



Therapeutic Importance of Extracts from Leaf and Bark of *Gymnacranthera farquhariana*

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Abstract

Background: *Gymnacranthera farquhariana* (Hook. f. and Thomson) Warb. is one of the endangered tree taxon of the Western Ghats, a biodiversity hot-spot in peninsular India. Very few reports are available on biological applications of the bark, leaf and seed of this plant. **Aim:** The present work deals with the screening of methanol and aqueous extracts of *G. farquhariana* from leaf and bark for therapeutic importance. **Methods:** Anti-bacterial activities of extracts were carried out using two bacterial strains viz., *Pseudomonas aeruginosa* and *Staphylococcus aureus* through the agar well diffusion method. The antifungal activities by poison bait method using *Aspergillus niger*, *Colletotrichum gloeosporioides* and *Fusarium solani*. Each experimental parameter was conducted in triplicate. **Results:** The results showed that *G. farquhariana* bark and leaf extracts exhibited promising activities against bacteria. Antifungal activity also showed good inhibition activity. The extracts showed a good anti-diabetic potential in α -amylase inhibitory assay. Bark aqueous extract showed 54.79% inhibition for α -amylase inhibition assay at 250 μ L. The extract also showed good anti-inflammatory potential in the Bovine Serum Albumin (BSA) denaturation assay. Bark aqueous extract showed 83.9% inhibition for BSA denaturation assay at 100 μ L. **Conclusion:** Both leaf and bark extracts of the plant showed good antioxidant, anti-diabetic and antimicrobial activity. This is the first such *in vitro* report concerning *G. farquhariana* plant parts.

Keywords: Anti-diabetic, Antimicrobial, Antioxidant Activities, *Gymnacranthera farquhariana*, Therapeutic Effect

1. Introduction

Various herbal formulations and plants are employed by local and folk-lore communities across the world including the Indian subcontinent for remedy of various diseases. World Health Organisation identified the use of Indian medicinal plants and their data documentation¹. From a scientific point of view, a rational relationship of folklore medicine is necessary for therapeutic, chemical and biological activities^{2,3}. The active principles present in plants and other biological communities pique the interest of scientists who seek to elucidate their impact on major infectious diseases. Presently researches are focussed on evaluating the plant extracts against various disease-causing bacteria⁴.

Indian biodiversity is rich with flora and fauna used since ancient times by traditional practitioners and local peoples for the treatment of different diseases. In India, among the estimated 18000 species of higher plants, 4500 species are found in the Western Ghats of which 30% are endemic species⁵. Most of the plants possess volatile oils, alkaloids and polyphenols as active ingredients that are used in folk medicines, while others are used as phytomedicines obtained from finished products⁶. The plant-based secondary metabolites lead to major research for new inventions for isolating pure active compounds from these plants to solve human health issues⁷. The antioxidant activity of different plants is due to a wide variety of metabolites including phenolics and flavonoids complexes. Among

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various methods, reducing power assay emerged as a good method for evaluating the total antioxidant power of medicinal plants⁸. These antioxidant agents are associated with the suppression of various chronic diseases like osteoarthritis, osteoporosis, Alzheimer's disease and cancer⁹.

Gymnacranthera farquhariana (Hook. f. and Thomson) Warb. syn. *G. canarica* Warb.,¹⁰ is one of the endemic tree taxon of the Western Ghats, one of the four biodiversity hotspots in India¹¹. The plant is classified under Myristicaceae, a family mostly known for its mace and seeds, which are of high medicinal importance¹². Myristicaceae, an evergreen tree family is composed of 400 species under 19 genera and found in the tropical evergreen forests¹³. Among this, four genera with 15 species are found in India, of which two species and a variety are endemic to peninsular India¹⁴. The fruits are globose with thick and fleshy pericarp. The seed conforms to the fruits, testa woody with bright red coloured aril¹⁰. In the Western Ghats region of Karnataka, the 5 species inhabited are *G. farquhariana*, *Knema attenuata*, *Myristica dactyloides*, *Myristica fatua* var. *magnifica* and *Myristica malabarica* excluding cultivated *Myristica fragrans*¹⁵.

The seeds are reported to be polyembryony¹⁶ and the rhizosphere region is harboured by several fungal communities including vesicular arbuscular mycorrhiza¹⁷⁻¹⁹. The freshwater *Myristica* swamps are associated with *G. farquhariana* as a co-dominant plant in the vegetation which presently faces habitat destruction¹⁵. The phytoconstituent natural compounds occurring in the herbs constitute respective colour and sensory properties. These phytochemicals have biological significance but are not reported as essential edible diet. Investigations lead to the occurrence of many phytochemicals having the capacity to overcome several deadly diseases such as cancers, and cardiac and metabolic disorders²⁰.

