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Studies on phytochemical properties, antimicrobial and antioxidant activities of seeds of *Gymnacranthera farquhariana* (Hook. f. & Thomson) Warb.

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ABSTRACT

In the present study, preliminary phytochemical screening has been made in methanol and aqueous extract of *Gymnacranthera farquhariana* seeds with standard protocols. The extractions were carried out using soxhlet. It showed the presence of phytoconstituents namely carbohydrates, proteins, phenolics, resins, tannins and alkaloids. Antimicrobial activities were tested against five bacterial species, such as *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Bacillus subtilis* by agar well diffusion method. The results of antimicrobial activity studies exhibited higher activity of methanol extract against all the microorganisms over aqueous extract. Similarly, the methanolic extracts showed good antioxidant activity. These results indicate that seeds of this endemic plant can be explored for treatment of some diseases.

Keywords: Phytochemical, antimicrobial properties, antioxidant activity, *Gymnacranthera farquhariana.*

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INTRODUCTION

India is rich with floras which were exploited for treatment of various diseases from ancient period to date. Of the 18000 species of flowering plants estimated in India 4500 species are found in the Western Ghats, of which one third are endemic (Shetty et al., 2002). Among the endemic flora one third were rare and threatened. Each plant may possess one or the other medicinal properties. Myristicaceae is one of the important families of flowering plants comprising 19 genera and 400 species widely distributed (Mabberley, 1987). There are 5 species of Myristicaceae belonging to 3 genera in the Western Ghats region of Karnataka viz., *Gymnacranthera farquhariana, Knema attenuata, Myristica dactyloides, Myristica fatua* var. *magnifica* and *Myristica malabarica* excluding cultivated *Myrictica fragrans* (Rama Bhat and Kaveriappa, 2009). Among these, *G. farquhariana* and *K. attenuata* are endemic and *M. malabarica* and *M. fatua* var. *magnifica* were rare and threatened to the Western Ghats of India (Ahmedullah and Nayar, 1986).The seeds showed polyembryony which is a rare process in the members of Myristicaceae family (Rama Bhat and Kaveriappa, 2002) and the rhizosphere region of this plant is inhabited by various fungi including vesicular arbuscular mycorrhiza [VAM] (Rama Bhat and Kaveriappa, 2005, 2007, 2009).

The populations of *Gymnacranthera farquhariana* found in *Myristica* swamps of Gersoppa, Shimoga district of Karnataka as well as in other swamp forests of Uttara Kannada and Dakshina Kannada districts. This species is co-dominant tree of the *Myristica* swamp vegetation. The species is exclusively associated with swampy conditions and habitat destruction seems to be the major threat (Rama Bhat and Kaveriappa, 2009). It is an evergreen tree up to 25 m tall with dark brown, warty, blaze reddish brown bark; branches are verticillate and are right angles to main trunk, young branchlets round, rusty velvety. The leaves simple, alternate, 1 to 2 cm long, slightly channeled and velvety when young. The flowers unisexual, dioecious: male flowers yellowish in axillary panicles, fulvous velvety (Figure 1a); female flowers larger, borne in racemes in leaf axils. Fruit is a spherical capsule, up to 3.2 cm across, hairless; seed one,

a **b**

Figure 1. a: Branch of *G. farquhariana* showing inflorescence; b: Dehisced capsules with red lacinate aril covering the seeds.

spherical, pale brown with red lacinate aril (Figure 1b). It is commonly known as Kanara nutmeg, in Kannada as Pindi and in Malayalam as Pintikkaya.

The chemical compounds that occur naturally in plants and responsible for color and organoleptic properties. These phytochemical have biological significance but are not established as essential nutrients. Scientists estimated that there may be as many as 10,000 different phytochemical having the potential power to overcome diseases such as cancer, stroke or metabolic syndrome, are abundant in fruits, vegetables and herbs (Sunil et al*.,* 2012).

