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Research Article

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *MYRISTICA FATUA* VAR. MAGNIFICA (BEDDOME) SINCLAIR

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ABSTRACT

Objective: To evaluate the antioxidant and antibacterial activities of different extracts of *Myristica fatua.*

Methods: Antioxidant potential of different extracts of *M. fatua* was analyzed by following 2, 2-diphenyl -1-picryl hydrazyl and reducing power assay. Extracts were also screened against two Gram-positive and three Gram-negative bacteria. The minimum inhibitory concentration of the extracts was determined by macrodilution technique and the minimum bactericidal concentration (MBC) values by subculturing method. Phenolics and flavonoids were determined by standard procedures. An attempt was made to analyze the correlation between antibacterial and antioxidant activity with phenols and flavonoids using Pearson's correlation.

Results: Bark hexane extract showed the significantly high amount of phenolics 195 (gallic acid equivalent[GAE]/mg), whereas testa methanolic extract showed significantly highest flavonoids (332 mg quercetin equivalent [QE]/g). Ethyl acetate extracts of testa and methanolic extracts of kernel and aril exhibited a better antioxidant activity in terms of IC_{50} values. The MBC assay revealed that chloroform extract of aril at the concentration of 5 mg/ml was enough to inhibit the growth of bacteria *Staphylococcus aureus*. Flavonoids were moderately correlated with reducing power.

Conclusion: Our study suggests that the possibility of using the aril and seed extracts as a natural food preservative and other parts as a new source of natural antioxidants for pharmaceutical industries.

Keywords: *Myristica fatua* var. magnifica, Flavonoids, Phenolics, Antioxidant, Antibacterial.

INTRODUCTION

Medicinal plants are a rich source of secondary metabolites, many of which are potentially used as drugs and bioactive products [1]. Secondary metabolites of plants are an alternative to chemically synthesized products and microbially originated antibiotics. Screening of plants used in ethnomedicine for the cure of infectious diseases is considered as a suitable strategy as those plants are known to possess medicinal properties and are regarded as safe [2]. The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial injections lead to the screening of several medicinal plants for their potential antimicrobial activity [1]. Natural antioxidants from plants have been shown to increase the antioxidant capacity of the plasma [3] while, synthetic antioxidants are reported to have toxic and carcinogenic effects in animal models [4].

Myristica fatua var. magnifica (Beddome) Sinclair, a wild relative of *Myristica fragrans*, endemic to the Western Ghats belongs to family Myristicaceae. It is a large arborescent tree having significant ecological importance and is restricted to freshwater swamps [5]. Although other members of this family were reported to have the antibacterial and antioxidant activities including other various pharmacological properties [6-12], there are no reports on the antibacterial activity and antioxidant properties of *M. fatua.* Hence, the present study was undertaken to report the antioxidant and antibacterial properties of various parts of *M. fatua.*

METHODS

Collection of plant material

Mature, split and aril exposed fruits, leaf, and bark of *M. fatua* var. magnifica were collected directly from the tree in the month of August 2014 from the Katthalekan forests (14° 15' 50" N latitude and 74° 45' 35" E longitude) of Uttara Kannada district of Karnataka, India. Fruit rind, aril, kernel, and testa were separated. All the plant parts were cleaned with tap water, and the parts without any damage or infection were selected for the study.

Processing and extraction

Plant parts were shade dried and coarsely powdered using a Warren blender. Soxhlet extraction of the powder was done using different polar and nonpolar solvents such as water, methanol, ethyl acetate, chloroform, hexane, and petroleum ether. Extracts were concentrated in a rotary evaporator (Superfit, Model-Supervac) and stored in airtight bottles at 4°C until use.