Myristica swamps were first described by Krishnamoorthy²¹ as a special type of freshwater niche from the Travancore area in Kerala. There is no documented evidence about the therapeutic activities of the seed, but the local people used it as wild nutmeg. The seeds contain large amounts of fat, probably as an adaptive mechanism for dispersal and survival under swampy conditions. From the past few decades, due to the conversion of swamps into areca gardens, teak

plantations and paddy fields, regeneration as well as propagation of *G. canarica* has been drastically affected²²⁻²⁴. Indian traditional medicine is widely used for asthma, fever and heart disease. Nutmeg has several medicinal uses and is commonly used to treat gastrointestinal disorders, lymphatic ailments and kidney diseases. *G. farquharian* is an evergreen medium-sized plant with smooth brownish bark and pale red or light brown wood. The fatty seeds are mashed, packed in bamboo splits and used by locals as crude candles²⁵⁻³⁰.

The present work aims to study *in vitro* antioxidant, anti-inflammatory, antidiabetic and *in vitro* antimicrobial properties of leaf and bark extracts of *G. farquhariana*.

2. Materials and Methods

2.1 Leaf and Bark Samples

The plant samples of *G. farquhariana* were procured from Panja reserve forests, Sullia Taluk, Dakshina Kannada, Karnataka. The samples were shade-dried for a week and coarsely powdered.

2.2 Extract Preparation

25g of leaf and bark powder of *G. farquhariana* was taken in a conical flask, which was mixed with 250mL distilled water and heated at 80°C for 3 hours with continuous agitation, filtered and concentrated by rotary evaporation method. The extract was then kept in a water bath at 50°C for 7 days and stored at 4°C. Similarly, methanol extract was also obtained.

2.3 Anti-oxidant Activity Study

2.3.1 DPPH Assay

The free radical scavenging activity of the bark and leaf extracts was evaluated by 1, 1-Diphenyl-2-Picryl-Hydrazyl (DPPH) assay as per Shen *et al.*,³¹. Ascorbic acid served as the standard with 1mg/1000µL concentrations. The DPPH solution (60µM) was prepared in methanol. Exactly, 3.9mL of DPPH stock was mixed with 100µL of test solution with varied concentrations like 250, 500, 750 and 1000 µg/mL. The standard and test sample tubes were incubated in the dark for 15 minutes and the absorbance was measured at 517nm using a UV-Vis spectrophotometer. Each experiment was conducted in triplicates. Methanol

without samples served as the control. The percentage of radical scavenging activity was calculated by the formula:

$$\text{Percentage Inhibition} = \frac{\text{OD Blank} - \text{OD Test}}{\text{OD Blank}} \times 100$$

Where, OD Blank = Optical Density of the DPPH radical + Solvent, OD Test = Optical Density of DPPH Radical+ Sample Extract/Standard.

2.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Exactly, 2.5mL each of 1% potassium ferricyanide and 200mM sodium phosphate buffer was mixed with various concentrations (250, 500, 750 and 1000µL/mL) of the sample followed by incubation for 20 minutes at 50°C and 2.5mL of 10% TCA was added³². Later, 2.5mL of distilled water and 1mL of freshly prepared 0.1% ferric chloride were added to this. The absorbance was measured at 700nm using a spectrophotometer. Ascorbic acid served as the standard.

2.4 Anti-diabetic Assay

2.4.1 Alpha Amylase Inhibitory Assay

The protocols of Worthington Enzyme Manual^{33,34} were employed for measuring this activity. A volume of 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/mL of alpha-amylase and varying concentrations (250, 500, 750 and 1000 µg/mL) of extract as inhibitor were incubated at 25°C for 10 minutes followed by 500 µL of 1% starch solution in 0.02M sodium phosphate buffer which was added to all the tubes. The reaction was stopped using 1.0mL of DNS. The test tubes were kept in a boiling water bath for 5 minutes and cooled. The volume was increased to 10mL using distilled water and the optical density was measured at 540nm using UV-Vis spectrophotometer.

$$\text{Percentage of Inhibition} = \frac{\text{OD Control} - \text{OD Test}}{\text{OD Control}} \times 100$$

2.5 Anti-Inflammatory Activity Assay

2.5.1 Bovine Serum Albumin Denaturation Assay

This method was employed for testing *in vitro* anti-inflammatory activity as per the standard protocols^{35,36}. Exactly, 0.5mL of reaction mixture containing 0.45 mL

