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Original Article

POTENTIAL ANTIMICROBIAL, ANTHELMINTIC AND ANTIOXIDANT ACTIVITIES OF MYRISTICA DACTYLOIDES GAETRN BARK

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ABSTRACT

Objective: The present study was undertaken to determine the antimicrobial, anthelmintic and antioxidant activities of bark extracts of *Myristica dactyloides Gaetrn*.

Methods: The antimicrobial activity of the petroleum ether, ethyl acetate and methanol extracts were evaluated by the Agar well diffusion method against different gram-positive, gram-negative bacteria and fungi. Different extracts of the plant were taken for anthelmintic activity against Indian earthworm *Pheretima Posthuma*. DPPH radical scavenging activity was measured by the DPPH antioxidant assay method using ascorbic acid as standard and the total phenolic content was estimated spectrophotometrically using Folin-Ciocalteu method.

Results: Petroleum ether extract exhibited significant antifungal activity, anthelmintic activity and considerable DPPH radical scavenging activity with an IC_{50} value of 10.97±0.07µg/ml. Whereas methanol extract exhibited significant antibacterial activity against both gram positive and gram negative bacteria and it is the richest source of phenolics with a total phenolic content of 95.11±2.14 mg of Catechol equivalents/100 mg dried extract. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins/phenolics, steroids/triterpenoids and saponins which may be the reason for its biological properties.

Conclusion: The findings of this study indicate that this plant is medicinal with prominent antioxidant, antimicrobial and anthelmintic property. The plant can be considered as promising plant species with high potential value for drug preparation.

Keywords: *Myristica dactyloides Gaetrn*, Antimicrobial, Anthelmintic, Antioxidant, DPPH and *Pheretima Posthuma*.

INTRODUCTION

Nature serves as the man's primary source for the cure of his ailments. Among the rich diversity of Indian medicinal plants, the majority of the plants are yet to be scientifically assessed for such properties. On the other hand, the potential of higher plants as a source for new drugs is still largely unexplored [1]. Infectious diseases caused by bacteria, fungi, viruses and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance [2]. Interest in natural products with antimicrobial properties has revived as a result of the current problems associated with the use of antibiotics [3]. Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health [4]. The indigenous system of medicine reports a number of natural sources for their anthelmintic efficacy.

Free radicals are found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species (ROS) are known to be implicated in many cell disorders and in the development of many diseases, including cardiovascular diseases, atherosclerosis, chronic inflammation etc [5, 6]. Although organisms have endogenous antioxidant defenses produced during normal cell aerobic respiration against ROS, other antioxidants are taken both from natural and synthetic origin [7]. Synthetic antioxidants are widely used, but their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly.

Myristica dactyloides Gaetrn belonging to *Myristicaceae* family is commonly known as "Kaadu Jayikayi". It is indigenous in Africa, Indo Malaysian region and cultivated all over India. Flowers are numerous pendent, 7.5 cm long and 3.8 cm wide. At first they are white in color and then they become deep red [8]. *Myristica dactyloides Gaetrn* is an indigenous medicinal plant and used very commonly in the management of various diseases like diabetes, bronchitis, constipation and in various skin ailments.

Myristica fragrans Houtt is an evergreen tree belongs to the family *Myristicaceae*, which has been supported by its strong antioxidant activity attributed to the phytochemicals present naturally [9]. *Myristica fragrans Houtt* has been shown to possess strong antioxidant activities, act as good preservative agent and offer benefits in some medical treatments. In the family of *Myristicaceae* species, the abundant flavonoid compounds retrieved were mostly chalcones, flavones, dihydroflavanols, flavonols, flavans, flavan-3-ols, virolanes, virolanols, dihydrochalcones, isoflavones and pterocarpans [10]. It is among the class of flavan-3-ols with the molecular formula of $C_{15}H_{14}O_6$, which may prevent substances in the bloodstream from oxidizing and clogging the arteries, known as heart healthy flavonols [11].