Determination of total phenolic content

The total phenolic content was measured using the Folin-Ciocalteu method [13]. 100 µl of the extract (10 mg/mL) was mixed with 2 mL of 2% Na_2CO_3 and allowed to stand for 2 minutes at room temperature, followed by the addition of 100 µl of 50% Folin-Ciocalteu's phenol reagent. After incubation for 30 minutes at room temperature in darkness, the absorbance was read at 720 nm using spectrophotometer (Systronics-166). The total phenolic contents of the samples were expressed as mg gallic acid equivalent per gram of the extract (mg GAE/g).

Determination of flavonoid content

Total flavonoid content was determined following the aluminum chloride method [14]. A known volume of each of the extract was made up to 4 ml using distilled water followed by the addition of 0.3 ml of $NaNO_2$ (1:20). After 5 minutes, 0.3 ml of 10% $AlCl_3.H_2O$ solution was added. After the 6th minute, 2 ml of 1 M NaOH solution was added, and the total volume was made up to 9 ml using distilled water. The absorbance against blank was determined at 510 nm. Results were expressed as mg quercetin equivalents (QE)/g of extract.

In vitro antioxidant assay

The antioxidant activity of plant extracts was determined by *in vitro* method: the 2, 2-diphenyl -1-picryl hydrazyl (DPPH) free radical scavenging activity and reducing power assay.

Evaluation of DPPH scavenging activity [15]

A solution of DPPH (0.135 mM) in methanol was prepared, and 1 ml of this solution was mixed with 1 ml of varying concentrations of the extracts. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using ascorbic acid as standard. The ability to scavenge DPPH free radical was calculated as:

$$\%\text{DPPH radical scavenging activty} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Reducing power assay [16]

About 100 µl of the extracts of varied concentrations were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and freshly prepared $FeCl_3$ solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Reducing power was expressed as ascorbic acid equivalent (AAE) in milligram per gram of the extract.

Antibacterial activity by disc diffusion method

Five bacterial cultures, *viz*., Two Gram-positive (*Bacillus subtilis* [BS], ATCC 6633 and *Staphylococcus aureus*[SA], NCIM 2079) and three Gram-negative (*Proteus vulgaris* [PV], NCIM 2813, *Pseudomonas aeruginosa* [PA], NCIM 2200, and *Escherichia coli* [EC], NCIM 2931) were obtained from National Chemical Laboratory, Pune, India and were maintained on nutrient agar slants. 200 µl of the overnight grown culture of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 4-5 hrs at 37°C to standardize the culture to 10^{-5} CFU/ml.

Antibacterial activity assay was carried out by disc diffusion method. For this, 0.1 ml (10^{-5} CFU/ml) of 24 hrs old bacterial culture was placed on Mueller-Hinton agar medium and spread throughout the plate by spread plate technique. Sterile paper discs (5 mm in diameter) obtained from Himedia, impregnated with 25 µl of the extract (10 mg/ml) was placed on the surface of the medium and incubated at 37°C for 24 hrs. Antibacterial activity was recorded by measuring the diameter of the zone of inhibition. Tetracycline was used as positive reference standard.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC of the DMSO extracts was determined using different concentrations of extracts in Mueller-Hinton broth for bacteria by macrodilution method [17]. The lowest concentration of the DMSO extract inhibiting the visible growth of microorganisms was considered as MIC. The MBC was determined by subculturing the test dilution on to a fresh drug-free solid medium and incubated for 18-24 hrs. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

Statistical analysis

All the data were expressed as means±standard deviation of triplicate values. One-way analysis of variance was applied using IBM SPSS Statistics 20 software to detect the significance between the inhibitory zones produced by bacteria. Correlation between both the antioxidant activities and phenols as well as flavonoids was carried out using Pearson's correlation (Microsoft Excel Version. 2007).

RESULTS

Total phenolics and flavonoid content

The total phenolics ranged between 14.67 and 195 mg GAE/mg, whereas, flavonoids ranged between 8.0 and 332 mg QE/g (Table 1).

