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ANTI-BACTERIAL, ANTI-OXIDANT AND CYTOTOXIC POTENTIAL OF VARIOUS EXTRACTS OF *MYRISTICA FRAGRANS*

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ABSTRACT

The scientists have identified number of herbs from different parts of India having medicinal properties. The aim of the study was to examine anti-microbial, anti-oxidant and cytotoxicity in fruit extract of *Myristica fragrans* (aqueous, cyclohexane, benzene, chloroform, acetone, ethyl acetate, ethanol and methanol). The phytochemical analysis revealed the presence of alkaloid, flavonoid, tannins, glycoside, reducing sugar, steroids and terpenoids that may be responsible for its anti-microbial, anti-oxidant and cytotoxicity in *M. fragrans* extracts. The methanolic extract showed considerable anti-microbial activity against *S. aureus* and the zone of inhibition was found to be 16 mm at the concentration of 40 mg/ml of plant extract by well and disc diffusion method. The anti-oxidant activity by different methods was screened and the highest activity was shown by methanol extract and gave IC₅₀ value of 56.83 ± 5.67 µg/ml by DPPH method. The superoxide radicals were scavenged strongly by *M. fragrans* acetone extract which showed IC₅₀ value of 321.2 ± 12.75 µg/ml. The nitric oxide radicals were scavenged strongly by *M. fragrans* methanol extract demonstrated IC₅₀ value of 45 ± 1.55 µg/ml. The hydroxyl radicals were strongly scavenged by *M. fragrans* benzene extract and showed IC₅₀ value of 181.3 ± 4.04 µg/ml. The extracts of *M. fragrans* were found cytotoxic to cancerous cell lines by MTT cell proliferation assay. The chloroform extract of *M. fragrans* showed cytotoxicity in A549 cell line with IC₅₀ value of 392 ± 6.51 µg/ml.

Keywords: *Myristica fragrans*, MTT assay, DPPH, anti-oxidant activity and cytotoxic

INTRODUCTION

The plant of *Myristica fragrans* is also known as nutmeg tree. It is one of the commercially important plant and its parts are used as food product and one of the several species found in genus *Myristica*. The *M. fragrans* tree is important for two spices derived from the fruit of the plant, they are nutmeg and mace. The nutmeg is known commonly as Jaiphal. The seed of the tree is known as nutmeg, the shape of the seed is roughly like egg and its size varies from 20 to 30 mm (0.8 to 1.2 in) long and 15 to 18 mm (0.6 to 0.7 in) wide. The weight of *M. fragrans* fruit is found between 5 and 10 g (0.2 and 0.4 oz) in the dry form. The fruit propagation in plant takes a long time and the first harvest of nutmeg takes place in 7–9 years after planting. *Myristica fragrans* are carminative, stimulating, astringent, digestive, stomachic, abortifacient and aphrodisiac, useful in flatulency, nausea, vomiting and also used in tonics. *M. fragrans* is a constituent for preparations of medicines for dysentery, diarrhea, stomachache, malaria, rheumatism and sciatica¹. The ethyl acetate and ethanol extracts of *M. fragrans* showed anti-microbial activity against three gram-positive bacteria *Streptococcus mutans*, *Streptococcus mitis* and *Streptococcus salivarius* and two gram negative bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*². The *M. fragrans* oil was found cytotoxic to MCF-7 breast cancer and A-357 epidermal skin cancer cell lines³. Another study shows anti-microbial activity against *Shigella dysenteriae* and cytotoxicity against Vero cell line⁴. The present study

includes the separation of crude aqueous and organic extracts (aqueous, cyclohexane, benzene, chloroform, acetone, ethyl acetate, ethanol and methanol) of *M. fragrans*. The extracts were screened for anti-microbial activity against different bacterial strains by well and disc diffusion method. The anti-oxidant activity of *M. fragrans* extracts were analyzed by DPPH, alkaline DMSO, nitric oxide and hydroxyl radical by Deoxyribose scavenging assay. The cytotoxicity was screened on cancerous (A549 and Jurkat) and normal (Hek 293 and MRC-5) cell lines.

MATERIALS AND METHODS

Collection and Identification of Plant

The leaves of plant were collected from in and around the campus of Integral University and were authenticated and sample vouchers were stored in NBRI, Lucknow, India. The identified plant parts were washed and air dried at room temperature and was powdered with the help of mortar and pestle. The plant extracts prepared using Soxhlet apparatus in different solvents.