The use of plant extracts and phytochemical, both with known antimicrobial properties are of great significance in ailment of diseases. Extracts of plants were used for the treatment of various diseases which included in Indian system of medicines. However, this area is not much developed when compared to modern system of medicines, mainly because of the lack of scientific documentation in this field. Some of the genera of this family possess antimicrobial and antioxidant activity which were scientifically proved (Helen et al., 2012; Manjunath et al., 2012; Gupta et al., 2013). The seeds contain fat which were used for making candles in earlier days but other uses have not been recorded (Anonymous, 1962; Hegnauer, 2000).

The objectives of the present study are i) to analyse the phytochemical constituents in both aqueous and methanolic extracts of *G. farquhariana* seed, ii) to evaluate antimicrobial activity of both methanolic and aqueous extracts on selected bacterial and fungal strains, and iii) to evaluate the antioxidant activity of both aqueous and methanolic extracts of *G. farquhariana* seed.

MATERIALS AND METHODS

Collection of sample

Gymnacranthera farquhariana seeds were collected from Kathalekane evergreen swamp forests of Shimoga district, near Jog falls, Karnataka during September to October 2015. The seeds were allowed to shade dry for a week. These were then kept in hot air oven at 60°C for 24 to 48 h until it was dried completely. These were then coarsely powdered and stored at laboratory temperature (30°C) in a closed container for further use.

Preparation of an aqueous and a methanolic extract

The seed extract was prepared from coarse powder of the seeds (40 g) by soxhlet method. The decoction thus obtained was extracted for 1hour with 150 ml of distilled water. This was followed by the distillation process. The extract was then dried in hot air oven at 40°C for a week. From this per cent yield in each solvent was calculated. The dried extract thus obtained was used for assessment of antimicrobial and antioxidant activities. For methanolic extract methanol was used.

Phytochemical screening of the seed extract

Phytochemical qualitative tests for carbohydrate, proteins, alkaloids, phenolics, tannins and resins were carried out of the aqueous and methanolic extracts using standard protocols. Quantitative analysis of total carbohydrates, proteins, phenolics and tannins were carried out using standard methods (Sadasivam and Manickam*,* 2008). The results were expressed as mean ± standard error.

Antibacterial screening of seed extract

Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and *Salmonella typhi* were used as test

organisms maintained in slants at P.G Department of Biotechnology, Alva's College, Moodbidri. Seed extract of different concentrations were prepared for antimicrobial assay.

Antibacterial activity test by agar well diffusion method

Petri dishes were plated with Muller Hinton Agar media and allowed to solidify for 30 min. The test organisms were then spread on the surface of the media using sterile ear buds. Cork borer (4 mm) was used to bore wells in media. The aqueous extract of different concentrations viz., 25, 75, 100, 300, 500 and 1000 µg/ml were dispensed into the wells using a micropipette. A negative control of distilled water and a positive control of streptomycin were kept and the extract was allowed to diffuse for half an hour. Similarly 25, 75, 100, 300, 500 and 1000 µg/ml of methanolic extract were dispensed into the wells along with a negative control of methanol and a positive control of streptomycin were kept and allowed to diffuse. The plates were incubated at 37°C for 24 h and zone of inhibitions were measured.

Antifungal activity by Poison Drop method

Fungal cultures of *Candida albicans, Aspergillus fumigatus* and *A. niger* were maintained for the experiment. Plant extracts were swabbed on to the Potato Dextrose Agar (PDA) plates and fungal cultures disk of 5mm diameter was taken and inoculated at the center of Petri plates containing plant extracts in aseptic condition. The PDA plates were incubated at 25 ± 3°C and radial growth of colony was measured after 7 days of incubation. Each test was performed in duplicates for both methanolic and aqueous extracts.