3% bovine serum albumin with varying concentrations of test samples (250, 500, 750 and 1000 µg/mL) with pH 6.3 were incubated for 20 minutes at 37°C followed by at 80°C for 2minutes in water bath. Once cooled, 2.5mL of phosphate buffer saline was added and OD was measured at 660nm using a spectrophotometer. The inhibition (%) of protein denaturation was determined by the formula:

$$\text{Inhibition (\%)} = \frac{\text{OD Control} - \text{OD Test}}{\text{OD Control}} \times 100$$

2.6 Antibacterial Activity Assay of the Plant Extracts by Well Diffusion Method³⁷

The bacterial strains used in the experiment are *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa* and *S. aureus*. Fresh cultures of inoculum were maintained before the experiment in nutrient agar broth. The 24-hour bacterial strains were inoculated over the surface of nutrient agar media, using a 9mm cork borer and four wells were made on inoculated media. Two wells were loaded with different concentrations of extracts (100 and 200 µg/mL). Distilled water or methanol was the negative control and streptomycin was the positive control. The zone of inhibition was measured after 24 hours of incubation at lab temperature.

2.7 Determination of Antifungal Activity by Poison Bait Method³⁸

The fungal strains used were *C. gloeosporioides*, *F. solani* and *A. niger*. Here the single mycelial discs of each test strain are inoculated in 100mL Erlenmeyer flask containing 25mL Potato Dextrose Broth (PDB) media and 1mL of fungal suspension. The flasks were kept at 282 °C for 5 days for fungal growth. The mycelial biomass was filtered through filter paper. Bavistin served as positive while methanol and water served as negative control. The mycelial biomass was dried in an oven at 60°C. The weight of the mycelial biomass was measured.

2.8 Statistical Analysis

In all the experiments for each parameter, three replicates were made. The data of experiments were analysed statistically by mean standard deviation and regression using MS Excel.

3. Results

3.1 Antioxidant Activity Assay

3.1.1 DPPH Assay

The per cent radical scavenging activity was found to range from 21.801.81 to 74.361.81 for leaf and bark extracts of *G. farquhariana* (Figure 1). The highest inhibition of 74.361.81% was observed for leaf methanol extract (500 μ g/mL) and bark methanol extract (250 μ g/mL). The bark aqueous extract had the lowest inhibition (1000 μ g/mL). The ascorbic acid per cent inhibition was found to be increased with concentration. The IC_{50}

value was 77.070.45 μ g/mL for ascorbic acid whereas for Leaf Aqueous Extract (LAE) and Bark Aqueous Extract (BAE) the IC_{50} values were 888.15 ± 0.83 μ g/mL and 644.96 ± 1.15 μ g/mL respectively.

3.1.2 FRAP Assay

The result indicated that *G. farquhariana* extract possessed strong *in vitro* antioxidant efficacy, which exhibits an efficient, free radical scavenger and enhances its potential medicinal value. In the present study, reducing power activity was found in the concentration ranges 250-1000 μ g/mL (Figure 2). A xc for Leaf

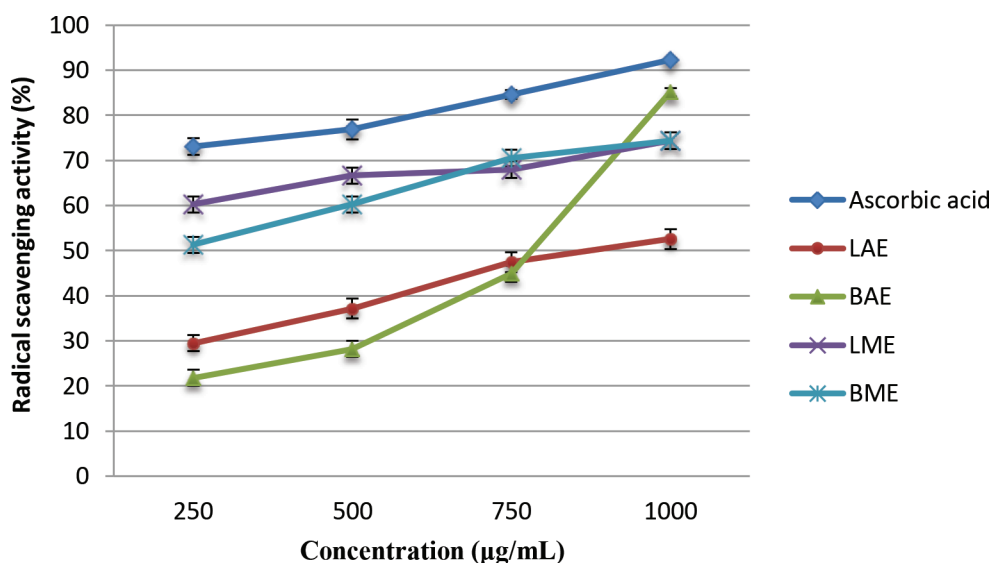


Figure 1. DPPH radical scavenging assay of *G. farquhariana* leaf and bark extracts with a standard at different concentrations.