Determination of Extraction Yield of Plant Extract (% yield)

The yield (% w/w) from all dried extracts was calculated by the formula given below.

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0} \times 100$$

Where, W₂ is the weight of the extract and the container, W₁ weight of the container alone and W₀ the weight of the plant powder⁵

Phytochemical Analysis (Qualitative) of Plant Extract

The qualitative phytochemical analysis was estimated on the basis of color formation by standard methods⁶⁻¹⁰.

Alkaloids

The plant extract was prepared by taking 500 mg of dry plant material in 500 ml of methanol on a water bath at 37° C for 20 min, the extract was filtered, cooled and few drops of Wagner's reagent added (2 g iodine and 6 g of potassium iodide in 100 ml distilled water). A reddish brown colored precipitate indicates the presence of alkaloids.

Flavonoids

The crude extract was heated with 10 ml of ethyl acetate for 3 min, filtered and 1 ml of ammonia solution was added to 4 ml filtrate, formation of yellow color indicates the presence of flavonoids.

Glycosides (Fehling's Test)

To the 2 ml of extract, 10 ml of 50% hydrochloric acid (HCl) was added and heated in water bath for 30 min and to it 5 ml of Fehling's solution was added. After 5 min formation of brick red precipitate indicates the presence of glycosides.

Terpenoids

The 0.2 g of dried plant material was mixed with 2 ml of chloroform and 3 ml of concentrated hydrochloric acid (HCl) was added carefully to form a layer. A reddish brown coloration of the interface formed indicates the presence of terpenoids.

Tannins (Ferric Chloride Test)

The 0.5 g of extract was dissolved in 10 ml of distilled water, filtered and ferric chloride reagent was added, a blue-black precipitate was taken as evidence for the presence of tannin.

Reducing Sugar

The 0.5 g of extract dissolved in 1 ml of distilled water and 2 to 8 drops of Fehling solution was added and boiled for few minutes. The presence of brick red precipitate indicates presence of reducing sugar.

Steroids (Salkowski test)

The extract was dissolved in methanol and to it 5 drops of concentrated hydrogen sulphoxide (H₂SO₄) was added. The formation of red color indicated the presence of steroids.

Anti-microbial activity

The anti-microbial activity was screened in extracts by disc diffusion and well diffusion method. The bacterial strains used were *Staphylococcus aureus* 2079, *Escherichia coli* 2065, *Proteus vulgaris* 2027, *Bacillus cereus* 2156, *Bacillus subtilis* 296, *Staphylococcus epidermis* 2493 and *Sacchromyces cereviceae* 3090. All the strains were obtained from the National Chemical Laboratory (NCL), Pune, India. Dried filter paper discs (4 mm) impregnated in known amount of test samples and for well diffusion method, the extracts were inoculated in well prepared using well cutter (0.6 cm). The dried plant extracts were prepared in dimethyl sulfoxide at a concentration of 40 mg/ml, 30 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml. The plates were incubated at 37°C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which dimethyl sulfoxide was used as a negative control instead of the extract and

the discs of Tetracycline (30 mcg/disc), Penicillin G (10 units/disc), Streptomycin (10 mcg/disc) and Amoxicillin (30 mcg/disc) were used as a positive control. The experiment was done three times and the mean values are presented¹¹.

Anti-oxidant assay

The different anti-oxidant assays used for the study were by DPPH method, superoxide radical with the alkaline DMSO (dimethyl sulfoxide) method, nitric oxide radial inhibition assay and hydroxyl radical in the deoxyribose method. L-ascorbic acid, butylated hydroxy toluene and quercetin were used as standard, while methanol or dimethyl sulfoxide was used in place of plant extract as control. The crude plant extracts were prepared at different concentrations varying from 1000 µg/ml to 0.46 µg/ml.

DPPH (2, 2 – Diphenyl – 1- Picryl Hydrazyl) radical scavenging activity method

DPPH radical scavenging activities of all the fractions were determined by the method of Blois with some modification¹². Initially 10 µl of plant extracts was mixed with 200 µl of 100 mM DPPH (dissolved in methanol). The reaction mixtures were incubated for 30 min at 37° C under dark condition. The absorbance was measured at 490 nm spectrophotometrically¹³.

$$\text{Scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract}) \times 100}{\text{Absorbance of control}}$$

Scavenging of Superoxide radical with the alkaline DMSO (dimethyl sulfoxide) method

