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EVALUATION OF *IN VITRO* ANTIOXIDANT POTENTIAL OF METHANOLIC EXTRACT OF *COCCULUS HIRSUTUS* (L.) DIELS

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ABSTRACT

Plants are a potential source of natural antioxidants. Antioxidants protect from the potentially damaging oxidative stress, which is a result of an imbalance between the formation of ROS (Reactive oxygen species) and the body antioxidant defense. The present study was directed to investigate the antioxidant activity of methanolic extract of different plant parts of *Cocculus hirsutus.* The antioxidant activity of the methanolic extracts of *in vivo* and *in vitro* plant parts of *Cocculus hirsutus* was determined on the basis of their scavenging activity of the stable 2, 2- diphenyl-2-picryl hydrazyl (DPPH) free radical. In the present study, methanolic extracts of the different plant parts and callus of *Cocculus hirsutus* showed potential free-radical scavenging activity. The methanolic extract of *Cocculus hirsutus* and Ascorbic acid (standard) showed DPPH free radical scavenging activity in a concentration range of 10-1000 µg/ml and its inhibition ranged from 36.92 - 84.1% and that of standard was 49.68 - 94.60%. Methanolic extracts of callus were found to have maximum IC_{50} value (151.65 µg/ml) followed by leaf (121.45 µg/ml) and stem (37.83 µg/ml). Standard antioxidant used was Ascorbic acid (1mg/ml) having 24.79 µg/ml IC_{50} value. The highest DPPH radical scavenging activity was detected in methanolic extract of stem followed by methanolic extract of leaf and callus respectively. These activities are less than that of standard ascorbic acid. The results of DPPH scavenging activity assay in this study indicates that the methanolic extract of stem was potently active than other plant parts of *Cocculus hirsutus* and the methanolic extract of the study species is effective in scavenging free radicals and has the potential to be powerful antioxidant.

Key words: *Cocculus hirsutus,* Antioxidant and DPPH free-radical scavenging assay

INTRODUCTION

Cocculus hirsutus (L.) Diels is an important medicinal plant belonging to the family Menispermaceae. It is commonly known as Jal-jamni. [1] Traditionally, the plant was patronized for its unique property of healing all types of cuts, wounds and boils. It is pungent in taste and has post digestive effect and hot potency. It alleviates 'kapha' and 'vata' doshas. It possesses light, oily and slimy attributes. It is an aphrodisiac and is used in diseases like arthritis, cystitis and diabetes mellitus. Root is bitter and used as alterative, laxative, diuretic, antiperiodic in fever, in malaria, joint pains, in the treatment of skin diseases, constipation and kidney problems.[2] It has mild laxative digesting and appetite stimulant properties. It is used in anorexia, with great benefit. It also works well in asthma, cough and cold. The plant of *Cocculus hirsutus* has been reported to contain essential oil, ginnol, glycosides, sterols and alkaloids. [3] Preliminary phytochemical analysis of the leaves showed presence of alkaloids, phenolic compounds, flavonoids, glycosides, and carbohydrates. The phytochemical studies showed the presence of bis-benzyl isoquinoline alkaloids; viz. shaheenine [4], cohirsinine, hirsutine, jamtinine, jamitine- *N* -oxide, cohirsine, cohirsitine and haiderine [5], which are isolated from stem and roots. The alkaloids present in the leaves of *C. hirsutus* are D-trilobine, DL-coclaurine, isotrilobine, (+) syringaresinol and protoquericitol [6]. Roots are reported to contain D-

trilobine and coclaurine, sterols and resins. [7] Plants are a potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are secondary metabolites of plants.[8] Living cells possess a protective system of antioxidants which prevents excessive formation and enables the inactivation of ROS (Reactive oxygen species). Antioxidants protect from the potentially damaging oxidative stress, which is a result of an imbalance between the formation of ROS and the body antioxidant defense. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods because of their potential in health promotion and disease prevention, and their high safety and consumer acceptability. [9] In search of novel sources of antioxidants in the last years, medicinal plants have been extensively studied for their antioxidant activity. As the role of free radicals has been documented in many plant species, the present study was directed to investigate the antioxidant activity of methanolic extract of different plant parts of *Cocculus hirsutus.*

MATERIALS AND METHODS

Collection of Plant material: Plant parts of *Cocculus hirsutus* were collected from the Kulish Smriti Van, Jaipur and specimen was compared with the voucher specimen available at the Herbarium of Department of Botany, University of Rajasthan, Jaipur. The fresh plant samples (*C. hirsutus*: leaf and stem) were collected and washed individually under running tap water to remove soil particles and other dirt. Furthermore, *in vitro* callus obtained on MS medium fortified with IAA (0.5 mg/l) and BAP (0.5 mg/l) was also taken for the present study. The *in vivo* leaf and stem were dried in the laboratory at room temperature for 7 days while the callus was dried at 60ºC for 2 days in an oven. All dried samples were ground well into a fine powder in a mixer grinder. The powder was stored in air tight bottles at room temperature before extraction.