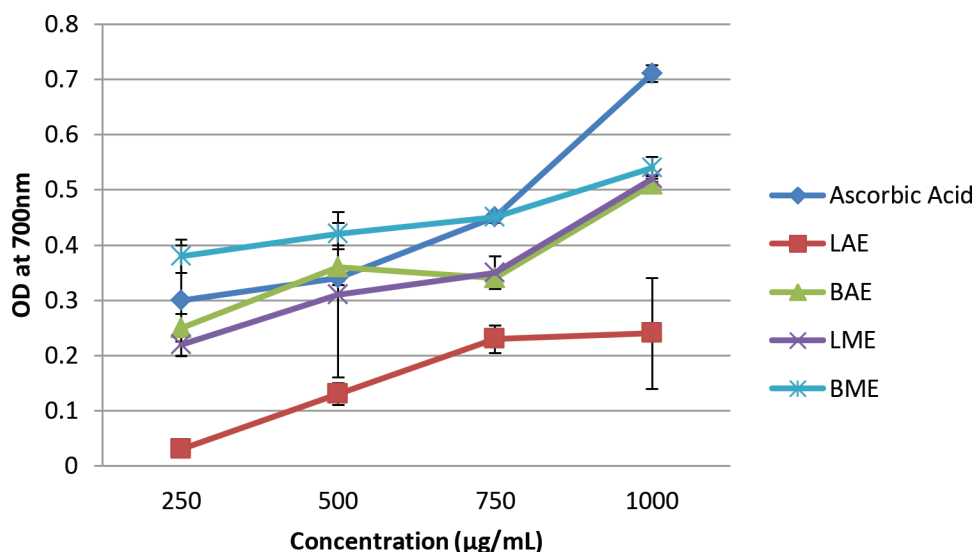


Figure 2. FRAP activity of different plant extracts and standards at different concentrations.

Methanol Extract (LME) (at 1000 $\mu\text{g/mL}$) at 1.09 and the lowest OD was observed for BAE (at 750 $\mu\text{g/mL}$) at 0.20.

3.2 In Vitro Antidiabetic Assay

3.2.1 α -Amylase Inhibitory Assay

G. farquhariana extracts were tested for enzyme inhibition and the percentage inhibition ranged from 1.631.89 to 94.240.71. The highest inhibition was observed in bark aqueous extract at 100 $\mu\text{g/mL}$ concentration which is 94.240.71 % (Figure 3). IC_{50} value for LAE, Leaf Methanol Extract (LME), and Bark Methanol Extract (BME) was found to be $67.18 \pm 1.16 \mu\text{g/mL}$, $102.77 \pm 1.25 \mu\text{g/mL}$ and $110.80 \pm 0.94 \mu\text{g/mL}$.

3.3 Anti-inflammatory Assay

Plant extract showed inhibition of BSA denaturation ranging from 0.550.5 % to 71.862.21 %. The maximum anti-inflammatory activity was found in bark aqueous extract at 1000 $\mu\text{g/mL}$ which is 85.71% and the lowest was observed for leaf methanol extract at 250 $\mu\text{g/mL}$ which is 0.68% (Figure 4). IC_{50} for LAE, BAE, LME and BME were calculated to be 797.071.25 $\mu\text{g/mL}$, 611.091.26 $\mu\text{g/mL}$, 2260.381.95 $\mu\text{g/mL}$, 1297.630.95 $\mu\text{g/mL}$ respectively.