Hexane extract of bark showed significantly the highest phenolics of 195 mg GAE/g followed by bark chloroform extract (149.33 mg GAE/mg), whereas water extract recorded least phenolics (14.67 mg GAE/mg). A significantly higher amount of flavonoid (332 mg QE) was recorded in the methanol extract of testa followed by the chloroform extract of aril (326 mg QE), whereas hexane extract of aril showed the least (8 mg GAE/mg).

Table 1: Phenols and flavonoid content of different extracts of *M. fatua*

Plant parts	Extracts	Phenolics	Flavonoids
Leaf	Water	114±0.26^{f}	23.33±0.60^{q}
	Methanol	98.5±0.70^{h}	11±1.10^{s}
	Hexane	114±0.26^{f}	35.33±0.15^{o}
	Petroleum ether	94.5±0.70^{i}	176±0^{f}
Bark	Water	14.67±0.54^{q}	114±0^{j}
	Methanol	93±0.14^{i}	34.67±0.57^{o}
	Chloroform	149.33±0.23^{b}	156.67±0.32^{h}
	Hexane	195±0.26^{a}	177.33±0.15^{f}
Kernel	Water	17±0.14^{q}	30±0^{p}
	Methanol	137±0.66^{c}	11.33±0.15^{s}
	Ethyl acetate	118±0^{e}	53.33±1.1^{m}
	Chloroform	104±0.28^{g}	326±0^{b}
	Hexane	61±0.14^{m}	252±0^{d}
	Petroleum ether	94±0^{i}	274.67±0.30^{c}
Aril	Water	58±0^{n}	124±0.20^{i}
	Methanol	95±0.15^{i}	85.33±0.15^{k}
	Ethyl acetate	64±0^{l}	18±0^{r}
	Chloroform	120±0de	252±0^{d}
	Hexane	43.33±0.33^{o}	8±0^{t}
	Petroleum ether	58±0^{n}	246±0^{e}
Testa	Water	122±0^{d}	46.67±0.15^{n}
	Methanol	118±0^{e}	332±0.60^{a}
	Ethyl acetate	62.67±0.11^{lm}	162±0^{g}
Fruit rind	Water	24.67±0.11^{p}	86±0.2^{k}
	Methanol	55.33±0.21^{n}	20±0^{r}
	Ethyl acetate	68±0^{k}	57±0.1^{l}
	Chloroform	81±0.71^{j}	246.67±0.15^{e}

Results expressed as Mean±SD (n=3) with different alphabets indicating the significant difference at p<0.01. SD: Standard deviation

Antioxidant activity

Significantly on par good IC_{50} value was observed in the extracts of bark, kernel, aril, and fruit rind (Table 2).

Methanolic and ethyl acetate extracts of kernel, methanolic extract of aril, and ethyl acetate extract of testa also shown significantly higher on par DPPH activity. However, leaf extracts did not show any significant DPPH scavenging activity. The IC_{50} values were significantly higher in ethyl acetate extract of testa and kernel, methanolic extract of kernel and aril compared to standard ascorbic acid.

A significantly higher reducing power of 90.4 mg AAE/g was observed in the ethyl acetate extracts of kernel followed by 81.1, 78.4, and 70.2 mg AAE/g was noticed in the kernel, petroleum ether extract of kernel, and methanolic extract of aril, respectively.

Antibacterial activity

Antibacterial activity of different extracts of *M. fatua* against Gram-positive and Gram-negative bacteria is given in Table 3.

Methanolic extract of kernel showed a good antibacterial activity against *B. subtilis,* which was significantly higher compared to other extracts. A significantly higher antibacterial activity was noticed in case of ethyl acetate extract of the kernel.

The water extract of aril showed a significantly higher inhibition zone of 12 mm, which is almost the half of the inhibition zone shown by standard tetracycline against PV. The chloroform extract of kernel, petroleum ether extract of aril, and water extract of leaf showed significantly a good zone of inhibition.