Preparation of the plant extracts: A fixed weight (10 gm) of each powdered material i.e. leaf, stem and callus was soxhlet extracted in 250 ml of 80 % methanol for 72 hours. At the end of extraction each extract was passed through Whatman No.1 filter paper and evaporated under vacuum. All extracts were stored at 4ºC in a refrigerator until used for further analysis.

Chemicals: DPPH (Sigma Aldrich, Mumbai), methanol, ascorbic acid (Merk, Germany). All other reagents were of analytical grade.

DPPH Assay: The antioxidant activity of the methanolic extracts of *in vivo* and *in vitro* plant parts of *C. hirsutus* was determined on the basis of their scavenging activity of the stable 2, 2- diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 1ml of each solution of different concentrations (10-1000 µg/ml) of the extracts was added to 2 ml of 0.002% methanolic DPPH free radical solution. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (10-1000 µg/ml). The method described by Hatano *et al*, [10] was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

$$\%\text{radical scavenging activity} = \frac{(\text{absorbance of blank} - \text{absorbance of sample})}{\text{absorbance of blank}} \times 100$$

From calibration curves, obtained from different concentrations of the extracts, the IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. [11]

Procedure:

1. At first, eight test tubes were taken to make aliquots of eight concentrations (10, 50, 100, 200, 400, 600, 800 and 1000µg/ml) with the samples.
2. Each plant extract and ascorbic acid were weighed accurately and dissolved in methanol to make required concentrations by dilution technique. Here ascorbic acid was taken as standard.
3. DPPH was weighed and dissolved in methanol to make 0.002% (w/v) solution. To dissolve homogeneously magnetic stirrer was used.
4. After making the desired concentrations 2 ml of 0.002% DPPH solution was applied on each test tube by pipette.
5. The room temperature was recorded and the test tubes were kept for 30 min in light to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only methanol was taken as blank.
6. After 30 minutes, the absorbance of each test tube were taken by a UV Spectrophotometer.
7. IC_{50}'s were measured from % Inhibition *vs.* Concentration graphs.

RESULTS AND DISCUSSION

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, methanolic extracts of the different plant parts and callus of *C. hirsutus* showed potential free-radical scavenging activity. Methanolic extracts of different plant parts of *C. hirsutus* showed different levels of DPPH activity. Results are summarized in Table-1, Figure 1. In DPPH radical-scavenging activity assay, the radical scavenging activity of the methanolic extract of the different plant parts of *C. hirsutus* increased with increasing concentration (Table-1). The methanolic extract of *C. hirsutus* and Ascorbic acid (standard) showed DPPH free radical scavenging activity in a concentration range of 10-1000 µg/ml and its inhibition ranged from 36.92 - 84.1% and that of standard was 49.68 - 94.60%. Methanolic extracts of callus were found to have maximum IC_{50} value (151.65 µg/ml) followed by leaf (121.45 µg/ml) and stem (37.83 µg/ml). Standard antioxidant used was Ascorbic acid (1mg/ml) having 24.79 µg/ml IC_{50} value. The results are presented in Figure 2. The highest DPPH radical scavenging activity was detected in methanolic extract of stem followed by methanolic extract of leaf and callus respectively. These activities are less than that of standard ascorbic acid. The results of DPPH scavenging activity assay in this study indicates that the methanolic extract of stem was potently active than other plant parts of *Cocculus hirsutus.* This suggests that the methanolic extract of *Cocculus hirsutus* contains compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Similar results in which methanolic extract possesses strong antioxidant activity was reported in *Ionidium suffruticosum.* [12] The antioxidant activities of medicinal plants are due to the active constituents present in them. It is also proved that *C. hirsutus* contains certain constituents like tannins which are responsible for its antioxidant activity. Many reports suggest that plants which are having more phenolic content show good antioxidant activity, hence, there is a direct correlation between total phenol content and antioxidant activity. [13-14] The antioxidant capacity possessed by phenolic compounds is mainly due to their redox properties, which permit them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity viz. *Persea americana* and *Cnidosculus aconitifolius* [15], *Pleurotus ostreatus* [16] and *Drosera indica.*[17]