3.4 Anti-bacterial Assay by Well Diffusion Method

The leaf and bark extracts of *G. farquhariana* showed good antibacterial activity in the well diffusion method

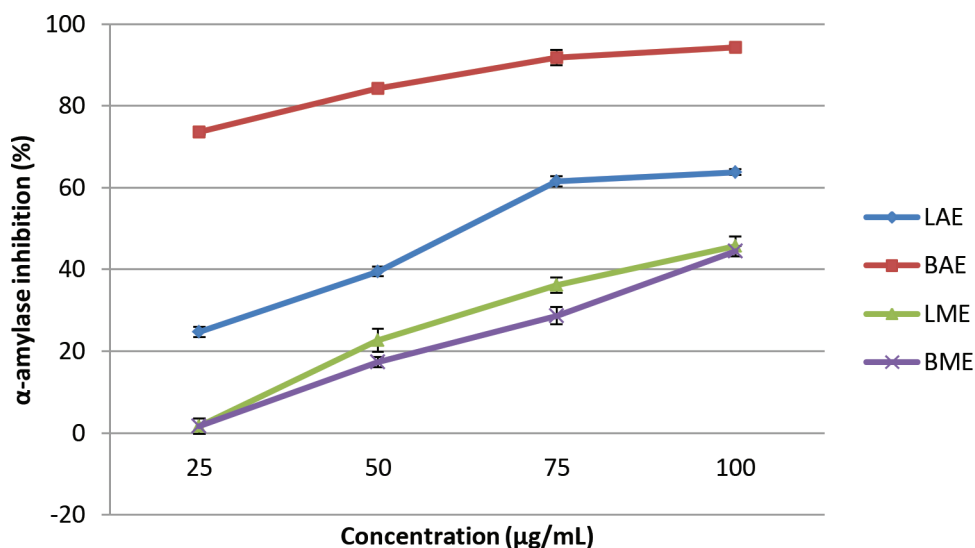


Figure 3. α -Amylase inhibitory activity of leaf and bark extract of *G. farquhariana*.

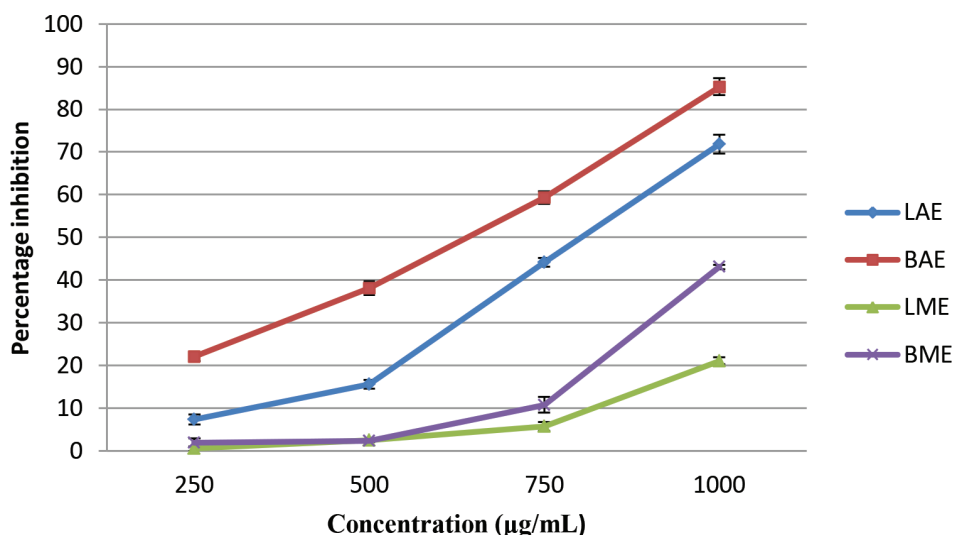


Figure 4. Bovine Serum Albumin (BSA) denaturation activity of extracts and standard at different concentrations.

with different bacterial strains used in the study (Table 1). LME showed a higher inhibition zone at 100 µg/mL at 270.14 mm against *K. pneumoniae* (Gram-negative). BAE showed a higher inhibition zone at 100 µg/mL which is 240.71 against *P. aeruginosa*. BAE showed a higher inhibition zone at 100 µg/mL against *E. coli* which is 380.42 mm. LAE showed a higher inhibition zone at 100 µg/mL against *S. aureus* which is 20.20.75 mm.

3.5 Antifungal Activity Using Poison Bait Method

In this study, LME demonstrated inhibition when compared to other extracts based on the dry weight of fungal mycelium was least in medium with LME that is 0.250.13 g against *C. gloeosporioides*. BME against *F. solani* had higher inhibition with the dry weight of mycelium which was 0.230.056g. *A. niger* was inhibited by LME and BME (Figure 5).