The fruit of *Myristica fragrans Houtt* is rich in phenolic constituents and demonstrated good antioxidant capacity. It is potentially used for the supplement and pharmaceutical exploration due to its high antioxidant properties. In view of this the exploration of antioxidant properties in *Myristica dactyloides Gaetrn* bark has been significant since consumption of plant based food is favorable for reduction of oxidative stress related diseases [12]. From the literature survey, it is clear that the plant *Myristica dactyloides Gaetrn* is an indigenous medicinal plant with significant medicinal properties and it is used very commonly in the management of various diseases. The antioxidant activity and anthelmintic activity on bark extract of the plant is not reported so far, therefore the present study was undertaken to evaluate its biological properties including antimicrobial, anthelmintic and antioxidant properties.

MATERIALS AND METHODS

Collection and identification plant material

The bark part of *Myristica dactyloides Gaetrn* plant was collected from Agumbe region, Shivamogga, Karnataka, during the month of April 2013. The plant was authenticated by taxonomist Dr. Gopal, Assistant Professor, Dept of Botany, Sahyadri Science College, Shivamogga and a voucher specimen representing herbarium no. AJ & MKC 009 has been deposited in the herbarium of the Department of Botany, Sahyadri Science College, Shivamogga, Karnataka, India, for reference.

Processing and extraction

The bark of the plant was shade dried coarsely powdered (2.5 kg) and was successively extracted with petroleum ether, ethyl acetate and methanol using Soxhlet apparatus by hot extraction method. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was preserved in an airtight bottle. The crude extracts thus obtained were stored at 4°C for further investigation of potential antimicrobial, anthelmintic and antioxidant properties.

Selection of worms

Indian adult earthworms (*Pheretima Posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocols.

General chemicals and instruments

All chemicals and solvents used in the study were of analytical grade. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) Folin-Ciocalteu reagent were purchased from Sigma Aldrich. Ascorbic acid and methanol are procured from Sd fine chem. Ltd, India. Double beam UV-Vis Spectrophotometer (Navyug, India). Vacuum rotary evaporator (Shivam Instruments, India), weighing balance (Contech, India) were the instruments used for the study. All other chemicals and reagent used were of analytical grade.

Preliminary phytochemical screening

Standard phytochemical screening tests were performed to identify the different phytochemical constituents present in petroleum ether, ethyl acetate and methanol extracts of the plant [13-14].

Antimicrobial activity

Organisms and culture media

The bacterial and fungal strains used to assess the antimicrobial property of the crude extracts of *Myristica dactyloides Gaetrn* bark were *Staphylococcus aureus* ATCC-6538, *Staphylococcus faecalis* ATCC-10541, *Streptococcus pyrogenus* ATCC-19615, *Shigella flexneri* ATCC-12022, *Enterobacter aerogenes* ATCC-13048, *Pseudomonas fluorescens* ATCC-13525, *Candida tropicalis* ATCC-456, *Trichophyton mentagrophytes* ATCC-9533, *Trichoderma viride* ATCC-13233. The bacterial cultures were maintained on nutrient agar (NA), while fungal cultures on Sabouraud dextrose agar (SDA).

Screening for antibacterial activity

Agar well diffusion method

Agar well radial diffusion technique was used for the assessment of antibacterial activity of the test samples. The sterilized nutrient agar medium was poured into sterilized petri dishes. Nutrient broth containing 100 µl of 24 h old cultures of respective bacterial strains was spread separately on the agar medium. Wells was made using a stainless steel sterilized cork borer under aseptic conditions. 25, 50, 100 µg/ml of petroleum ether, ethyl acetate and methanol crude extracts were loaded into corresponding wells. The antibiotic Tetracycline was used as standard (1µg/ml of sterile water). The plates were incubated for 24 h at 37 °C and the diameter of the zone of bacterial growth inhibition was measured and the readings were recorded in millimeter [15]. The tests were carried out in triplicates and the results were recorded as mean±SEM (Standard Error Mean).

