

ANTI OXIDATIVE ACTIVITY OF THE ETHANOL EXTRACT OF *DESMODIUM GYRANS* DC. LEAVESS. GOPALAKRISHNAN*, R. RAJAMEENA¹

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Received: 02 Aug 2013, Revised and Accepted: 01 Oct 2013

ABSTRACT

Objective: *Desmodium gyrans* DC is commonly known as "Thozhukanni" in Tamil and "Namaskari" in Sanskrit. It is widely used as an antidote, cardiac-tonic and for wound healing. The antioxidant activity of the ethanolic extract of *D. gyrans* has been studied for its free radical scavenging property on different *in-vitro* models and biochemical parameters on *in-vivo* models.

Methods: Four groups of rats were used in each method. Two test groups received *D. gyrans* ethanolic extract of the leaves at doses of 200 mg/kg and 400 mg/kg; standard group received silymarin (25mg/kg), and the control group received only vehicle in *in-vivo*. The standard group received ascorbic acid (100µg/kg). Control group received carboxy methyl cellulose in *in-vitro* model. On the 8th day animals were sacrificed and antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) were estimated in liver homogenate. In free radical scavenging activity assay based on neutralization of DPPH, superoxide radical, hydroxyl radical were determined spectrophotometrically.

Results: The extract showed good dose dependent antioxidant activity as compared to standard. It exhibits low IC₅₀ value 36.6 ± 1.47 for DPPH, 55.3 ± 1.29 for superoxide radical, 43.7 ± 2.43 for hydroxyl radical. A significant (p<0.001) increase in SOD and CAT were noticed.

Conclusion: The results showed that *Desmodium gyrans* ethanolic extract of the leaves has potent antioxidant activity.

Keywords: *Desmodium gyrans* DC, Superoxide dismutase, Catalase, DPPH, Antioxidant.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals [1] like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second [2,3]. When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates [4-6] and this leads to a number of physiological disorders. Free radicals are involved in the development of degenerative diseases. They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorder, and in the process of aging [7].

Antioxidant is a molecule which terminates the chain reaction by removing free radical intermediates. Plants and animals maintain complex system of multiple type of antioxidant. The natural plant based antioxidants have played an important role in the maintenance of human health for the past three decades [8].

Desmodium gyrans DC (Fam. *Fabaceae*) is commonly known as 'Thozhukanni' in Tamil and 'Namaskari' in Sanskrit. It is a very useful remedy in adhering the cut ends of flesh and healing it. This is also used in curing snake bite poisons. It is used as an antidote, cardiac-tonic and for wound healing. The leaves and roots of the plant are used for wound healing [9]. The related spices are used as herbal medicines. For example, *Desmodium gangeticum* has been demonstrated to possess antioxidant, anti-nociceptive, anti-inflammatory [10], anti-emetic [11], cardio-protective [12] and anti-ulcer effects [13]. Gangetin [14], salicylic acid, rutin [15], desmodin, gangetinin [16], chlorogenic acid and caffeic acid have been isolated from *Desmodium gangeticum* [17]. *Desmodium triflorum* has been reported to possess antioxidant [18], analgesic and anti-inflammatory activities [19] have been isolated and identified (7 alkaloids) from the roots of *Desmodium gangeticum* [20].

The present study has been directed to investigate the antioxidant activity of *Desmodium gyrans* DC the ethanolic extract of in different *in vitro* and *in vivo* models.

MATERIALS AND METHODS

Plant materials

The plant was collected in the month of September from Trivandrum, Kerala, India and was identified by Dr. V.Chelladurai, Research Officer (Botany), Central Council of Research in Ayurveda and Siddha, Government Siddha Medical College, Palayamkottai, Tamilnadu, India. A voucher specimen (MSU/PHAR/HER-139) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli - 627 012.

Extraction of plant material

The leaves of *Desmodium gyrans* DC were dried under shade and powdered. The dried powder (500g) was successively extracted using petroleum ether (40°-60°C), benzene, chloroform, ethanol and water by using a Soxhlet apparatus. The last trace of the solvent was removed under reduced pressure by rotary evaporator. The dried crude ethanol extract has been used for the study [21].