Antioxidant activity by DPPH method

The free radical scavenging activity of the extract was measured *in vitro* by 2,2'-diphenyl-1-picrylhydrazyl (DPPH method) (Dev et al*.,* 2015). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20°C until required. From the stock solution, three ml aliquot used to make 100 µl of the sample of various concentration (20, 60, 100, 500 and 1000 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room, instead of absorbance was recorded at 570 nm. The control was prepared as described without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Control absorbance } (A_0) - \text{Sample absorbance } (A)}{\text{Control absorbance}} \times 100$$

RESULTS

The percent yields of methanolic and aqueous seed extract of *G. farquhariana* were 57.5 ± 0.68 and 10.0 ± 0.06 respectively. The aqueous seed extract showed positive results for the presence of carbohydrates, proteins, alkaloids whereas methanolic extract showed positive result for carbohydrates, proteins, alkaloids, tannins, phenolic compounds and resins (Table 1).

The estimated carbohydrate from the seed extract of *G. farquhariana* was 0.31± 0.0011 mg/g, phenol 0.085 ± 0.0021 mg/g, protein content 0.23 ± 0.0051 mg/g and tannin contents was 0.062 ± 0.0031 mg/g.

Table 1. Photochemical analysis for methanol and aqueous extract of the plant *Gymnacranthera farquhariana.*

Phytochemicals	Inference	
	Aqueous extract	Methanol extract
Carbohydrates	+	+
Proteins	+	+
Alkaloids	+	+
Tannins	-	+
Phenolics	-	+
Resins	-	+

+ present, - absent.

The bacterial cultures showed varied levels of sensitivity towards different concentrations of methanolic extracts while they did not showed levels of sensitivity towards aqueous extracts. Among these, *Escherichia coli* and *Salmonella typhi* showed higher sensitivity against methanolic plant extract (Table 2).

The antifungal activity of the various concentrations of methanolic extracts of *G. farquhariana* was carried out against the various strains of fungi such as *Aspergillus fumigatus, A. niger* and *Candida albicans*. The methanolic extract showed activity against *A. niger* and *Candida albicans*, but no clear zone of inhibition was showed against *A. fumigatus* (Table 3), thus indicates *A. fumigatus* resistant.

The scavenging effects of the methanolic extract on DPPH radical were higher as compared to aqueous extract. Though the antioxidant potential of the methanolic extract was found to be lower than the ascorbic acid (Figure 2), the study revealed that the methanolic extract have prominent antioxidant activity; the presence of phenolic compounds are mainly found in this extract and could be attributable to the antiradical properties of this extract.

DISCUSSION

The qualitative test for phytochemicals showed the presence of carbohydrates, proteins, alkaloids, tannins, phenolics and resins of which carbohydrate, protein, phenolics and tannins were quantified. One of the study on phytochemical analysis of dried seeds of *Myristica fragrans* using different solvent extracts such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether, acetone and aqueous revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids (Thomas and Krishnakumari, 2015). The ethanolic, methanolic and aqueous extract revealed the maximum presence of phytoconstituents whereas chloroform, petroleum ether and ethyl acetate extracts showed minimal amounts. One of the earlier report (Rama Bhat

Table 2. Antibacterial activity for the methanolic seed extract of *G. farquhariana* at different concentrations.

Test organisms	Zone of inhibition (mm) for different concentrations (µg/ml) of methanolic seed extract							
	25	75	100	300	500	1000	Control 1	Control 2
Staphyllococcus aureus	11.0	10.0	12.0	14.0	15.5	21.5	35.0	10.0
Klebsiella pnuemoniae	10.5	11.0	13.0	13.0	15.0	21.0	35.0	10.5
Escherichia coli	11.0	12.5	15.5	16.5	18.5	22.5	34.0	10.5
Salmonella typhi	11.5	13.0	15.8	16.0	20.5	24.5	35.0	10.5
Bacillus subtilis	9.5	10.0	12.0	14.5	16.0	18.5	34.0	10.0

Control 1: Streptomycin, Control 2: Methanol.

Table 3. *In vitro* antifungal activity of *G. farquhariana* by Poison Drop method.