Table 1. Anti-bacterial activity of extracts and standard at different concentration against bacterial strains

Extract	<i>Klebsiella pneumoniae</i>		<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>	
	Conc. (µg/mL)	Zone of inhibition*	Conc. (µg/mL)	Zone of inhibition*	Conc. (µg/mL)	Zone of inhibition*
Leaf aqueous extract	50	11.5±0.21	50	17.5±0.71	50	16.6±0.35
	100	14.5±0.071	100	22.5±0.71	100	20.2±0.75
	Streptomycin	35±0.71	Streptomycin	31.5±0.81	Streptomycin	38±0.82
	Neg. control	-	Neg. control	-	Neg. control	-
Bark aqueous extract	50	11.5±0.21	50	22.5±0.14	50	13.3±0.42
	100	12±0.28	100	24±0.71	100	18.7±0.92
	Streptomycin	38±0.0	Streptomycin	31.5±0.21	Streptomycin	39±0.42
	Neg. control	-	Neg. control	-	Neg. control	-
Leaf methanol extract	50	16.5±0.21	50	2.3±0.21	50	9.6±0.32
	100	27±0.14	100	2.5±0.0	100	13.7±0.42
	Streptomycin	39±0.14	Streptomycin	28.5±0.71	Streptomycin	39.8±0.42
	Neg. control	-	Neg. control	-	Neg. control	-
Bark methanol extract	50	15.2±0.14	50	7±0.14	50	4.6±0.12
	100	23±0.14	100	14±0.71	100	13.4±0.62
	Streptomycin	37±0.71	Streptomycin	31.5±0.71	Streptomycin	27.8±0.42
	Neg. control	-	Neg. control	-	Neg. control	-

* (mm), Mean ± standard deviation, N=3

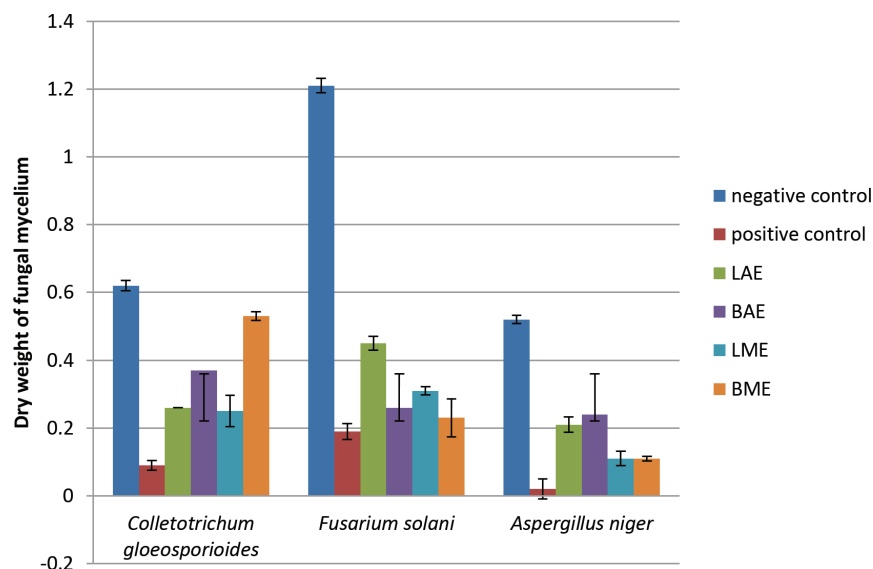


Figure 5. Antifungal activity of extracts and standard at different concentrations against fungi.

4. Discussion

In the present study, the highest inhibition of 74.361.81 % was observed for leaf methanol extract (500 µg/mL) and bark methanol extract (250 µg/mL) of *G. farquhariana*, while the bark aqueous extract (1000 µg/mL) exhibited the lowest inhibition. The DPPH radical scavenging activity ranged between 21.80 1.81 to 74.36 1.81 for leaf and bark extracts. Similarly, the IC₅₀ value was 77.07 0.45 µg/mL for ascorbic acid whereas in the leaf and bark extracts, the IC₅₀ values were 888.15 0.83µg/mL and 644.96 1.15 µg/mL respectively. Ngoua *et al.*,³⁹ observed that higher antioxidant activity in a water-ethanol extract of *Scyphocephalium ochocoa* was revealed by a greater inhibition of DPPH radical with IC₅₀ = 4.969 ± 0.263 µg/mL. The ethyl acetate and acetate extracts of *Virola venosa* (Myristicaceae) had EC₅₀ = 18.6 ± 1.20 and 27.1 ± 1.38, respectively, as shown by Fernandes *et al.*⁴⁰. Similarly, *M. fragrans* acetone extract (1 mg/mL) showed DPPH radical scavenging activity and chelating activity of 63.04 ± 1.56 % and 64.11 ± 2.21 % respectively⁴¹. The DPPH scavenging activity of purified natural lignin dimmer at the concentration 100,10,1 and 0.1µg/mL extracted from *M. fragrans*, exhibited scavenging activity of 76.7%, 65%, 28% and 8% respectively while the partially purified lignan had the scavenging activity of 44.3%, 18.5%, 11% and 0% respectively⁴². Gagana⁴³ found higher antioxidant activity in *G. farquhariana* methanol seed, leaf and bark extracts compared with other solvent extracts. Among plant sources, seed extract showed a maximum of 87.99% inhibition at 1000µg/mL concentration. Another report by Chowdhury *et al.*⁴⁴, found that *M. fragrans* methanol seed and mace extract have DPPH free radical scavenging activity of 49.69 ± 0.06% at 80 µg/mL. Similarly, Vivek and Chandrashekar⁴⁵ reported higher DPPH radical scavenging activity in *M. fatua* methanol fruit extracts. Another study with clove essential oil, by Ghadermazi *et al.*,⁴⁶ showed a more pronounced inhibitory percentage with concentration and at 200µg/mL concentration which exhibited complete antioxidant efficacy of 100%.

