 546nm. The experiment was repeated in triplicate.

RESULTS AND DISCUSSION

The effects of methanolic extracts of aerial parts and tubers of *S. wightii* was evaluated for its antioxidant activity on different *in vitro* models like DPPH, Hydroxyl radical and Nitric acid radical scavenging activities in a concentration dependent manner. Comparing aerial part extract, there was an increased activity found in tuber extract. The aerial and tuber parts of *S. wightii* were studied for DPPH radical scavenging activity. The DPPH radical scavenging activity increased with increasing concentrations of 10,20,30,40 and 50 µg/ml respectively. The aerial parts have 48.71 %, 62.38 %, 68.29 %, 74.53 %

scavenging activity and the tuber part have 46.52%, 69.47%, 75.19%, 80% and 83.78% scavenging activity (**Table 1**). The IC50 values of aerial parts and tuber were found to be 22.52 and 16.40µg/ml respectively. These results indicated that *S. wightii* methanolic extract of tuber part has exhibited the high ability to quench the DPPH radical, which indicated that tuber extract was good antioxidant with radical scavenging activity.

Table 1: DPPH radical-scavenging activity of different concentrations of *Stephania wightii.*

S.No	Plant parts used	Concentrations of sample (µg/ml)	% inhibition ± SD	IC 50 value (µg/ml)
1	Aerial parts	10	19.52±0.71	22.32±0.28
		20	48.71±0.24	
		30	62.38±0.62	
		40	68.29±0.34	
		50	74.53±0.73	
2	Tuber	10	46.52±0.72	16.49±0.12
		20	69.47±0.36	
		30	75.19±0.47	
		40	80±0.14	
		50	83.78±0.26	

Hydroxyl radical scavenging activity of aerial and tuber extracts of *S. wightii* were presented in (**Table 2**). Both the extracts have got profound antioxidant activity. The percentages of Hydroxyl radical scavenging activity of aerial and tuber extracts were increased with increasing concentration. The activity of extract was found to be increased in a dose-dependent manner from 21 % to 65 % at a concentration of 10-50 µg/ml in aerial parts, whereas in the tuber extract has 44 % to 82 % activity. The extracts exhibited an IC50 value of 27.23 µg/mL and 12.76 µg/mL respectively. *In vitro* antioxidant studies of the two extracts aerial and tuber parts, the extent of nitric oxide radical scavenging at different concentrations (10-50 µg/ml) of *S. wightii* extracts was also measured. The radical scavenging effect was found to increase with increasing concentrations. The plant aerial extract showed their activity of 92.49 % with IC50 values of 12.23 µg/ml. The tuber extract of *S. wightii* indicated that it had more radical scavenging ability at 98.72 % at 50 µg/ml. IC 50 value of tuber extract found to be 9.47 µg/ml (**Table 3**).

The stable radical DPPH has been used widely for the determination of primary antioxidant activity [11, 12]. The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of anti-oxidants [13]. The present study indicates that the IC50 value of methanol tuber extract is quite high when compared to the standard which may be attributed to its poor proton study carried out by Hasan *et al*. [14] has shown the DPPH radical scavenging activity of *T. cordifolia* aerial parts with an EC50 value of 0.02 mg/ml. The difference in the EC50 value can be attributed to the distribution of secondary metabolites that may fluctuate between different plant organs [15].

The alkaloidal fraction of roots of *Cissampelos pareira* (Menispermaceae) was screened for *in vitro* antioxidant activity in mice. It possess strong antioxidant activity which was revealed by its ability to scavenge the stable free radical DPPH, superoxide ion and to inhibit lipid peroxidation in rat liver

homogenate induced by iron/ADP/Ascorbate complex [16]. The stem bark of *Tinospora crispa* was extracted with methanol and the extract was partitioned with petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions. The extracts were subjected to antioxidant screening by DPPH free radical scavenging activity. In this butylated hydroxytoluene (BHT) and ascorbic acid were used as antioxidant standard. By DPPH assay, it is found that the carbon tetrachloride soluble fraction of *T. crispa* showed strong antioxidant activity with the IC50 value 30 µg/ml. Besides petroleum ether and chloroform soluble fractions also showed free radical scavenging activity with the IC50 value 70 and 75 µg/ml, respectively [17].

Table 2: Hydroxyl radical-scavenging activity of different concentrations of *Stephania wightii.*

S.No	Plant parts used	Concentrations of sample (µg/ml)	% inhibition ± SD	IC 50 value (µg/ml)
1	Aerial parts	10	21.46±0.45	27.23±0.37
		20	33.47±0.46	
		30	52.91±0.13	
		40	64.12±0.05	
		50	65.49±0.84	
2	Tuber	10	44.17±0.79	12.76±0.26
		20	58.73±0.48	
		30	67.41±0.33	
		40	79.32±0.67	
		50	82.79±0.07	

Table 3: Nitric oxide scavenging activity of different concentrations of *Stephania wightii.*

S.No	Plant parts used	Concentrations of sample (µg/ml)	% inhibition ± SD	IC 50 value (µg/ml)
1	Aerial parts	10	48.76±0.03	12.23±0.32
		20	64.81±0.48	
		30	78.42±0.59	
		40	88.63±0.58	
		50	92.49±0.43	
2	Tuber	10	55.52±0.12	9.47±0.51
		20	62.76±0.27	
		30	79.84±0.13	
		40	85.34±0.07	
		50	98.72±0.14	

CONCLUSION

In present study, antioxidant activities of the methanolic extract of aerial parts and tuber of *S. wightii* was investigated. The extracts were found to possess radical scavenging and antioxidant activities, as determined by scavenging effect on the DPPH, OH radical and Nitric oxide radical scavenging activity. Generally, IC50 values of lower than 100 µg/ml indicated that the extracts were effective in antioxidant properties. Methanol extract is the most effective DPPH, hydroxyl radical and Nitric oxide scavengers. This holds promise to identify the potential sources of natural compounds with promising antioxidant activity. Further research is needed for the isolation and identification of the active components in these extracts can serve as natural sources to develop beneficial herbal therapies in future.

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