CONCLUSION

The results of DPPH scavenging activity assay in this study indicate that the methanolic extract of stem was potently active than other plant parts of *Cocculus hirsutus.* Maximum antioxidant potential was found in methanolic extract of stem while minimum was found in methanolic extract of callus. In the present study, it is found that methanolic extract of *Cocculus hirsutus* showed concentration dependent free radical scavenging activity and this antioxidant effect may be due to tannins and saponins, highly responsible secondary metabolite for antioxidant activities. Thus, the *Cocculus hirsutus,* methanolic extract as promising natural sources of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free radical-mediated diseases. However, pharmacognostical studies are suggested to confirm the antioxidant ability before going for commercialization.

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Table 1: Percentage DPPH radical scavenging activity of methanolic extract of *Cocculus hirsutus* plant parts *in vivo* and *in vitro*

S. No	Conc. (µg/ml	Percentage inhibition by methanolic extracts of *Cocculus hirsutus* ± S.E.M			
		Standard	**Stem**	**Leaf**	**Callus**
1	10	49.68±1.092	39.6±1.112	36.92±1.114	49.1±1.105
2	50	63.25±1.122	49.60±1.107	58.31±1.118	57.8±1.110
3	100	63.75±1.104	52.10±1.115	64.50±1.122	63.7±1.120
4	200	86.87±1.124	59.90±1.078	65.30±1.126	70.3±1.112
5	400	89.93±1.086	64.30±1.121	66.30±1.130	79.7±1.130
6	600	90.29±1.120	67.70±1.086	67.47±1.082	81.3±1.134
7	800	91.55±1.103	76.60±1.128	69.09±1.109	83.9±1.136
8	1000	94.60±1.107	80.10±1.131	69.90±1.111	84.1±1.138
		IC_{50}=24.79 µg/ml	**IC_{50}=37.835 µg/ml**	**IC_{50}=121.455 µg/ml**	**IC_{50}=151.65 µg/ml**

**Data presented as the mean ± SEM of three measurements.*

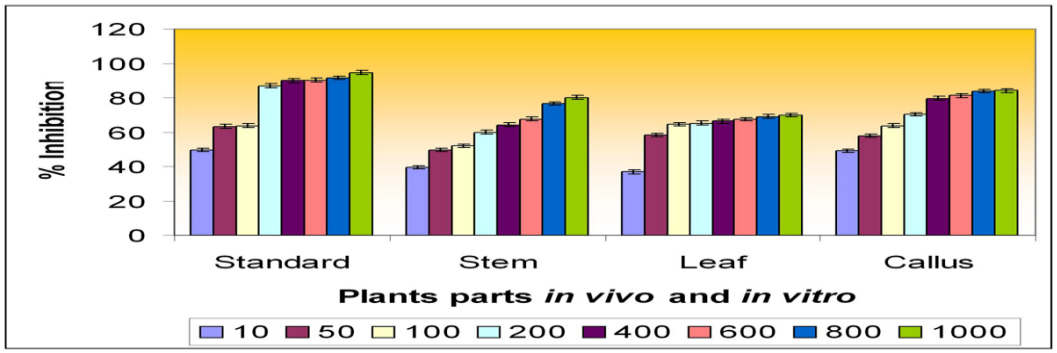

Figure 1. Estimation of DPPH free radical scavenging activity.

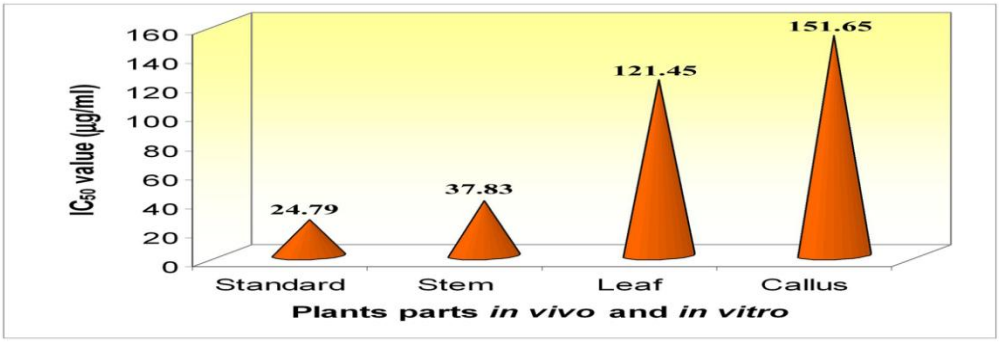

Figure 2. Estimation of IC_{50} value.

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