The findings of the present study indicate the efficiency of *G. farquhariana* extracts as a good *in vitro* antioxidant, which proves it as a strong, free radical scavenger thereby increasing its medicinal applications. The reducing power in the FRAP assay

was observed over the concentration range of 250-1000 µg/mL. Higher OD of 1.09 was observed at 1000 µg/mL for leaf methanol extract and the lowest OD of 0.20 was observed at 750 µg/mL for bark aqueous extract. *M. fragrans* mace extract showed a free radical assay value near to BHT (positive control) (68.7 µM Fe (II) g⁻¹ vs. 63.2 lM Fe(II) g⁻¹) as reported by Loizzo *et al.*⁴⁷. FRAP value 113.1 ± 0.28 (Bark); 99.9 ± 1.97 (Leaf); 21.88 ± 2.86 (pericarp); 43.025 ± 1.59 (mace) 57.9 ± 1.69 (seed) in *Myristica beddomei* observed by Joy and Mohan⁴⁸. In another experiment, the FRAP assay showed scavenging activity in the range of 250 to 2600 mM Fe (II)/g in the bark extracts of *Pajanelia longifolia* with the highest antioxidant activity in 70% methanol extract compared among four extracts⁴⁹.

4.1 In Vitro Antidiabetic Assay

4.1.1 α-Amylase Inhibitory Assay

In the present study, the percentage inhibition ranges between 1.63 1.89 to 94.240.71. The highest inhibition was observed in bark aqueous extract at 100µg/mL concentration which is 94.240.71%. The IC₅₀ value for leaf aqueous, leaf methanol and bark methanol extracts was found to be 67.18 1.16 µg/mL, 102.77 1.25 µg/mL and 110.80 0.94µg/mL respectively. Among different compounds of *M. fatua* tested for their porcine pancreatic α-amylase inhibitory activity, Malabaricone A, B and C showed higher α-amylase inhibitory activity with IC₅₀ values 19.07 ± 0.517, 12.89 ± 0.068 and 10.63 ± 0.171 µM⁵⁰. *G. canarica* seed extracts showed inhibitions of 8.46%, 24.84%, 55.09% and 74.68% at different concentrations viz., 25, 50, 75 and 100 µg/mL respectively. Methanol seed extract shows IC₅₀ values of 72.633%⁵¹.

4.2 Anti-Inflammatory Activity Assay

Different extracts of *G. farquhariana* showed inhibition of BSA denaturation ranging from 0.55 0.5 % to 71.86 2.21 %. The highest anti-inflammatory activity of 85.71% was observed in bark aqueous extract at 1000 µg/mL while it was lowest (0.68%) for leaf methanol extract at 250 µg/mL. Similarly, the IC₅₀ for LAE, BAE, LME and BME was 797.07 1.25 µg/mL, 611.09 1.26 µg/mL, 2260.38 1.95 µg/mL, 1297.63 0.95 µg/mL respectively. Ngoua *et al.*,³⁹ found anti-inflammatory activity with all plant extracts of *S. ochocoa* Warb. The IC₅₀ ranged between 34.775 ± 2.543 to 74.577 ± 3.456 µg/mL for

protein denaturation inhibition. In another experiment, methanol extract of *Justicia secunda* leaves showed $IC_{50} = 186.20 \pm 2.25 \mu\text{g/mL}$ for BSA denaturation assay as reported by Anyasor⁵². There are reports on maximum inhibition of egg albumin denaturation up to 80% at 100 $\mu\text{g/mL}$ of clove oil and similarly with cinnamon oil^{53,54}.