Screening for antifungal activity

The antifungal activity was screened against *Candida tropicalis, Trichophyton mentagrophytes* and *Trichoderma viride.* Spore suspension of all the three organisms was prepared by washing one or two colonies using five milliliters of sterile phosphate buffer solution (pH 7.0). One milliliter of inoculum was added to 10 ml of molten potato dextrose agar, mixed and poured into petri dishes. After solidification at room temperature for a maximum of 20 minutes, wells were made in the agar with sterile stainless steel cork borer. The petroleum ether, ethyl acetate, and methanol extract were dissolved in each of 10% DMF 25, 50 and 100 µg/ml of the extracts were loaded in the corresponding wells. Petri dishes were incubated for 48 hours at 26°C. The standard Fluconazole was used as a reference antifungal substance (1µg/ml of sterilized distilled water). The diameters of clear zones around wells were measured and expressed in millimeter [16]. The tests were carried out in triplicates and the results were recorded as mean±SEM (Standard Error Mean).

Evaluation of anthelmintic activity

The standard Albendazole (25 mg/ml) and the test solutions of *Myristica dactyloides Gaetrn* (50, 100 mg/ml) were evaluated for anthelmintic activity with Indian adult earthworm *Pheretima Posthuma*. Observations were made for the time taken for paralysis and death of individual worms up to four hours of the test period. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time of death of the worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water of 50°C [17]. The tests were carried out in triplicates and the results were recorded as mean±SEM (Standard Error Mean).

In vitro antioxidant assay

The antioxidant activity of plant extracts was determined by *in vitro* method, the DPPH (2,2-Diphenyl-1-picryl hydrazyl) free radical scavenging activity and total phenolic content assay.

DPPH free radical scavenging activity

DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al.* [18]. The different concentrations of each of the extracts were prepared in methanol and were added to 3 ml of 0.1 mm methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \text{ Inhibition} = \frac{(A0 - A1)}{A0} \times 100$$

Where, A_0 is the absorbance of the control (without test samples)

A_1 is the absorbance of test samples.

Each experiment was carried out in triplicates and the results were recorded as a mean % antiradical activity±SD.

Estimation of total phenolic content

The total phenolic content of the *Myristica dactyloides Gaetrn* bark extract was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth [19]. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 ml of the plant extract (100 µg/ml) was mixed with 2 ml of the Folin-Ciocalteu regent (diluted 1:10 with de-ionized water) and was neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The optical density of the resulting blue color was measured at 765 nm using double beam UV-VIS spectrophotometer. A calibration curve was constructed using Catechol solution as standard and total phenolic content of the extract was expressed in terms of milligrams of Catechol per gram of dry weight of extract. Experiments were

performed in triplicates and a result was recorded as mean±SEM (Standard Error Mean).

Statistical analysis

Results are expressed as the standard error mean of three independent experiments. Student's t-test was used for statistical analysis; P values>0.05 were considered to be significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical analysis of various solvent extracts such petroleum ether, ethyl acetate and methanol extract of *Myristica dactyloides Gaetrn* indicated the presence of certain secondary metabolites.

The petroleum ether extract showed the presence of phytoconstituents alkaloids, flavonoids, tannins/phenolics, steroids/triterpenoids and saponins. While ethyl acetate and methanolic extracts showed the presence of phytoconstituents such as flavonoids, tannins/phenolics, steroids/triterpenoids and saponins. The results of phytochemical analysis are as shown in table 1.

Table 1: It shows phytochemical screening of various solvent extracts of *Myristica dactyloides Gaetrn* bark

Phytoconstituents	Pet. ether extract	Ethyl acetate extract	Methanolic extract
Alkaloids	+ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Tannins/Phenolics	+ve	-ve	+ve
Steroids/Triterpenoids	+ve	+ve	+ve
Saponins	+ve	+ve	-ve

Antibacterial and antifungal activity

The antibacterial activity was determined by measuring the diameter of the zone of inhibition recorded. The different extracts of the plant *Myristica dactyloides Gaetrn* bark were found to have maximum antibacterial activity. The results of the antibacterial activity of different extracts against some bacterial strains are depicted in table 2 (a), 2 (b) and 2 (c) for petroleum ether, ethyl acetate and methanol respectively.

