Antioxidant and antimicrobial potential of methanolic extract of Indian sacred grove *Gymnostachyum febrifugum* Benth. root

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Summary

The methanolic extract of root from *Gymnostachyum febrifugum* was analyzed for the quantification of total phenolics, tannins and flavonoids. The antioxidant activity was evaluated using various free radical scavenging assays and antibacterial activity against clinically important bacterial strains. Free radical scavenging assays such as hydroxyl, superoxide anion radicals, 2,2- diphenyl-1-picryl hydrazyl (DPPH) and 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays were performed. It was observed that the extract effectively scavenged hydroxyl and superoxide anion radicals. It also scavenges DPPH and ABTS radicals. All the concentrations of root extract showed free radical scavenging and antioxidant power and the preventive effects were in a dose-dependent manner. Those various antioxidant activities were compared to standard antioxidants such as ascorbic acid, butylated hydroxytoluene (BHT) and α-tocopherol. The agar disk diffusion method was used to study the antibacterial activity of *G. febrifugum* methanol extract of root against five bacterial strains.

Keywords: *Antioxidant activity, Antimicrobial activity, Streptomycin, Gymnostachyum febrifugum.*

Introduction

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. The main objective of this study is, to search for Indian plants with strong antioxidant and antimicrobial activity which could serve as good candidates for the development of standardized phytomedicine. 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 (1). 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 febrifugum* is the sacred groves of Dakshina Kannada and Udupi districts. This medicinal plant is rare and endemic to the Western Ghats of India. The root is used by the local people as a febrifuge and applied to the tongue to remove blisters and sores. It contains a bitter principle of a resinoid nature and small quantities of tannin and sugar (2,3). Since, there is no other previous study in this plant for this aspect, the present study was undertaken to assess the antioxidant and antimicrobial activities of the methanolic extract from the root.

Material and Methods

Plant material and extraction procedure

The plant of *Gymnostachyum febrifugum* was collected from Kakkayam, Calicut district, Kerala. Fresh plants were collected during the month of October 2009. The taxonomic identity of the plant was confirmed from the Kerala Forest Research Institute, Peechi, Trichur, Kerala and the voucher specimen (BUBH-2893) was deposited in the Botany department herbarium, Bharathiar University, Coimbatore, India. The dried and powdered plant root was extracted successfully with 600 ml of methanol (1:6 w/v) by using Soxhlet extractor.

Estimation of total phenolics, tannins and flavonoids

The total phenolic and tannin content of the extract was determined by Folin ciocalteu method (4). The total flavonoid content of sample extracts was determined by the method described by Zhishen, Mengecheng and Jianming (5).

Hydroxyl, Superoxide anion, DPPH radical, Evaluation of total antioxidant scavenging assays

The hydroxyl radical scavenging activity of the extract was determined by the method of Halliwell, Gutteridge, & Aruoma (6). Superoxide anion radical scavenging activity of the extract was determined by the method of Nishimiki, Rao & Yagi (7). The $DPPH^{\cdot}$ scavenging activity of extract was determined by the method of Williams, Cuverlier & Berset, (8). The method of Wolfenden & Willson (9) was used for determining the scavenging activity of the extract.

Antimicrobial activity, Disc diffusion method

The antibacterial activity of the extract was investigated against, *Staphylococcus aureus* (MTCC 3381), *Pseudomonas aeruginosa* (MTCC 5210), *Klebsiella pneumonia* (MTCC 3384), *Escherichia coli* (MTCC 119) and *Proteus mirabilis* (MTCC 1429) using Mueller Hinton Agar medium.The antimicrobial susceptibility for the extract was tested by agar diffusion method (10). .

Results and discussion

Quantification of total phenolics, total tannins and total flavonoids

The amount of total phenolics, tannins and flavonoids of methanol extract of root of *G. febrifugum* was analyzed and tabulated in Table 1. The total phenolics and tannin content were found to be 197.5 and 132.2mg GAE/ g extract respectively whereas total flavonoids was 122.3 mg RE/ g extract. It has already been investigated in many plant species that the total phenolics could significantly contribute to the antioxidant capacity of species (11). In view of the fact that since *G. febrifugum* root possesses good phenolic, tannin and flavonoid content, it could be assumed that it can have a higher free radical scavenging activity.