Alkaline DMSO radical scavenging assay were determined by the method of Kunchandy and Rao¹⁴ with slight modification^{15, 16}. The reaction mixture prepared of 0.1 ml of nitro blue tetrazolium (1 mg/ml in DMSO) and 1 ml of alkaline DMSO (1 ml of DMSO containing sodium hydroxide 5 mM in 0.1 ml of water). To the reaction mixture 0.3 ml of the crude extract prepared in DMSO was added. To give a final volume of 1.4 ml and the absorbance was measured at 560 nm spectrophotometrically.

$$\text{Percentage super oxide scavenging activity} = \frac{\text{Test absorbance} - \text{Control absorbance} \times 100}{\text{Test absorbance}}$$

Anti-oxidant assay by Nitric oxide radial inhibition assay

The 1 ml of plant extracts was mixed with 1 ml phosphate buffer saline and 4 ml (10 mM) sodium nitroprusside and was kept for incubation at room temperature at 25° C for 150 min. After incubation, 0.5 ml of reaction mixture and 1 ml sulphanic acid reagent (0.33% in 20% glacial acetic acid) were added and incubated for 5 min at room temperature (for diazotization reaction). Then 1 ml N-(1-naphthyl) ethylenediamine dihydrochloride was added and kept in diffused light for 30 min and absorbance was measured at 540 nm¹⁷.

$$\text{Scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract}) \times 100}{\text{Absorbance of control}}$$

Scavenging of Hydroxyl radical in the Deoxyribose method

Scavenging of hydroxyl free radical was measured by the method of Halliwell¹⁸ and co-workers with minor changes. The reaction mixture was prepared containing deoxyribose (3mM) 0.2 ml, ferric chloride (0.1mM) 0.2 ml, ethylenediaminetetraacetic acid disodium salt (EDTA) (0.1 mM) 0.2 ml, ascorbic acid (0.1 mM) 0.2 ml and hydrogen peroxide (2 mM) 0.2 ml in phosphate buffer (pH, 7.4, 20 mM). To the reaction mixture 0.2 ml of various concentrations of the extract or standard in DMSO were added to give total volume of 1.2 ml. The solution was then incubated for 30 min at 37° C. After incubation, ice-cold trichloroacetic acid (0.2 ml, 15% w/v), and thiobarbituric acid (0.2 ml, 1% w/v) in 0.25 N hydrochloric acid were added. The reaction mixture was then kept in a boiling water bath for 30 min, cooled, and the absorbance was measured at 532 nm^{19, 16}.

$$\text{Scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract}) \times 100}{\text{Absorbance of control}}$$

Cytotoxicity by MTT cell proliferation assay

Cell proliferation were measured by using MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide) assay that colorimetrically measures a purple formazan compound produced by viable cells²⁰. The cell lines used for the study were Jurkat (human lymphoblastic leukaemia), Hek 293 (human kidney), A549 (human alveolar adenocarcinoma) and MRC-5 (human lung). The cells were plated 0.5 x 10⁴ cells for A549, Hek 293 and 1 x 10⁴ cells for MRC-5 and Jurkat in 96 well plates. After 24 h of plating, cells were treated with crude plant extracts of different solvents at the concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml. The treated cells were incubated for 24 h at 37° C. After 24 h of treatment, MTT was added and cells were kept for 4 h at 37° C. The formation of purple formazan compound produced by viable cells was dissolved in dimethyl sulphoxide and the plates were read at 570 nm wavelength using ELISA reader. Results are expressed as percentage inhibition of growth treated with plant extracts. All the assays were performed in triplicate. The percentage inhibition was calculated in cancerous and normal cell lines and IC₅₀ values were determined.

RESULTS

Myristica fragrans had a value in herbal medicine for the treatment of different diseases. The crude extracts were prepared in different solvents. The presence of alkaloid, flavonoid, tannins, glycoside, reducing sugar, steroids and terpenoids which were estimated qualitatively and may be responsible for its anti-microbial, anti-oxidant and cytotoxicity of *M. fragrans* extracts. The initial weight of the dried plant was taken that is 20 gm in 200 ml of solvent. The percentage yield obtained in different extracts were found 3% in aqueous extract, 0.2% in cyclohexane extract, 0.4 % in benzene extract, 6.55 % in chloroform extract, 10.7 % in acetone extract, 7.45 % in ethyl acetate extract, 1.1 % in ethanol extract and 0.1 %

in methanol extract. A wide range of the yields among extracts was observed depending on the extraction solvent.