Table 2 (a): It shows antibacterial activity of pet. ether extract of *Myristica dactyloides Gaetrn* bark with standard

S. No.	Bacterial strains	Inhibition zone in mm			
		Pet. ether			Tetracycline
		25(µg/ml)	50(µg/ml)	100(µg/ml)	1(µg/ml)
1	*Staphylococcus aureus*	19.00±0.06	21.03±0.09	21.93±0.12	19.23±0.15
2	*Staphylococcus faecalis*	16.10±0.06	18.00±0.06	20.07±0.12	21.10±0.15
3	*Streptococcus pyrogenus*	19.10±0.06	20.07±0.12	20.10±0.06	22.13±0.19
4	*Shigella flexneri*	12.97±0.09	14.87±0.09	16.97±0.15	19.07±0.07
5	*Enterobacter aerogenes*	15.93±0.12	16.97±0.03	18.00±0.12	20.07±0.12
6	*Pseudomonas fluorescens*	16.97±0.15	17.93±0.12	19.17±0.12	18.07±0.18

Table 2 (b): It shows antibacterial activity of ethyl acetate extract of *Myristica dactyloides Gaetrn* bark with standard

S. No.	Bacterial strains	Inhibition zone in mm			
		Ethyl acetate			Tetracycline
		25(µg/ml)	50(µg/ml)	100(µg/ml)	1(µg/ml)
1	*Staphylococcus aureus*	11.87±0.09	13.03±0.03	14.10±0.15	19.23±0.15
2	*Staphylococcus faecalis*	10.07±0.12	10.83±0.09	11.00±0.00	21.10±0.15
3	*Streptococcus pyrogenus*	10.23±0.12	10.03±0.15	11.97±0.09	22.13±0.19
4	*Shigella flexneri*	07.10±0.15	08.97±0.03	09.10±0.06	19.07±0.07
5	*Enterobacter aerogenes*	07.97±0.09	08.13±0.13	09.07±0.12	20.07±0.12
6	*Pseudomonas fluorescens*	10.00±0.17	11.13±0.09	13.07±0.09	18.07±0.18

Table 2 (c): It shows antibacterial activity of methanol extract of *Myristica dactyloides Gaetrn* bark with standard

S. No.	Bacterial strains	Inhibition zone in mm			
		Methanol			Tetracycline
		25(µg/ml)	50(µg/ml)	100(µg/ml)	1(µg/ml)
1	*Staphylococcus aureus*	20.23±0.12	21.03±0.03	23.10±0.15	19.23±0.15
2	*Staphylococcus faecalis*	17.97±0.09	20.00±0.17	21.00±0.06	21.10±0.15
3	*Streptococcus pyrogenus*	18.10±0.06	16.97±0.09	19.00±0.00	22.13±0.19
4	*Shigella flexneri*	15.03±0.15	17.07±0.12	17.97±0.09	19.07±0.07
5	*Enterobacter aerogenes*	16.97±0.09	19.03±0.03	19.07±0.12	20.07±0.12
6	*Pseudomonas fluorescens*	17.97±0.09	19.83±0.12	21.03±0.03	18.07±0.18

All the three extracts of *Myristica dactyloides Gaetrn* bark viz., petroleum ether, ethyl acetate and methanol have showed a significant inhibitory activity against almost all bacterial strains. Among the extracts, maximum activity was observed in methanol extract; next to it is petroleum ether extract, whereas the ethyl acetate extract has showed the least activity against all the bacterial strains. Both gram-positive and gram-negative bacteria were susceptible to the plant extracts. The inhibition zones produced were significantly higher for the methanol extract when compared to petroleum ether and ethyl acetate extracts respectively. In the present work, among gram-positive bacteria, *Staphylococcus aureus* was the most susceptible when compared to *Staphylococcus faecalis* and *Streptococcus pyrogenus* with inhibition zones of 21.93±0.12 mm, 14.10±0.15 mm and 23.10±0.15 mm for 100µg/ml in petroleum ether, ethyl acetate and methanol extracts, respectively. Whereas in case of gram negative-bacteria, *Pseudomonas fluorescens* was the most susceptible when compared to *Shigella flexneri* and *Enterobacter aerogenes* with inhibition zones of 19.17±0.12 mm, 13.07±0.07 mm and 21.03±0.03 mm for 100µg/ml, in petroleum ether, ethyl acetate and methanol extracts respectively.