The hexane extract of the kernel followed by water extract of leaf recorded a good antibacterial activity against PA while water

Table 2: Antioxidant activity of different extracts of *M. fatua*

Plant parts	Extracts	Reducing power assay (A700/20 minutes/100 mg)	DPPH (concentration at IC_{50} value (mg/ml)
Leaf	Water	5.2±0.2^{u}	0.5933±0.003^{h}
	Methanol	16.85±0.74^{q}	0.6866±0.001^{i}
	Hexane	26.9±0.36^{n}	2.63±0.105^{m}
	Petroleum ether	51.1±0^{i}	0.535±0.005^{g}
Bark	Water	8.9±0.2^{s}	0.125±0.003^{c}
	Methanol	7.8±0.2^{s}	0.049±0.002^{ab}
	Chloroform	6.5±0.5^{t}	0.075±0.003^{a-c}
	Hexane	58.9±0.9^{h}	0.068±0.002^{ab}
Kernel	Water	36.6±0.35^{l}	0.2941±0.004^{e}
	Methanol	81.1±0.1^{c}	0.0355±0.0035^{a}
	Ethyl acetate	90.4±0.37^{b}	0.0324±0.002^{a}
	Chloroform	47.2±0.9^{j}	0.0656±0.004^{ab}
	Hexane	21.2±0.15^{o}	0.10056±0.009^{bc}
	Petroleum ether	78.4±0.36^{d}	0.077±0.006^{a-c}
Aril	Water	65.3±0.5^{g}	0.0496±0.001^{ab}
	Methanol	70.2±0.04^{e}	0.038±0.006^{a}
	Ethyl acetate	40±0^{k}	0.054±0.006^{ab}
	Chloroform	32.3±0.5^{m}	0.0496±0.003^{ab}
	Hexane	68.7±0.35^{f}	0.2068±0.0008^{d}
	Petroleum ether	66.43±0.51^{g}	0.3636±0.0024^{f}
Testa	Water	8.6±0.2^{s}	0.8333±0.012^{j}
	Methanol	68.9±0.05^{f}	0.089±0.005^{a-c}
	Ethyl acetate	65.8±0.7^{g}	0.0315±0.004^{a}
Fruit rind	Water	32±0.1^{m}	0.625±0.008^{h}
	Methanol	14.8±0.66^{r}	1.23±0.045^{k}
	Ethyl acetate	21.2±0.2^{o}	2.02±0.011^{l}
	Chloroform	19.1±0.15^{p}	0.050±0.005^{ab}
Standard Ascorbic acid		116±0.2^{a}	0.070±0.003^{ab}

Results expressed as mean±SD (n=3) with different alphabets indicating the significant difference at p<0.01. DPPH: 2, 2-diphenyl-1-picryl hydrazyl, SD: Standard deviation