Organisms	Zone of inhibition (mm)	
	Control	Methanolic extract – 500 µg/ml
Candida albicans	6.0	2.0
Aspergillus fumigatus	5.0	9.0
Aspergillus niger	9.0	6.5

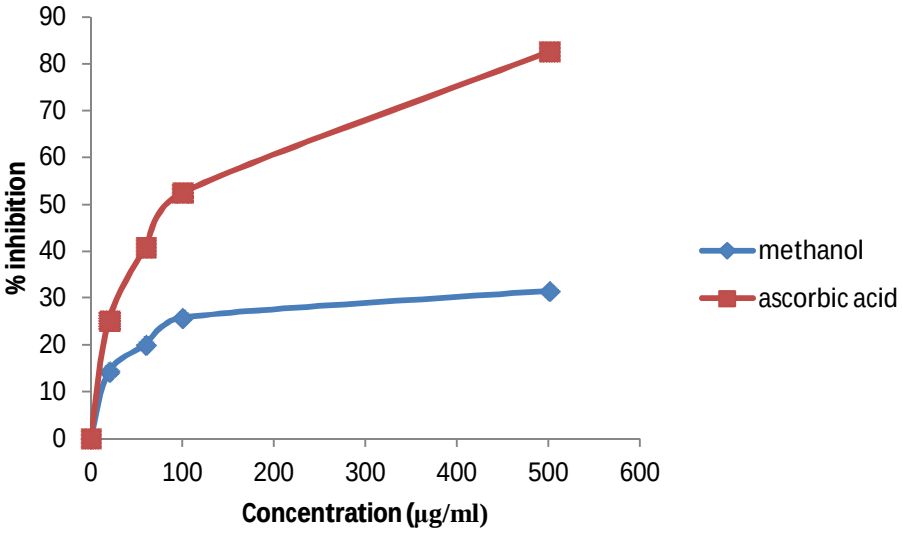

Figure 2. DPPH radical scavenging activity of methanolic extract of *G. farquhariana* compared with standard ascorbic acid.

and Kaveriappa, 1998) on proximate analysis of kernel and mace of *M. fatua* var. *magnifica* showed total sugar content 9.8% for kernel and 19.3% for mace, protein 14.1 for kernel and 11.6 for mace, tannin 8.9% for kernel and 8.5% for mace. Similar results were obtained during the analysis of *Centella asiatica* with the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoids, saponins and proteins, reducing sugar, carbohydrates and cardiac glycosides in methanolic extracts (Singh et al*.,* 2012). The above observations indicate different plant species possess various phytoconstituents at different levels which are responsible for their therapeutic use.

In the present study of the two solvents used for extraction methanol yielded more extract as compared to the aqueous system. Deepa et al. (2015) found greater in methanol as compared to the aqueous extract of fruit, bark, leaf of *Samadera indica*. There are some earlier reports conducted experiments on bark and root extracts which supports the present work on extract yield (Akhila Zainab et al., 2013; Ashutosh Yende et al., 2013; Prajna and Rama Bhat, 2015).

In the present study, methanolic extract was found to be more resistant to all the organisms than the aqueous extract. The highest zone of inhibition was found in the concentration of 1000 µg/ml for *Salmonella typhi* (24.5

mm), *Escherichia coli* (22.5 mm) in methanolic extract. Ameen (2012) observed the effectiveness of aqueous and ethanolic extracts of *Myristica fragrans* against *E. coli* with inhibition zones of 16 and 19 mm respectively in the 100% concentration, while in 75% concentration, the inhibition zones were 14 and 19 mm, respectively. The 100% concentrations, the aqueous and methanolic extract of nutmeg were effective against *Staphylococcus aureus* with inhibition zones of 12 and 13 mm, respectively.