Animals

Wistar albino rats of either sex weighing between 180 g and 200 g were selected for the acute toxicity and antioxidant activity studies. The study was approved by the Institutional Ethics Committee for animal experimentation (KMCRET/PH.D/17/2012-13), KMCH college of Pharmacy, Coimbatore. The animals were stabilized for 1 week. They were maintained in standard conditions at room temperature, 60 ± 5% relative humidity and 12 h light dark cycle. They were given standard pellet diet supplied by Hindustan Lever Co., Mumbai and water *ad libitum* throughout the course of the study.

Drugs and chemicals

Silymerin (Silybin 140) was purchased from Microlabs Limited, Goa and Thioacetamide from Lova Laboratories Pvt. Ltd., Mumbai. All others chemicals used in this study were of analytical grade.

Antioxidant activity studies

In-vivo biochemical parameter models

Rats were randomly divided into four groups of six animals each and each group was kept in a separate cage. All the groups were treated orally for 7 days [22].

Group I served as control and was treated with vehicle (0.5% carboxyl methyl cellulose). Group II served as standard and was treated with silymarin (25 mg/kg bw). Group III was treated with ethanol extract of the leaves of *Desmodium gyrans* (200 mg/kg bw). Group IV was treated with ethanol extract of the leaves of *Desmodium gyrans* (400 mg/kg bw).

On the 8th day, the rats were sacrificed for the determination of antioxidant enzymes. Liver was dissected out, washed in the ice-cold saline, and the liver homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and supernatant was used for the assay of antioxidant enzymes, namely glutathione peroxidase (GPX) [23], glutathione S-transferase (GST) [24], glutathione reductase (GRD) [25], superoxide dismutase [26], catalase (CAT) [27], and lipid peroxidation (LPO) [28].

In-vitro free radical scavenging models

Determination of DPPH radical scavenging activity

The capacity of the samples in scavenging 1,1 diphenyl -2- picryl hydrazyl radical (DPPH) was measured according to the method of Yamaguchi *et al*[29]. The stock solutions (10 mg/ml) were diluted with 70% ethanol into different concentrations, 200 µg/ml, 400 µg/ml. The wells in a 96 well plate were pipetted with 25 µl of sample solutions and 171 µl of 0.3 mM DPPH solution, and then left to stand at room temperature for 30 min in the dark before the absorbance was measured at 517 nm. The inhibition percentage (%) of radical scavenging activity was calculated according to the following equation:-

$$\text{Inhibition (\%)} = (A_0 - A_s) / A_0 \times 100$$

Where A_0 is the absorbance of the control and A_s is the absorbance of the sample at 517 nm.

IC₅₀ value was the attentive concentration at which 50% of DPPH radicals were scavenged and obtained by interpolation from linear regression analysis. A lower IC₅₀ value indicated a greater antioxidant activity.

Determination of superoxide radical scavenging activity

Superoxide scavenging was determined by the nitro blue tetrazolium reduction method [30]. The reaction mixture consisted of ethylene diamine tetra acetic acid (EDTA; 6µM), sodium cyanide (µg), riboflavin (2µM), nitro blue tetrazolium (50 µM), various concentrations of *Desmodium gyrans* ethanolic extracts (200 µg/ml, 400 µg/ml) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent visible light for 15 minutes, and the optical density was measured at 530 nm before and after the illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and experimental tubes.

Determination of hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to a modified method of Yamaguchi *et al*[29] Stock solutions of EDTA (1mM), FeCl₃ (10mM), ascorbic acid (1 mM), H₂O₂ (10 mM) and deoxy ribose (10 mM) were prepared in distilled

deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of FeCl₃, 0.1 ml of H₂O₂, 0.36 ml of deoxy ribose, 1.0 ml of *Desmodium gyrans* ethanolic extract (200 µg/ml, 400 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid sequence. The mixture was then incubated at 37°C for 1 hour. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10g/100g TCA and 1.0 ml of 0.5g/100g TBA (in 0.025M NaOH containing 0.025g/100g TBA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as percentage inhibition of deoxyribose degradation.

Statistical analysis

Results are presented as mean ± standard error. Statistical analysis of experimental result was based on analysis of variance followed by Dunnett's 't' test.