Table 3: Antibacterial activity of different extracts of *M. fatua*

Plant parts	Extracts	Zone of inhibition (mm)				
		Gram-positive bacteria		Gram-negative bacteria		
		BS	SA	PV	PA	EC
Leaf	Water	8±0^{g}	8.3±0.1^{d-g}	10.36±0.11^{e}	10.3±0.1^{c}	7.3±0^{h}
	Methanol	-	-	-	-	-
	Hexane	6.26±0.05^{j}	8.2±0.1^{d-g}	8.36±0.05^{m}	8.3±0^{h}	8.36±0.05^{g}
	Petroleum ether	6.3±0.05^{j}	7.3±0.1^{f-h}	8.67±0.11^{kl}	8±0.00^{c}	7.0±0.00^{i}
Bark	Water	9.0±0.00^{f}	7.3±0.1^{f-h}	9.0±0.0^{j}	5.93±0.3^{l}	11.0±0.0^{b}
	Methanol	-	-	-	-	-
	Chloroform	7.0±00^{i}	8.6±0.00^{d-g}	7.5±0.1^{n}	9.0±0.0^{e}	9.5±0.3^{e}
	Hexane	6.0±0.0^{k}	9.0±0.0^{b-f}	10.00±0.0^{f}	9.0±0.0^{e}	11.0±0.0^{b}
Kernel	Water	-	-	7.5±0.8^{n}	-	-
	Methanol	14.7±0.1^{b}	9±0^{b-f}	9.7±0.2^{g}	9±0^{e}	10±0^{d}
	Ethyl acetate	8.67±0.4^{f}	10.6±0.1^{b}	9.3±0.1^{i}	9.0±0.3^{e}	6.67±0.2^{i}
	Chloroform	12±0^{c}	10.5±0.2^{bc}	11.7±0.3^{c}	9.7±0.1^{d}	10±0^{d}
	Hexane	11.67±0^{d}	9.5±0.1^{b-d}	8.4±0.2^{m}	10.7±0.1^{b}	9.3±0.3^{e}
	Petroleum ether	11±0e	9±0^{b-f}	9.5±0.3^{h}	8.5±0^{g}	10.5±0.1^{c}
Aril	Water	9±0^{f}	8.67±0.2^{c-g}	12±0^{b}	6.3±0.3^{k}	10±0^{d}
	Methanol	7±0^{i}	7.5±0.1^{e-h}	8.7±0.1^{k}	7.3±0.3^{j}	7±0^{i}
	Ethyl acetate	7±0^{i}	7.5±0.3^{e-h}	8.5±0.2^{lm}	8.6±0.67^{f}	7±0^{i}
	Chloroform	8±0^{g}	8.5±0.5^{d-g}	9.3±0.1^{i}	8.6±0.67^{f}	9±0^{f}
	Hexane	-	-	-	-	-
	Petroleum ether	7.67±0.7^{h}	9.3±0.1^{b-e}	11±0^{d}	8.6±0.6^{f}	9.0±0^{f}
Testa	Water	-	-	-	-	-
	Methanol	-	6±0^{ij}	6±0^{o}	-	-
	Ethyl acetate	6±0^{k}	8±0.7^{d-g}	6±0^{o}	-	6±0^{j}
Fruit rind	Water	6±0^{k}	6±0^{ij}	-	6±0^{l}	6±0^{j}
	Methanol	-	-	-	-	-
	Ethyl acetate	7±0^{i}	7±0^{g-i}	6±0^{o}	-	6±0^{j}
	Chloroform	6.1±0.1^{k}	7.1±0.1^{f-h}	-	-	6±0^{j}
Standard tetracycline		24.1±0.2^{a}	22±0.00^{a}	23±0.1^{a}	22.4±0.1^{a}	21.8±0.1^{a}

The diameter of zone of inhibition (mm) including disc diameter of 5 mm. Results with different alphabets indicating the significant difference at p<0.01. BS: *Bacillus subtilis*, SA: *Staphylococcus aureus,* PV: *Proteus vulgaris*, PA: *Pseudomonas aeruginosa*, EC: *Escherichia coli*, -: No activity

and hexane extract of bark exhibited on par higher antibacterial activity against EC. However, the antibacterial activity of the crude extract was more than 50 times lower compared to standard drug tetracycline.

Petroleum ether extract of aril inhibited the visible growth of *B. subtilis* at an MIC of 1.25 mg (Table 4). Similarly, the methanol extract of kernel showed an inhibition of S. aureus at the concentration of 1.25 mg.

The MBC assay revealed the MBC of 5 mg and 10 mg of the chloroform extract of aril and methanolic extract of kernel, respectively (Table 5).

DISCUSSION

The hexane extract of stem bark showed the moderately higher amount of total phenolics compared to extracts of other parts. Similarly, the crude methanolic extracts of *Myristica dactyloides* bark showed high phenolics [18]. In *Monodora myristica* and *M. fragrans,* the seed extracts showed a high amount of total phenolics as reported by Enabulele *et al.* [19] and Hou *et al.* [20].