Table 3 (a): It shows antifungal activity of pet. ether extract of *Myristica dactyloides Gaetrn* bark with standard

S. No.	Fungal strains	Inhibition zone in mm			
		Pet. ether			Fluconazole
		25(µg/ml)	50(µg/ml)	100(µg/ml)	1(µg/ml)
1	*Candida tropicalis*	17.17±0.09	18.93±0.12	22.97±0.15	22.10±0.21
2	*Trichophyton mentagrophytes*	17.90±0.06	19.00±0.06	22.03±0.03	21.20±0.15
3	*Trichoderma viride*	22.87±0.09	23.97±0.03	25.90±0.06	23.03±0.09

Table 3 (b): It shows antifungal activity of ethyl acetate extract of *Myristica dactyloides Gaetrn* bark with standard

S. No.	Fungal strains	Inhibition zone in mm			
		Ethyl acetate			Fluconazole
		25(µg/ml)	50(µg/ml)	100(µg/ml)	1(µg/ml)
1	*Candida tropicalis*	11.93±0.12	13.83±0.09	13.87±0.09	22.10±0.21
2	*Trichophyton mentagrophytes*	06.97±0.15	07.97±0.15	10.93±0.12	21.20±0.15
3	*Trichoderma viride*	13.17±0.09	14.00±0.06	14.13±0.03	23.03±0.09

Table 3 (c): It shows antifungal activity of methanol extract of *Myristica dactyloides Gaetrn* bark with standard

S. No.	Fungal strains	Inhibition zone in mm			
		Methanol			Fluconazole
		25(µg/ml)	50(µg/ml)	100(µg/ml)	1(µg/ml)
1	*Candida tropicalis*	07.01±0.03	09.37±0.03	10.93±0.12	22.10±0.21
2	*Trichophyton mentagrophytes*	08.97±0.15	08.97±0.15	09.90±0.06	21.20±0.15
3	*Trichoderma viride*	10.93±0.12	13.17±0.09	12.97±0.15	23.03±0.09

The results of the antifungal activity of different extracts against some fungal strains are depicted in table 3 (a), 3 (b) and 3 (c) for petroleum ether, ethyl acetate and methanol respectively. In case of antifungal activity of different extracts on three pathogenic fungi it was found that the petroleum ether extract has shown more activity than ethyl acetate and methanol extracts. In the present work, among the three fungi used *Trichoderma viride* was the most susceptible when compared to *Candida tropicalis and Trichophyton mentagrophytes* with inhibition zones of 25.90±0.06 mm, 14.13±0.03 mm and 12.97±0.15 mm for 100µg/ml in petroleum ether, ethyl acetate and methanol extracts, respectively. Results were compared with standard antibiotic Fluconazole.

Thus, the present findings of antimicrobial activity of *Myristica dactyloides Gaetrn* have fairly good degree of correlation with ethno medicinal uses of the plant. Preliminary results of this investigation appear to indicate that the bark of *Myristica dactyloides Gaetrn* has high potential antimicrobial activity. Novel bioactive compounds from the bark need to be isolated and screened for their pharmaceutical and biotechnological applications in order to cure chronic and infectious diseases.

Three different solvent extracts of the bark were tested for antimicrobial activity. Among them, methanol extract exhibited the highest antibacterial activity when compared to petroleum ether and ethyl acetate extracts. The maximum activity was recorded against gram-positive than gram-negative bacteria. In the case of antifungal activity petroleum ether extract showed more antifungal activity than ethyl acetate and petroleum ether extracts. Due to the reported development of resistance by bacteria and fungi to a various commercially available antimicrobial agents, the plant extracts are potential sources of new compounds which may be developed as effective drugs against microorganisms.

Generally the plants do not express activity to fungi even when it expresses, it is at the low profile. But in the present study, the bark extract exhibited a wide range of activities to both the bacteria and fungi. Hence, this plant has the potential for high throughput research in the process of drug development for several diseases.