Antioxidant assays

Inhibition of hydroxyl radical

The scavenging ability of *G. febrifugum* methanolic extract of root on $OH^{\cdot}$ compared with ascorbic acid are shown in Figure 1. The result showed that the extract exhibited more pronounced hydroxyl radical

scavenging activity compared to ascorbic acid in a dose dependent manner. The IC_{50} of the extract was 93.61 µg/mL whereas that of ascorbic acid was 94.16µg/mL. Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides (12).

Inhibition of superoxide anion radical

The superoxide anion scavenging ability of methanolic extract *G. febrifugum* root has been presented in Figure 2. The IC_{50} of extract and BHT on inhibition of superoxide generation was 83.92 µg/mL and 86.58µg/mL respectively. Since, the conversion of superoxide and H_2O_2 into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals (13) the extract can be used for better inhibition of superoxide radicals.

Inhibition of DPPH radical

The scavenging ability of methanolic extract *G. febrifugum* of root on DPPH radical compared with standard vitamin E is shown in Figure 3. The scavenging effect of extract and standards on the DPPH radical was found to be concentration dependent and was in the order of GFRM > vitamin-E. The IC_{50} value of the extract and vitamin E was 206.74 and 295.15µg/mL respectively. The better effect of extract on DPPH radical scavenging is thought to be due to its better hydrogen donating ability.

Total antioxidant activity - ABTS radical cation decolourization assay

The scavenging of various concentrations of extract on ABTS radical is shown in Figure 4. The IC_{50} value of the extract and BHT were 92.06 and 99.94 µg/mL respectively.The inhibition was found to be concentration dependent and was better than BHT. Hagerman *et al*., (14) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{\cdot+}$) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl groups' substitution than the specific groups. Therefore, it can be correlated that the higher phenolics and tannins in the extract, the higher the $ABTS^{\cdot+}$ scavenging is.

Antibacterial activity

The result of antibacterial activity of *G. febrifugum* methanolic extract of root is presented in Table 2. The mean zone of inhibition for the extract against gram positive and gram negative bacteria was found to increase with the increasing concentration of the extract and ranged between 18.0 and 7.0 mm. The highest mean zone of inhibition (18.0 mm) was recorded against *Proteus mirabilis.* The blind control 5% DMSO did not produce any zone of inhibition whereas the positive control *Streptomycein* produced zone of inhibition ranging between 21.0 and 15.0 mm. It can be also concluded that the plant extract have great potential as antimicrobial compounds, especially in the treatment of infectious diseases caused by resistant microorganisms.

Conclusions

G. febrifugum exhibiting a powerful antioxidant activity against various *in vitro* oxidative systems and can be used as accessible source of natural antioxidants and as a possible food supplement. The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate of possible synergism among extract components for their antioxidant and antimicrobial activity.

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Table 1. Total phenolics, tannins and flavonoid content in methanol extract of *G. febrifugum* root

Phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)	Flavonoids (mg RE/g extract)
197.5 ± 33.3	132.2 ± 5.0	122.3 ± 9.9

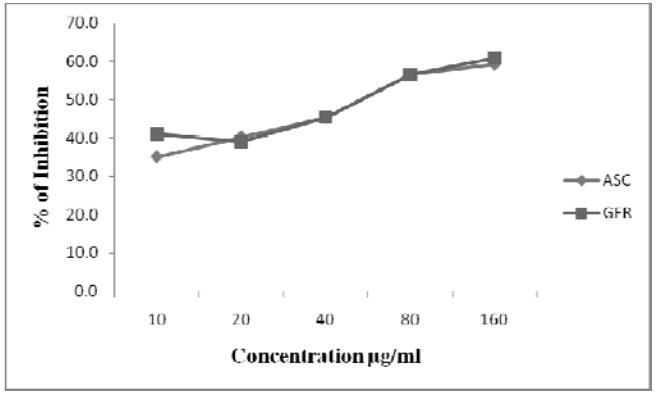
Values are mean of three replicate determinations ± Standard deviation

