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Free radical scavenging activity of *Gymnostachyum warriearanum* leaves extracts

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Abstract
The present study is to evaluate the *in vitro* free radical Scavenging activity of methanolic extract of *Gymnostachyum warrieranum* leaves. Here we study the *in vitro* free radical scavenging activity by using DPPH radical scavenging and hydroxyl radical scavenging methods. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. The present findings exhibited a concentration dependent free radical scavenging activity and it can be concluded that *Gymnostachyum warrieranum* has *in vitro* antioxidant activity.

Keywords: Acanthaceae, free radical, *Gymnostachyum warrieranum*, DPPH

1. Introduction
Medicinal plants have been playing a vital role on the health and healing of man since down of human civilization. In spite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine [1].
There are several reasons for researching medicinal plants: to gain knowledge about the medicinal potential of native plant diversity, to establish a rational basis for the medicinal use of particular plant species, to develop herbal medicines that are low-cost and exhibit relevant activity, to discover new prototypes for drugs and to gain information regarding traditional medicines [2].
The genus Gymnostachyum of family Acanthaceae consist of beautiful flowering shrubs and herbs. It can be used as an ornamental plant as well as ground cover plant in landscape designing. Antioxidant supplements or foods rich in medicinal plants may be used to help the human body in reducing the oxidative damage by free radicals and active oxygen [3]. Since the imbalance between antioxidants and free radicals leads to oxidative stress which may result in tissue injury and subsequent diseases such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension etc., the development and utilization of more effective antioxidants of natural origin are desired. *Gymnostachyum warrieranum* is rare and endemic to the Western Ghats of India. Some of the plants like *Gymnostachyum febrifugum*, Gymnostachyum *ceylanicum, Gymnostachyum latifolium* were reported scientifically for hepatoprotective and antioxidant properties [4]. Since, there is no other previous study in this plant for this aspect, the present study was undertaken to assess the antioxidant activity of the methanolic extract from the leaves.

2. Materials and methods
2.1 Plant material
The leaves of *Gymnostachyum warrieranum* were collected from Aralam, Kannur district, Kerala, India, in the month of December, 2015. The plant material was identified and authenticated by Dr. M. V. Krishnaraj, M.Sc., B.Ed., Ph.D., department of botany, Baselius College, Kottayam, Kerala.

2.2 Preparation of the plant material
Plant part was cleaned thoroughly with distilled water and the desired plant parts were dried under shade. The shade dried leaves were pulverized in a mechanical grinder to obtain coarse powder.

2.3 Preparation of the crude extracts
A weight of 50 grams of the coarsely powdered shade – dried sample was successively extracted by Soxhlet method using methyl alcohol as solvent.

2.4 Phytochemical screening
Phytochemical screening were performed using standard procedures [5, 6].

2.5 *In vitro* free radical scavenging activity
DPPH radical scavenging activity [7]
The radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference. 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol. Different volumes (1.25-20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm.3ml of DPPH was taken as control.
% scavenging of the DPPH free radical was measured using the following equation.

$$\% \text{ inhibition} = \frac{\text{Absorbance of the control-Absorbance of the test}}{\text{Absorbance of the control}} \times 100$$

The inhibition curve was plotted for triplicate experiments and represented as % of mean inhibition ± SD. IC_{50} values were obtained from the graph.

Hydroxyl Radical Scavenging Activity [8]
This assay is based on the qualification of the degradation product of 2 deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe3+ - ascorbate- EDTA –H2O2 system (The Fenton reaction). The reaction mixture contained in the final volume of 1 mL.2 deoxy 2 ribose (2.8mM) KH_2PO_4 —KOH buffer (20 mM pH 7.4), $FeCl_3$ (100µm), EDTA (100µm), H_2O_2 (1.0mM), ascorbic acid (100µm) and various concentrations (0-200µg/ml) of the test sample. After incubation for 1hour at 37 °C, 0.5 ml of the reaction mixture was added to 1ml of 2.8% TCA, then 1ml aqueous TBA was added and the mixture was incubated at 90 °C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532nm against an appropriate blank solution.

$$\% \text{ inhibition} = \frac{\text{Absorbance of the control – Absorbance of the test}}{\text{Absorbance of control}} \times 100$$

3. Results
3.1 Percentage yield
The yield was found to be 6% w/w

3.2 Phytochemical Screening of Plant Extracts
Phytochemical Screening of *Gymnostachyum warrieranum* plant revealed the presence of the following metabolites as indicated in table 1. These groups might be responsible for the observed antioxidant activity of these plant.
The extract showed the presence of flavanoids, steroids, phenolic compounds and tannins.

Table 1: The Chemical Constituents of *Gymnostachyum warrieranum* leaves

Type of Extract	Phytochemical	Results
Methanol Extract	Carbohydrates	-
	Proteins and amino acids	-
	Phenolic compounds and tannins	+
	Alkaloids	-
	Glycosides	-
	Flavonoids	+
	Saponins	-
	Coumarins	-
	Steroids and triterpenoids	+

Key: (+): present, (-): absent.