Anthelmintic property

It was seen that the petroleum ether extract of *Myristica dactyloides Gaetrn* possess dose dependent anthelmintic activity as compared to the standard drug Albendazole, whereas ethyl acetate and methanol extract has showed moderate activity. The mean paralyzing time of *Pheretima Posthuma* with the dose of 50 and 100 mg/ml for petroleum ether extract were found to be 22.83±0.09 and 10.97±0.15 minutes respectively. Albendazole in the concentration of 25 mg/ml has taken 31.00±0.0 minutes for getting paralysis.

The mean death time of *Pheretima Posthuma* with the dose of 50 and 100 mg/ml for petroleum ether extract were found to be 25.10±0.21 and 13.97±0.20 minutes. In the case of Albendazole at a dose of 25 mg/ml cause paralysis only, no death was observed during the experimental period of 4 hours.

The results of anthelmintic activity are depicted in table 4 for petroleum ether, ethyl acetate and methanol extracts respectively. The results obtained depicts that the petroleum ether extract exhibited significant activity when compared to ethyl acetate and methanol extracts. Therefore, this traditional drug is more effective than the commercially available drug Albendazole. Phytochemical screening of the leaves and stem of *Myristica dactyloides Gaetrn* revealed the presence of various valuable secondary metabolites, among them phenolic compounds may contribute [20] to the maximum percentage of anthelmintic property.

Table 4: It shows anthelmintic activity of different extracts of *Myristica dactyloides Gaetrn* bark with standard

S. No.	*Pheretima Posthuma*	Distilled water	Pet. ether		Ethyl acetate		Methanol		Albendazole
			Concentration of extract (mg/ml)						
			50	100	50	100	50	100	25
1	Time taken for paralysis (min)	-	22.83±0.09	10.97±0.15	29.33±0.20	34.10±0.26	29.77±0.19	42.50±0.29	31.00±0.0
2	Time taken for death (min)	-	25.10±0.21	13.97±0.20	36.07±0.23	41.43±0.26	36.97±0.32	50.87±0.41	-

DPPH radical scavenging activity

DPPH radical is one of the few stable and commercially available organic nitrogen radicals [21-23]. This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH radical in test samples. DPPH radical shows a strong absorption maximum at 517 nm (purple). A freshly prepared DPPH solution exhibit a deep purple color with an absorption maximum at 517 nm. The purple color generally fades or disappears when an antioxidant is present in the medium [24-25]. Among the extracts Petroleum ether extract has showed a potent antioxidant activity at the concentration of 10µg/ml, showing an IC_{50} value of 10.97±0.07µg/ml, while ethyl acetate and methanol extracts IC_{50} value of 21.41±0.13µg/ml and 34.34±0.18µg/ml respectively. The similar activity was 5.54±0.03µg/ml for standard ascorbic acid (fig. 1). The results revealed that, dose dependent radical scavenging activity in terms of IC_{50} values.

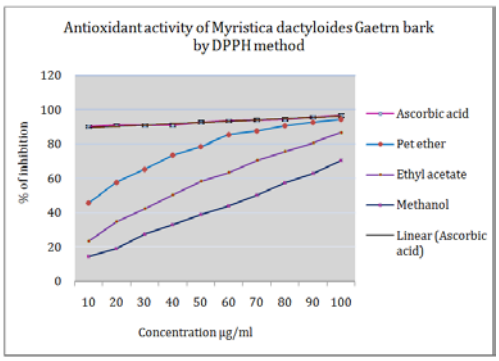

Fig. 1: It shows DPPH radical scavenging activity of *Myristica dactyloides Gaetrn* bark with standard

Estimation of total phenolic content

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators [26]. Phenolic compounds are commonly found in both edible and inedible plants and have been reported to have multiple biological effects, including antioxidant activity. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as the number and location of hydroxyl groups [27]. The phenolic compounds such a phenolic acid and flavonoids are most important antioxidant food source. The quantitative analysis of phenolic acids and flavonoids by the measurement of UV absorption is well known [28]. In the present study, the total phenolic content of *Myristica dactyloides Gaetrn* bark extract was analyzed. The total phenolic content was determined using Folin-Ciocalteu method and total phenolic content of the extract was expressed in terms of milligrams of Catechol per gram of dry weight by reference to standard curve fig. 2. ($y = 0.207x+0.444$ and $R^2 = 0.978$).