A good amount of flavonoids were recorded in different extracts of aril while leaf extracts recorded the least. Chloroform extract of kernel exhibited 274.67 mg QE/g which was four-fold higher than the flavonoid content of the same extract in *Knema attenuata* as reported by Vinayachandra and Chandrashekar [21].

Ethyl acetate extract of testa showed significantly higher IC_{50} at 0.0315 mg which is half the IC_{50} value of standard ascorbic acid (0.0708 mg). Methanolic extracts of kernel, aril, and stem bark exhibited a good response to the DPPH activity by showing IC_{50} values at 0.0355, 0.038, and 0.049 mg which is two-fold of the IC_{50} value of *Myristica malabarica* stem bark methanolic extract (0.020 mg/ml) as reported by Manjunatha *et al.* [22].

There was no correlation between phenols and DPPH assay as reported by Vinayachandra and Chandrasekhar [21] in *K. attenuata*. Even in the present study, there was no correlation between total phenolic content and antioxidant activities (Fig. 1a).

However, flavonoids are moderately correlated with reducing power (R=0.6813), while with DPPH a negative correlation (R=−0.3479) was observed (Fig. 1b).

Negative correlation between DPPH activity and flavonoid was reported in citrus species earlier by Ghafar *et al*. [23]. Benavente *et al*. [24] reported that flavonoids are responsible for the antioxidant properties of the medicinal plants, which according to them may be through scavenging of free radicals, through chelation of ions.

Variations in the correlation among antioxidant assays observed in this study indicate that a single assay may not be sufficient to evaluate the total antioxidant activity [25]. According to Hou *et al*., [20] phenolic compounds with more methoxy groups and fewer hydroxyl groups have a lower antioxidant property than the compounds possessing ortho-hydroxyl groups as found in *M. fargrans,* which may be the reason for the difference in the antioxidant properties of extracts.

Gram-positive bacteria were appeared to be more sensitive to plant extracts compared to Gram-negative bacteria. Aril petroleum ether extract and kernel methanolic extract showed moderately good MIC value of 1.25 mg/ml towards *B. subtilis* and *S. aureus,* respectively. Mahady *et al*. [9] reported that the MIC value of the methanol extract

Table 4: MIC

Part	Extract	Bacteria	MIC (mg)
Aril	Petroleum ether	*B. subtilis*	1.25
Aril	Methanol	*B. subtilis*	10
Aril	Ethyl acetate	*B. subtilis*	2.5
Aril	Chloroform	*S. aureus*	2.5
Kernel	Methanol	*S. aureus*	1.25
Standard tetracycline		*B. subtilis*	0.0025
Standard tetracycline		*S. aureus*	0.0025

MIC: Minimum inhibitory concentration, *B. subtilis*: *Bacillus subtilis, S. aureus: Staphylococcus aureus*

Table 5: MBC

Part	Extract	Bacteria	MBC (mg)
Aril	Chloroform	*S. aureus*	5
Kernel	Methanol	*S. aureus*	10
Standard tetracycline		*S. aureus*	0.0025