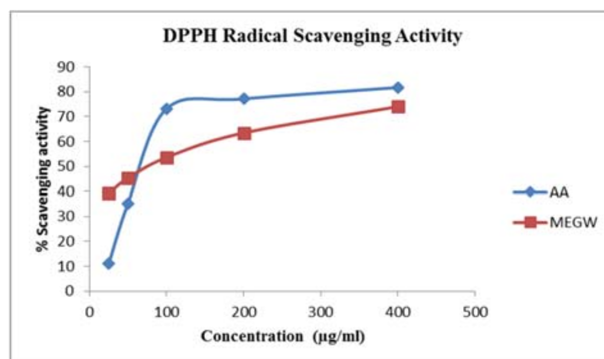
3.3 Free radical scavenging activity
3.3.1 DPPH Radical scavenging activity

Table 2: DPPH Radical scavenging activity of AA and MEGW

Sl. No	Concentration (µg/ml)	%Free radical scavenging activity	
		Ascorbic acid(AA)	MEGW
1	25	10.99 ± .282	39.30 ± .037
2	50	35.25 ± .184	45.33 ± .579
3	100	73.17 ± .282	53.59 ± .593
4	200	77.13 ± .384	63.43 ± .639
5	400	81.72 ± .741	74.02 ± .064

Values are expressed as mean ± SD for triplicates

The DPPH radical scavenging activity of the *Gymnostachyum warrieranum* extract at different concentrations (25-400µg/ml) were compared with ascorbic acid at varying concentrations (25-400µg/ml).Graph 1 showed a significant increase in the antioxidant action due to the scavenging ability of extracts and ascorbic acid. The extract showed maximum activity of 74.02% at 400µg/ml, whereas ascorbic acid at the same concentration exhibited 81.72% inhibition. The IC_{50} values were found to be 60µg/ml and 80µg/ml for ascorbic acid and *Gymnostachyum warrieranum* extract respectively.



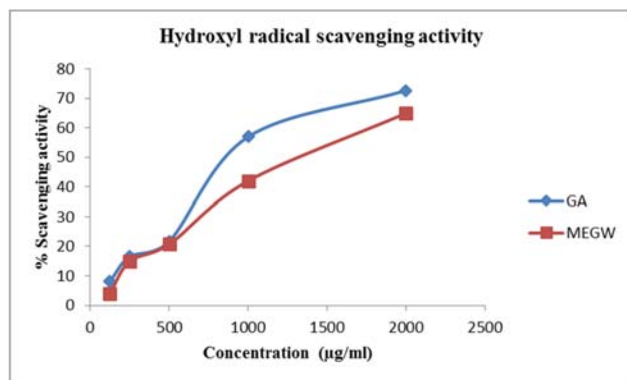
Graph 1: DPPH Radical Scavenging Activity

3.3.2 Hydroxyl radical scavenging activity

Table 3: Hydroxyl radical scavenging activity of GA and MEGW

SL.NO	Concentration (µg/ml)	% Free radical scavenging activity	
		Gallic acid(GA)	MEGW
1	125	8.12 ± .585	4.09 ± .176
2	250	16.6 ± .462	14.99 ± .286
3	500	21.70 ± .822	20.74 ± .227
4	1000	56.99 ± .566	42.03 ± .556
5	2000	72.67 ± .527	64.99 ± .463

Values are expressed as mean ± SD for triplicates



Graph 2: Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the *Gymnostachyum warrieranum* extract at different concentrations (125 – 2000 µg/ml) were compared with gallic acid at varying concentrations (125 – 2000 µg/ml). Graph 2 showed a significant increase in the free radical scavenging activity. The extract showed maximum activity of 64.99% at 2000µg/ml, whereas gallic acid at same concentration exhibited 72.67% inhibition. The IC_{50} values were found to be 800µg/ml and 1400µg/ml for gallic acid and *Gymnostachyum warrieranum* extract.

4. Discussion

To assess the free radical scavenging activity, DPPH and hydroxyl radical scavenging methods are used. The DPPH radical scavenging method is widely used to evaluate the free radical scavenging ability of natural antioxidants [9]. DPPH is a stable nitrogen-based free radical which has a violet colour that changes to yellow after reduction by either the process of hydrogen- or electron-transfer. Substances which are able to execute this reaction can be considered as antioxidants and therefore radical scavengers [10].

Hydroxyl radicals are generated by the Fenton reaction (Fe3+ - ascorbate – EDTA - H_2O_2 system). Hydrogen peroxide is able to undergo a set of reaction known as the Fenton reaction to release the hydroxyl radical in the presence of iron. Hydroxyl radical scavenging activity is based on the quantification of the 2-deoxyribose degradation product, malondialdehyde, by its condensation with thiobarbituric acid (TBA) to give a yellow colour which absorbs at 532 nm [11].

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds [12].

In this work the extract showed a dose dependant *in vitro* antioxidant activity in both methods. Phytochemical screening showed the presence of flavanoids, steroids, phenolic compounds and tannins. The present antioxidant activity of plant extracts may be due to their phenolics and flavonoid contents.

As this drug is unexplored further studies on its active constituents, its isolation, purification and characterization along with investigations is needed to provide some additional insight into the mechanism of action by which it exhibit these activity.

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