Anti-microbial activity of aqueous and organic extracts

The *M. fragrans* aqueous and organic extracts showed significant anti-microbial activity against *B. subtilis*, *E. coli*, *S. epidermis*, *S. cereviceae*, *P. vulgaris*, *B. cereus* and *S. aureus*. The anti-bacterial activity by well diffusion method was found to be in the order of methanol > acetone > chloroform > ethanol > ethyl acetate extract. The aqueous, cyclohexane and benzene extracts did not showed any activity against any of the strains used. The results of anti-microbial activity of *M. fragrans* showing zone of inhibition by well diffusion method are given in Table 1. The methanolic extract showed considerable activity against *S. aureus* and the zone of inhibition by well diffusion method was found to be 16 mm at the concentration of 40 mg/ml of plant extract. The anti-bacterial activity by disc diffusion method was found to be in the order of methanol > acetone > chloroform > ethanol > ethyl acetate extract. The aqueous, chloroform and benzene extracts did not showed any activity against any of the strains used. The methanolic extract showed considerable activity against *S. aureus* and the zone of inhibition was found to be 16 mm at the concentration of 40 mg/ml by disc diffusion method. The plant extracts when compared with the antibiotics for their anti-bacterial activity they showed significant activity and zone of inhibition in them were found equivalent to standard antibiotics. The zone of inhibition of standard antibiotics and negative control dimethyl sulfoxide were given in Table 3.

Comparative IC₅₀ values of various extracts by different anti-oxidant assay

The IC₅₀ values of the aqueous and organic extracts were calculated by different anti-oxidant assay. The crude extracts of *M. fragrans* showed 50 percent inhibition by different anti-oxidant assays as shown in Table 4. The data reveals *M. fragrans* methanolic extract showed considerable scavenging activity of DPPH free radicals and gave IC₅₀ value of 56.83 ± 5.67 µg/ml by DPPH method. The superoxide radicals were scavenged strongly by *M. fragrans* acetone and methanolic extracts and the acetone extract gave IC₅₀ value of 321.2 ± 12.75 µg/ml. The nitric oxide radicals were scavenged strongly by *M. fragrans* chloroform, ethyl acetate, ethanol and methanolic extracts. The methanol extract gave IC₅₀ value of 45 ± 1.55 µg/ml by nitric oxide method. The hydroxyl radicals were strongly scavenged by *M. fragrans* aqueous, chloroform, ethanol and methanolic extracts and the chloroform extract gave IC₅₀ value of 181.3 ± 4.04 µg/ml. The data obtained from scavenging assay clearly establish the anti-oxidant potency of all the extracts and the presence of alkaloid and flavonoid proves to be responsible for anti-oxidant activity of plant extract.

Table 1: Zone of inhibition (mm) of *Myristica fragrans* extract in different solvents by agar well diffusion method

Plant extract	Conc. of extract	Zone of Inhibition (mm)						
		<i>B. subtilis</i>	<i>E. coli</i>	<i>S. epidermis</i>	<i>S. cereviceae</i>	<i>P. vulgaris</i>	<i>B. cereus</i>	<i>S. aureus</i>
Aqueous	40 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Cyclohexane	40 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Benzene	40 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Chloroform	40 mg/ml	13±0.1	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	12 ±0.05	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	11 ±2.55	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	10 ±4.67	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Acetone	40 mg/ml	29 ±0.1	NZ	12±0	NZ	NZ	NZ	NZ
	30 mg/ml	20 ±0.05	NZ	11±0.22	NZ	NZ	NZ	NZ
	20 mg/ml	19 ±0.05	NZ	10 ±0.1	NZ	NZ	NZ	NZ
	10 mg/ml	18 ±0.05	NZ	9 ±0.76	NZ	NZ	NZ	NZ
	5 mg/ml	10 ±0.5	NZ	NZ	NZ	NZ	NZ	NZ
Ethyl acetate	40 mg/ml	NZ	8 ±0	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	7 ±1	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	4 ±1.1	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	3 ±0	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ethanol	40 mg/ml	10 ±1	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	9 ±2	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	7 ±2	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	6 ±0.1	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Methanol	40 mg/ml	13 ±2.3	15 ±0	13 ±0.34	14 ±0	13 ±5.6	15 ±0.5	16 ±1
	30 mg/ml	11 ±1.1	14 ±0	12 ±0.1	13 ±3.56	12 ±0	14 ±0.56	15 ±3.4
	20 mg/ml	9 ±0	14 ±5.0	11 ±0	12 ±0.95	11 ±0.1	13 ±0.1	10 ±0
	10 mg/ml	8 ±1.6	13 ±2.2	10 ±0	11 ±4.5	11 ±0	12 ±0.2	7 ±3.67
	5 mg/ml	NZ	4 ±0.5	NZ	NZ	NZ	4 ±0.05	5 ±0.05

The values are mean ± standard deviation (n=3) and NZ- No Zone.