4.3 Anti-bacterial Assay by Well Diffusion Method

The leaf and bark extracts of *G. farquhariana* showed good antibacterial activity in the well diffusion method with different bacterial strains used in the study. LME showed a higher inhibition zone of 27 0.14 mm at 100 $\mu\text{g/mL}$ against *K. pneumoniae* (Gram-negative). While BAE showed a higher inhibition zone of 24 0.71 mm at 100 $\mu\text{g/mL}$ against *P. aeruginosa*, BAE showed a higher inhibition zone of 38 0.42 mm at 100 $\mu\text{g/mL}$ against *E. coli* whereas LAE showed 20.2 0.75 mm inhibition zone at 100 $\mu\text{g/mL}$ against *S. aureus*. Chaithanneya and Bhat⁵⁵ found higher antibacterial activities against tested organisms in methanol extract of seeds of *G. farquhariana* over aqueous extract. The highest zone of inhibition of 22.5mm was found at 1000 $\mu\text{g/mL}$ methanol extract for *E. coli*. Ameen⁵⁶ found the antibacterial activity of *M. fragrans* aqueous and ethanol extracts against *E. coli* with 16 and 19 mm zones of inhibition at 100% concentration, while at 75% concentration, it was 14 and 19 mm respectively. The aqueous and methanol extract of nutmeg at 100% concentrations showed 12 and 13 mm zones of inhibition respectively against *S. aureus*. Similarly, Gupta *et al.*,⁴¹ reported the effectiveness of *M. fragrans* acetone extract against *Bacillus subtilis*, *P. aeruginosa*, *P. putida* and *S. aureus*. Tantry and Bhat⁵⁷ observed a significant antibacterial activity of *M. fatua* var. *magnifica* methanol extract at 1000 $\mu\text{g/mL}$ against *K. pneumoniae* (21.5mm), *Salmonella typhi* (19.5mm) and *S. aureus* (20.0mm). In another study by Ameen⁵⁶, aqueous and ethanol extracts of *M. fragrans* were 100% effective against *E. coli* with inhibition zones of 16mm and 19mm respectively while at 75% concentration, it was 14mm and 19mm respectively. At 100% concentration, the aqueous and methanol extracts were effective against *S. aureus* with inhibition zones of 12mm and 13mm respectively. Joseph and George⁵⁸ observed the antibacterial activities of *M. fragrans* pericarp methanol and ethanol extracts against *S. aureus*

and *S. typhi* in comparison to hexane extracts. In another study, different solvent extracts of *M. fragrans* showed effectiveness against *S. aureus* and *E. coli* with a zone of inhibition and Minimum Inhibitory Concentration (MIC) ranging from 8 - 20mm and 512 $\mu\text{g/mL}$ - 128 $\mu\text{g/mL}$ respectively⁵⁹.

4.4 Antifungal Activity Using Poison Bait Method

In the present study, higher inhibition was noted in LME compared to the other extracts i.e. dry weight of fungal mycelium was least in medium with LME that is 0.25 0.13 g against *C. gloeosporioides*. BME against *F. solani* had higher inhibition the dry weight of mycelium was 0.23 0.056g. *A. niger* was inhibited by LME and BME (Figure 5). Chaithanneya and Bhat⁵⁵ studied the antifungal activity of methanol and aqueous extracts against two fungi that showed different degrees of inhibition. At 500 $\mu\text{g/mL}$ concentration, the inhibition zone was maximum for both *A. niger* and *Candida albicans*. Gupta *et al.*,⁴¹ reported the highest antifungal activity in acetone extract with *A. niger* (14.4 \pm 0.37 mm). Similarly, Helen *et al.*,⁶⁰ reported the inhibitory activity of essential oil from the leaves of *M. fragrans* against *Candida tropicalis* (1.3cm), *C. albicans* (0.8cm), *Rhizomucor miehei* (0.6cm) and *Candida glabrata* (0.6cm). *A. niger* and *A. fumigatus* did not show any inhibitory activity of the oil.

5. Conclusion

This research suggested that *G. farquhariana* possesses the anti-inflammatory potential shown by the inhibition of BSA denaturation at higher concentrations. It leads to the belief that bark aqueous extract has high anti-diabetic activity. It also confirms the greater antioxidant and antibacterial activity in the bark extracts. This study indicates the methanol extract of bark has high antifungal activities. This study further enlightens the medicinal potential of different parts of *G. farquhariana* which was not reported earlier.

6. References

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