RESULT AND DISCUSSION

Free radical scavenging activity

The extract showed good antioxidant activity in *in-vitro* free radical scavenging models. Various concentrations ranging from 200 µg/ml and 400 µg/ml of ethanol extract of the leaves of *D. gyrans* were tested for their antioxidant activity in various *in-vitro* models (Table 1). Free radicals were scavenged by the test compounds in a concentration dependent manner with in the given range of concentration (IC₅₀) in the DPPH (36.30 ± 1.47%), superoxide scavenging activity (55.30 ± 1.29%), and hydroxide scavenging activity (43.70 ± 2.43%).

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals [31]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc.

Antioxidant can act by converting the unpaired electrons to paired ones. The dose dependent inhibition of DPPH (Figure 1) indicates that the ethanolic extract of the leaves of *D. gyrans* causes reduction of DPPH radical [32]. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of *D. gyrans* extract towards generation of superoxide in the *in vitro* reaction mixture[33].

Biochemical parameter

Enzymatic antioxidants like, SOD, CAT activities in liver were found significantly reduced (p<0.001) when compared to that of the control and the standard. There is a significant increase in activity from lower to higher dose. The dose of 400 µg/ml treated group was found to be effective similar to silymarin treated group (Table 2). The SOD and CAT activities of 400 µg/ml dose were found to be almost the same as that of silymarin.

A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom [34,35]. The reducing property of *Desmodium gyrans* implies that it is capable of donating hydrogen atom in a dose dependent manner.

Table 1: Free radical scavenging activities of ethanol extract of the leaves of *Desmodium gyrans* DC

Extract Concentration (µg/ml)	Inhibition (%)		
	DPPH	Superoxide	Hydroxyl radical
400µg/ml	86.49 ± 3.46	87.66 ± 3.51	78.63 ± 4.62
200µg/ml	81.67 ± 2.34	79.41 ± 3.65	74.41 ± 4.43
Ascorbic acid (100µg/ml)	95.11 ± 4.22	87.32 ± 0.87	94.44 ± 4.71
IC ₅₀	36.6 ± 1.47	55.3 ± 1.29	43.7 ± 2.43

Values are mean ± SD in each group (n=6)

Table 2: Enzymatic biochemical parameter activities of ethanol extract of *Desmodium gyrans* DC

Groups	SOD	CAT
Control	9.06 ± 0.06	55.93 ± 1.57
Silymarin (25 µg/ml)	8.65 ± 0.12***	55.31 ± 1.64
200 µg/ml	5.15 ± 0.09***	35.39 ± 1.60***
400 µg/ml	7.18 ± 0.13***	42.79 ± 17.8***

Values are mean ± SD in each group (n=3) ***p<0.001, compared with control

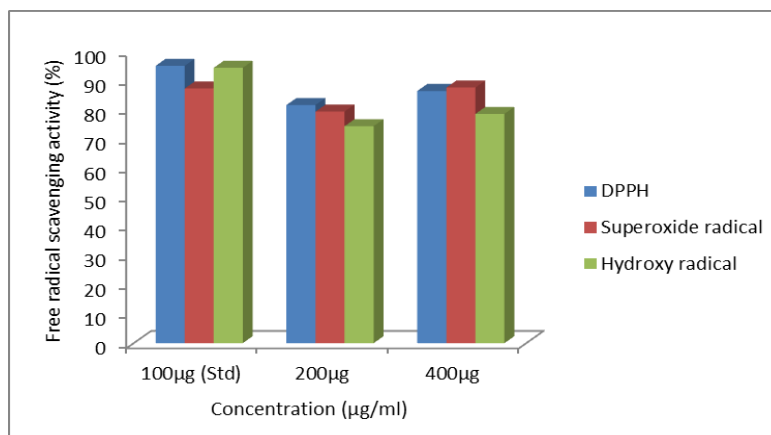


Fig. 1: Free radical scavenging activity of different concentration of the ethanolic extract of the leaves of *D. gyrans* DC and the reference antioxidant, ascorbic acid.

CONCLUSIONS

The results obtained thus indicate that the ethanolic extract of the leaves of *Desmodium gyrans* has potent antioxidant activity, achieved by scavenging abilities observed against DPPH, superoxide radical and hydroxyl radical. Also the wide use of the plant in the Indian System of Medicine for wound healing may be in part due to the antioxidant potential of the ethanolic extract of the leaves.

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