Table 2: Zone of inhibition (mm) of *Myristica fragrans* extract in different solvents by disc diffusion method

Plant extract	Conc. of extract	Zone of Inhibition (mm)						
		<i>B. subtilis</i>	<i>E. coli</i>	<i>S. epidermis</i>	<i>S. cereviceae</i>	<i>P. vulgaris</i>	<i>B. cereus</i>	<i>S. aureus</i>
Aqueous	40 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Cyclohexane	40 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Benzene	40 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Chloroform	40 mg/ml	13 ±0.05	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	12 ±0.5	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	11 ±0.1	NZ	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	10 ±0.1	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Acetone	40 mg/ml	13 ±0	NZ	10 ±0.2	NZ	NZ	NZ	NZ
	30 mg/ml	10 ±4.2	NZ	8 ±0.05	NZ	NZ	NZ	NZ
	20 mg/ml	9 ±0.05	NZ	3 ±0.05	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ

	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ethyl acetate	40 mg/ml	NZ	8 ±0	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	NZ	7 ±0.5	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	NZ	4 ±0.1	NZ	NZ	NZ	NZ	NZ
	10 mg/ml	NZ	3 ±0.2	NZ	NZ	NZ	NZ	NZ
Ethanol	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	40 mg/ml	9 ±0.05	NZ	NZ	NZ	NZ	NZ	NZ
	30 mg/ml	7 ±0.5	NZ	NZ	NZ	NZ	NZ	NZ
	20 mg/ml	7 ±3.1	NZ	NZ	NZ	NZ	NZ	NZ
Methanol	10 mg/ml	6 ±4.22	NZ	NZ	NZ	NZ	NZ	NZ
	5 mg/ml	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	40 mg/ml	10 ±0	13 ±4.2	NZ	13 ±1.22	12 ±0.5	8 ±0.5	16 ±2.33
	30 mg/ml	9 ±4.22	12 ±2.1	NZ	12 ±0.05	10 ±0	7 ±0.05	15 ±3.1
	20 mg/ml	7 ±0.05	10 ±0	NZ	10 ±0.2	8 ±0.5	3 ±0.5	10 ±6.32
	10 mg/ml	NZ	9 ±0.1	NZ	8 ±0.05	NZ	NZ	7 ±0.05
	5 mg/ml	NZ	4 ±0.5	NZ	NZ	NZ	NZ	5 ±0.05

The values are mean ± standard deviation (n=3) and NZ- No Zone.

Table 3: The data represents zone of inhibition (mm) of the standards.

Standards	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. epidermis</i>	<i>S. cereviceae</i>	<i>P. vulgaris</i>	<i>B. cereus</i>	<i>S. aureus</i>
Amoxicillin	NZ	NZ	12 ±3.21	NZ	NZ	NZ	NZ
Penicillin G	NZ	NZ	NZ	NZ	20 ±0.91	NZ	NZ
Tetracycline	27 ± 0.05	24 ± 0.1	28 ±0	23 ±0.2	25 ±0.43	16 ±0.5	25 ±0.1
Streptomycin	20 ±0.5	16 ±0	NZ	20 ±0.05	20 ±0.1	21 ±0.63	15 ±0.11
Dimethyl Sulfoxide	NZ	NZ	NZ	NZ	NZ	NZ	NZ

Dimethyl sulphoxide was used as a negative control. The values are mean ± standard deviation (n=3).