MBC: Minimum bactericidal concentration. S. *aureus: Staphylococcus aureus*

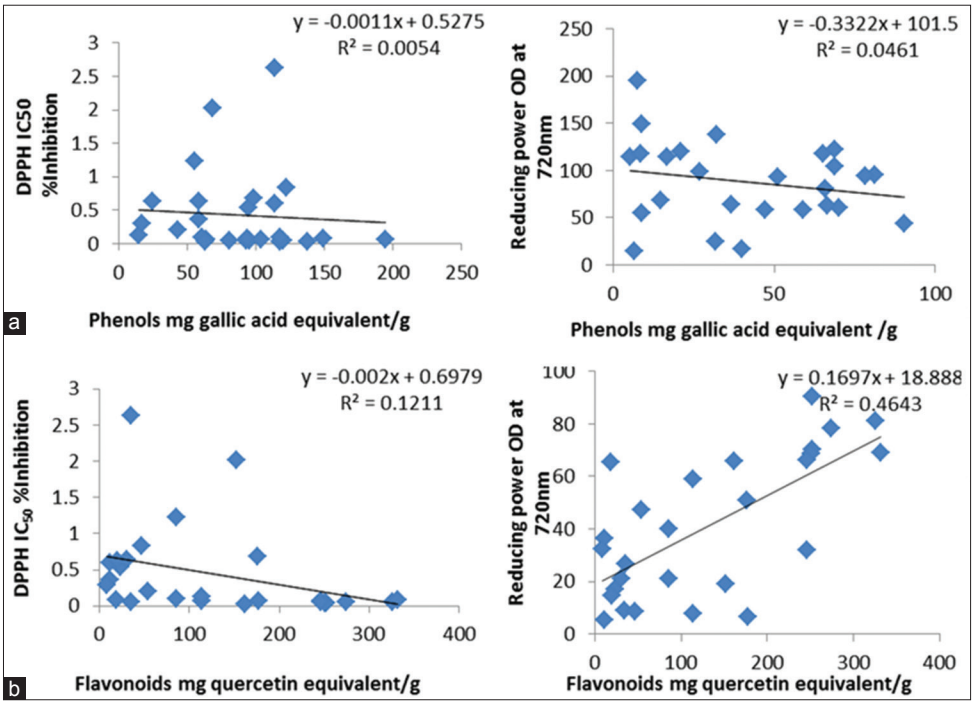

Fig. 1: (a) Linear correlation between, (1) phenols and 2, 2-diphenyl -1-picryl hydrazyl (DPPH) (2) phenols and reducing power, (b) linear correlation between, (1) flavonoids and DPPH (2) flavonoids and reducing power

of the seed was 12.5 µg/ml against *Helicobacter pylori.* In case of *M. myristica* seeds, MIC for extract ranged between 2.5-3.0 mg/ml, whereas that of the ethanolic extract ranged between 2.5 and 3.5 mg/ml as reported by Enabulele *et al*. [19].

In the present study, *S. aureus* showed MBC value at 5 and 10 mg/ml by the action of the chloroform extract of aril and methanolic extract of kernel, respectively. These values are comparable with MBC values obtained for the water extract (3-3.5 mg/ml) and ethanolic extract (3.5-4 mg/ml) for the seeds of *M. myristica.* [19].

Phenols, alcohols, aldehydes, ketones, ethers, and hydrocarbons have been recognized as major antimicrobial components in spices [26]. The antimicrobial activity of extracts was closely associated with their phenolic contents reported by Shan *et al*. [8]. Phenolic compounds act by causing the leakage of cytoplasmic constituents from bacteria which may be due to the disruption of cell peptidoglycan or damage of cell membrane. The presences of hydroxyl group in phenolic compounds make them antimicrobial by binding to the active sites and altering the metabolism and lipid solubility [26].

The cell wall structure of Gram-negative enteric bacteria may be responsible for observed resistance. For instance, the cell wall of Gram-negative bacteria contains 15-20% polysaccharides and 10-20% lipids, whereas cell wall of Gram-positive bacteria contains 35-60% polysaccharides and only 0-2% lipids [27]. The observed difference in their effectiveness could be due to the crude nature of the plant extracts which may contain some impure substances that may be inert and do not have antimicrobial property [28].

CONCLUSION

The results of the present study suggest that the extract of *M. fatua* contains compounds with the antimicrobial property that can be used as the antimicrobial agent for the therapy of infectious diseases caused by pathogens. The results also suggest the possible use of the aril and seed extracts as a natural food preservative and other parts as a new source of natural antioxidants to be used in pharmaceutical industries.

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