Table 2. Antibacterial activities of methanol extract *G. febrifugum* root and *Streptomycin*

S.No	Name of the microorganism	Mean zone of inhibition(mm)*			*Streptomycin*
		50 (µg/disc)	100 (µg/disc)	200 (µg/disc)	30 (µg/disc)
1.	*Staphylococcoccus aureus*	10	12	12	22
2.	*Pseudomonas aeruginosa*	11	15	13	20
3.	*Klebsiella pneumoniae*	7	7	11	16
4.	*Escherichia coli*	9	11	16	14
5.	*Proteus mirabilis*	13	18	13	20

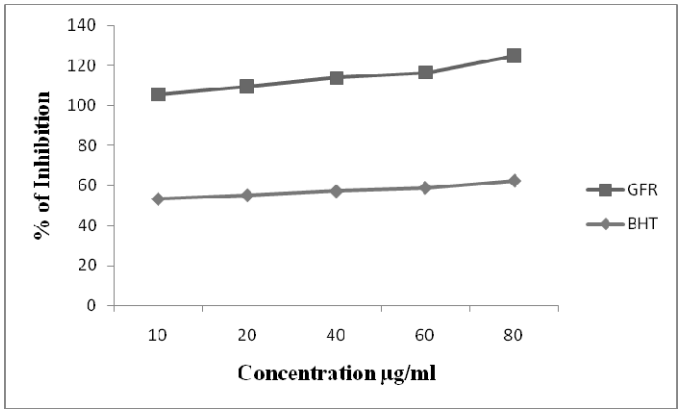
*Mean zone of three assays.

Figure 1. Hydroxyl radical scavenging activity



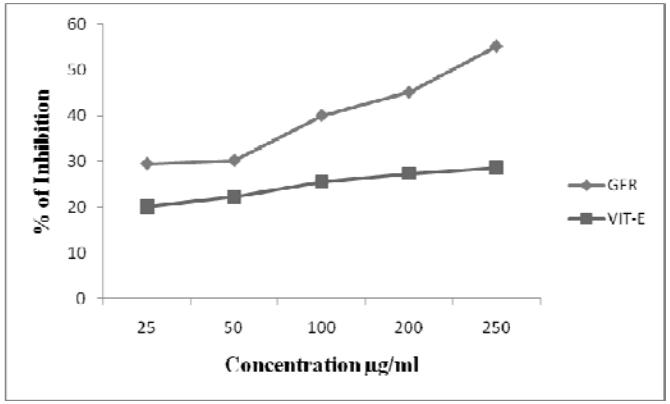
Values are mean (n=3) ± Standard deviation
ASC-Ascorbic acid, GFR- *G. febrifugum* root

Figure 2. Superoxide radical scavenging activity



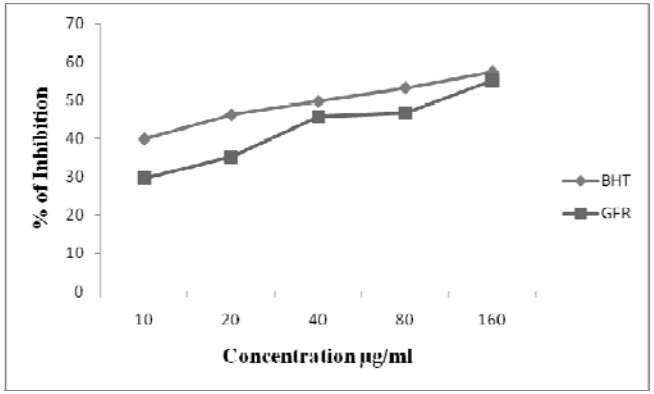
Values are mean (n=3) ± Standard deviation
GFR- *G. febrifugum* root BHT- Butylated hydroxytoluene

Figure 3. DPPH radical scavenging activity



Values are mean (n=3) ± Standard deviation
GFR- *G. febrifugum* root VIT-E – Vitamin – E

Figure 4. ABTS radical scavenging activity



Values are mean (n=3) ± Standard deviation
GFR- *G. febrifugum* root BHT- Butylated hydroxytoluene