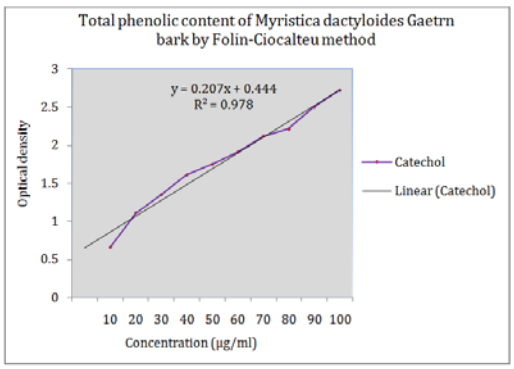

Fig. 2: It shows total phenolic content of *Myristica dactyloides Gaetrn* bark with standard

Total phenolic content in different extracts of plant used in the present study is presented in table 5. It is clear that the level of polyphenols in the methanolic extract of *Myristica dactyloides Gaetrn* was higher when compared to petroleum ether and ethyl acetate extracts of *Myristica dactyloides Gaetrn*.

Table 5: It shows total phenolic content of *Myristica dactyloides Gaetrn*

Extracts	mg of catechol equivalents /100 mg dried extract
Pet. ether	35.15±1.25
Ethyl acetate	60.10±1.65
Methanol	95.11±2.14

Medicinal plants contain various phytochemical compounds that attribute to their medicinal properties. A perusal of the literature reveals that, the majority of the antioxidant activity is due to the polyphenols, flavones, isoflavones, flavonoids, anthocyanin, coumarins, lignans, catechins and isocatechins [29]. The medicinal value of polyphenols in the plants is due to their higher antioxidant nature. Phenolic compounds are a class of antioxidant agents, which act as free radical terminators [30]. It is recognized that, flavonoid shows antioxidant activity and their effects on human nutrition and health was considerable. Flavonoids are a group of polyphenolic compounds with known properties, which includes free radical scavenging, inhibition of hydrolytic, oxidative enzymes and anti-inflammatory action [31, 32].

The preliminary phytochemical analysis of the petroleum ether, ethyl acetate and methanol extracts of bark showed the presence of alkaloids, flavonoids, phenolics, steroids, saponins, terpenoids and tannins this may account for the antioxidant potential of the extracts. The free radical scavenging activity of the plant extract contributes to the neutralization of free radicals, thereby inhibiting chain reaction and stops cellular damage within body cells. Hence, *In vitro* antioxidant activity was determined by the DPPH radical scavenging method and total phenolic content assay. The results confirmed that the petroleum ether extract exhibited a potent antioxidant activity in comparison to that of ethyl acetate and methanol extracts. Among the extracts petroleum ether extract has showed a potent antioxidant activity at the concentration of 10µg/ml, an IC_{50} value of 10.97±0.07µg/ml, while ethyl acetate and methanol extracts showed an IC_{50} value of 21.41±0.13µg/ml and 34.34±0.18 µg/ml respectively. The similar activity was 5.54±0.03µg/ml for standard ascorbic acid (fig. 1). It was found that the minimum is the value of IC_{50}, maximum is the antioxidant activity. The results revealed that, dose dependent radical scavenging activity in terms of IC_{50} values. Total phenolic content in methanol extract was found to be 95.11±2.14 followed by ethyl acetate extract having 60.10±1.65 and petroleum ether extract having 35.15±1.25 expressed as mg of Catechol equivalents/100 mg dried extract. The results of the two procedures are correlated to each other and confirmed the use of the plant as natural antioxidant.

CONCLUSION

The present study reveals that the crude drug posses prominent antimicrobial, anthelmintic and antioxidant properties, which supports its folk claim. Phytochemical studies portray the presence of several biologically active secondary metabolites. Therefore, there is no doubt that this plant is a reservoir of potentially useful chemical compounds, which serve as drugs, provide newer leads and clues for modern drug design.

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CONFLICT OF INTERESTS

Declared None

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