In the present study the methanolic and aqueous extracts showed varied levels of antifungal activity against different fungal species. *Candida albicans* and *A. niger* gave the highest zone of inhibition at the concentration 500 µg/ml. On the other hand, Gupta et al. (2013), reported that acetone extract of *Myristica fragrans* has shown the strongest antimicrobial activity than other solvent extracts of nutmeg against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas putida* and *P. aeruginosa.* The antifungal activity was also found highest in acetone extract with *A. niger* (14.4 ± 0.37 mm). In another study reported by Helen et al*.* (2012), it was observed that the essential oil from the leaves of *M. fragrans* showed significant inhibitory activity against the bacteria *Enterococcus faecalis* (1.3 cm), *Lactococcus plantarum* (0.9 cm), *Proteus vulgaris* (0.6 cm) and the fungus *Candida tropicalis* (1.3 cm), *C. albicans* (0.8 cm)*, Rhizomucor miehei* (0.6 cm) and *C. glabrata* (0.6 cm). No inhibitory activity was observed against the bacteria *Clostridium perfringens, Klebsiella pneumoniae* and *Bacillus megaterium*. There is no inhibitory activity of oil against the fungi *A. niger* and *A. fumigatus.* Similar reports made by Joseph and George (2014), where the methanolic and ethanolic extracts of *M. fragrans* pericarp showed remarkable activities against *Staphylococcus aureus* and *Salmonella typhi* respectively as compared to the hexane extracts.

In one of the study carried out by Ashutosh Yende et al. (2013) on antimicrobial activities of *Holigarna arnottiana* of the seven bacterial and two fungal species and observed, *Vibrio parahaemolyticus* and *Staphylococcus aureus* were found more susceptible to the extracts. Only methanolic extract showed antifungal activity against *Aspergillus niger* and *Penicillium chrysogenum*. Similarly, antimicrobial activities of *Pajanelia longifolia* showed a significant levels of antimicrobial activity against *Vibrio parahaemolyticus* and *Bacillus subtilis.* Only methanolic extract showed antifungal activity against *A. niger* and *P. chrysogenum* (Akhila Zainab et al., 2013). Viswanad et al. (2011) observed a significant antimicrobial activity of methanolic extract of *Samadera indica* against Gram positive, Gram negative bacteria and *Candida albicans,* but was resistant against *A. niger* and *A. fumigatus.*

The methanolic extract of *G. farquhariana* is showing antioxidant activity which is comparable to standard ascorbic acid. Since the extract contains number of phytoconstituents the reducing power lowered as compare to pure ascorbic acid. Among the different concentration of the extract, 500µg/ml showed 31.5% inhibition. Similar study was conducted by Manjunatha et al. (2012) in seed, whereby the *in vitro* antioxidant activity of *Myristica malabarica* by DPPH method. The methanolic extract exhibited good I_C values at 0.02mg/ml in DPPH radical scavenging assay, 0.107mg/ml in scavenging of Hydrogen peroxide assay, 1.6 µg/ml in ABTS radical cation decolourization assay (ABTS method) and 0.5 mg/ml in nitric oxide scavenging assay respectively.

On the other hand, Al-Jumaily et al. (2015) studied the free radical scavenging activity of purified natural lignin dimmer isolated from *M. fragrans* using DPPH. Results showed that 100, 10, 1 and 0.1 µg/ml of purified lignan had 76.7, 65, 28 and 8% scavenging activity respectively while the same concentrations of partial purified lignan had 44.3, 18.5, 11 and 0% scavenging activity respectively.

Similar results were observed in methanolic extracts of different plant species. The total antioxidant activity of the bark extracts of *Pajanelia longifolia* was found to be in the range of 250 to 2600 mM Fe (II)/g by FRAP assay. Among the four extracts used, 70% methanol extract showed the highest antioxidant activity (Akhila Zainab et al., 2013). In another study, the total antioxidant activity of the bark extract of *H. arnottiana* exhibited in the range of 150 to 1360 mM Fe (II)/g and also only 70% methanol extract showed the highest antioxidant activity among the four extracts used (Ashutosh Yende et al., 2013). There are other reports which support the present work based on antimicrobial and antioxidant activities (Viswanad et al., 2011; Kabbashi*,* 2014; Parameshwar et al., 2015). The present study confirmed that the methanolic extract have the prominent antioxidant activity compared to aqueous extract. The presence of phenolic compounds are mainly found in this extract of the seeds of this plant could be attributable to the higher antiradical properties of this extract. Further studies are needed to test the pure extract with different antioxidant assay as well as with cell lines and animal models.

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