Table 4: Comparative chart of IC₅₀ values of aqueous and organic extracts of *Myristica fragrans* and standard L-ascorbic acid, BHT and quercetin

Plant name	Extract in different solvents	IC ₅₀ values ± SD (µg/ml) of different anti-oxidant assay			
		DPPH	Alkaline DMSO	Nitric oxide	Deoxyribose
<i>M. fragrans</i>	Aqueous	-	-	-	777.2±0
	Chloroform	-	-	105.8±0	181.3±4.04
	Acetone	-	321.2±12.75	-	-
	Ethyl acetate	-	-	106.7±3.17	-
	Ethanol	-	-	56.8±1.55	446.3±8.48
	Methanol	56.83±5.67	665.9±0	45±1.55	668.5±16.22
L-ascorbic acid		61.4±1.55	537.7±14.33	54.97±4.73	865.2±1.50*
BHT		50.8±3.85	801.5±0	461.3±2.54*	958.8±0
Quercetin		27.9±1.55	316.5±1.21*	47.57±10.68	419.9±1.2*

Unit for IC₅₀ for all the activities are µg/ml. Data are expressed as mean ± SD (n=3). *p< 0.0001 vs 0 µg/ml.

Table 5: Comparative chart of IC₅₀ values of different cancerous and normal cell lines

Plant Name	Extract in different solvents	Jurkat	Hek-293	A-549	MRC-5
<i>Myristica fragrans</i>	Chloroform	-	-	392±6.51* µg/ml	-

Data represents the mean ± SD (n=3). *p<0.0001 versus 0 µg/ml.

Cytotoxicity in different cell lines by crude plant extracts using MTT assay

The *M. fragrans* chloroform extract showed maximum cytotoxicity to A549 cells as compared to other extracts with IC₅₀ value of 392 ± 6.51 µg/ml. Whereas, *M. fragrans* extracts showed less cytotoxicity on Jurkat cell line. The IC₅₀ values of different extracts were given in Table 5. The cytotoxicity on A549 cell line indicates the anti-cancer activity of the crude plant extracts.

DISCUSSION

In the present study it was found that the methanolic extract of *M. fragrans* showed considerable activity against gram negative bacteria *Escherichia coli* with MIC values of 45 µg/ml. Some of the studies carried out by Gupta²¹ and co-workers, the acetone extract of *M. fragrans* showed anti-bacterial activity against gram positive bacteria *B. subtilis* and *S. aureus* and against

gram negative *P. putida* and *P. aeruginosae* bacteria using disc diffusion method. The antioxidant activity in *M. fragrans* was shown considerable in methanolic and other extracts by different methods. The strongest activity was found in methanolic extract with IC₅₀ value of 45 ± 1.55 µg/ml by nitric oxide method. Akinboro²² and co-workers reported anti-oxidant activity in aqueous extract *M. fragrans* by DPPH scavenging assay having IC₅₀ value of 0.20 mg/ml. The anti-oxidant property in *M. fragrans* acetone extract were reported by DPPH radical scavenging activity it was found to be 0.66 ± 0.015 mg/ml⁷. Tan²³ and co-workers, reported anti-oxidant activity in seed of *M. fragrans* by FRAP and TEAC values of 190.83 ± 17.33 and 50.99 ± 0.23 respectively. *M. fragrans* showed considerable anti-oxidant activity by different methods. The methanolic extract of *Caesalpinia crista* leaves also showed similar anti-oxidant scavenging activity by different methods with IC₅₀ value of 0.44 ± 0.1 mg/ml, 24.9 ± 0.98 µg/ml, 33.72 ± 0.85 µg/ml, 61.13 ±

3.24 µg/ml and 170.51 ± 4.68 µg/ml for hydroxyl, superoxide, nitric oxide, singlet oxygen and hypochlorous acid respectively²⁴. Prakash and Gupta²⁵ reported ethanolic extracts of *M. fragrans* inhibited growth of human cancer cell lines. The cytotoxicity was evaluated by SRB assay at the concentration of 100 µg/ml and the percentage cytotoxicity obtained in different cell lines were 97% in ovary cell line (OVCAR-5), 86% in Colon 502713 and prostate (PC-5), 73% in Central nervous system (SF-295) and Colo205 and 72% in HepG2. While in another study showed methanolic extracts of *M. fragrans* were found cytotoxic to Jurkat cells at the concentration of 50 and 100 µg/ml by MTT cell proliferation assay. Apoptosis was induced in Jurkat cells by *M. fragrans* in a dose dependent manner, the concentration ranges from 20-100 µg/ml by annexin V staining and further evaluated by SIRT1 gene down-regulation at the concentration of 10 µg/ml²⁶.

CONCLUSION

The present study showed potent anti-microbial, anti-oxidant and cytotoxicity in *M. fragrans* extracts. The methanolic extract of *M. fragrans* was found to have the maximum activity and can be used as a therapeutic agent for treating number of diseases. Further studies could be carried out for further purification and characterization of its compounds.

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