



## Tumorolytic Activity of *Myristicafragrans* Metabolic Compounds *M. fragrans* in cancer studies

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### Abstract

*The aqueous and methanol extract of Myristicafragrans (seed and mace) were accessed for their antibacterial activity. The extracts of Myristicafragrans were found to be more or less active against almost all tested pathogenic strains. The phytochemical analysis showed the presence of Flavonoids, Saponins, Alkaloids, Amino acids, Tanins and Terpenoids in both aqueous and methanol extracts. The active components present in the extract were found to be amino acids, Alkaloids, Lipids and terpanoids. The antibacterial activity of active components present in the seed and mace extracts showed inhibition of Salmonella and Klebsiella. The aim is to stimulate research in this field, prevent and treat osteosarcoma cancer by initiating detailed studies in this field. It will be a novel agent, safe and effective for humans suffering with cancer in general and as a specific agent. Myristicafragrans possesses significant anticancer activity in in-vitro in human osteosarcoma cancer cell line (MG-63) and it was found effective.*

**Key words:** *Myristicafragrans, Saponins, Salmonella, Klebsiella and osteosarcoma.*

### I. INTRODUCTION

Cancer is often deadly and affects a considerable number of people worldwide. Ongoing research is being done throughout the world to seek out effective treatments for cancer, including the use of plants to relieve and treat cancer patients. The use of botanical when treating cancer patients is considered a natural alternative, because some plants may contain properties that naturally have the ability to prevent the spread or risk of developing various forms of cancer. As in all medical testing, careful precautions and considerations are taken when studying the different compounds present in plants that are known to treat cancer.

Ayurveda, Siddha and Unani systems of medicine provide good base for scientific exploration of medicinally important molecules from nature. Traditional medicine has a long history of serving peoples all over the world. The ethno botany provides a rich resource for natural drug research and development. According to the World Health Organization (WHO), about three quarters of the world's population currently use herbs and other forms of traditional medicines to treat diseases. Traditional medicines are widely used in India. Even in USA, use of plants and phyto medicines has increased dramatically in the last two decades. It has been also reported that more than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to include apoptosis in various cancer cells of human origin.

There are around 460 species of plants that can be used as herb for remedy, including plant healer various types of cancer. Various types of anticancer plant are Zedoary (*Curcuma zedoaria*), Rodent Tuber (*Typhonium flagelliforme*), God's Crown (*Phaleria macrocarpa*), Madagascar Periwinkle (*Catharanthus roseus*), Artocarpus Integer (*Selaginella corymbosa*), Bamboo Grass (*Loathatreum Gracies*), handsome (*Taraxacum mongolicum*), fruit makasar (*Bruccajavanica*), Garlic (*Allium sativum*), Echo China (*Smilax china*), Sunflower (*Helianthus annus*), Leunca (*Solanum nigrum*), Job's Tears (*Coix Lachryma-Jobi*), Bamboo Rope (*Asparagus cochinchinensis*), and others.

*Myristicafragrans* is a perennial edible plant of the *Annonaceae* family is a berry that grows wild in the evergreen forests of West Africa. The seeds are economically and medicinally important. The kernel obtained from the seeds is a popular condiment used as a spicing agent. The seeds are embedded in a white sweet-smelling pulp and are most economically important part of the tree. They are aromatic and are used after grinding to a powder as a condiment in food providing flavour resembling that of nutmeg (*Myristicafragrans*).

Medicinal plants have been used for centuries as remedies for human diseases because they contain bioactive components of therapeutic value. *Myristicafragrans* is getting attention as a new avenue in treating various diseases. Nutmeg has been shown to possess a spectrum of pharmacological activities, including antibacterial, anti-inflammatory, anticancer, antidiabetes and hepato protective activities.

*Myristicafragrans* is an aromatic green tree usually growing to around 5-13 meters high, and their seeds (nutmegs) is firm broadly ovoid. The objectives of the study were to find the concentration of nutmeg oil that has maximum antibacterial action against *Staphylococcus aureus*. Different concentrations of nutmeg oil were prepared and their antibacterial activity against *Staphylococcus aureus* was determined by disk diffusion method. The concentration (5%) showed maximum inhibitory zone (16.8mm) for the micro-organism which was parallel to the effects of amoxicillin (positive control), both with highly significant action ( $P < 0.05$ ) [15].

The antimicrobial activity of oil was tested against human and plant pathogenic bacteria and fungi. The oil showed significant inhibitory activity against the bacteria, *Enterococcus faecalis* (1.3cm), *Lactococcus plantarum* (0.9cm) and *Proteus vulgaris* (0.6cm) and the fungus *Candida tropicalis* (1.3cm), *Candida albicans* (0.8cm), *Rhizomucormiehei* (0.6cm) and *Candida glabrata* (0.6cm). 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, *Aspergillus niger* and *Aspergillus fumigates*. Using fluorescent stains localize cortex region, phloem fibres, oil ducts, phenol secreting cells and vascular cambial cells. *Myristicafragrans* showed 100% larvicide activity. The addition of various concentrations of essential oil of *Myristicafragrans* in the MCF-7 breast cancer cell line and A-357 epidermal skin cancer cell line showed cytotoxic activity [11].

## **II. MATERIALS AND METHODS**

### **2.1. Collection of samples and extraction**

Seeds and Mace of *Myristicafragrans* were collected from AyurvedyaSala, Ukkadam, Coimbatore. The seeds and mace were washed under running tap water to eliminate dust and other foreign particles. The fresh seeds and mace were trodden into small pieces, powdered, and mixed in 1:10 ratio with distilled water and methanol separately. The extractions were obtained through continuous grind using mortar and pestle followed by filtration using Whatman No.1 filter paper. The residues were re-dissolved with the appropriate solvents for the further analysis.

### **2.2. Phytochemical Components**

Phytochemical analyses were carried out [19] of the extracts of mace and seed for the identification of phytochemicals like, alkaloids, saponin, steroids, flavonoids, Cynogenic glycosides, Cardiacglycosides, Phlobatannins and phenols.

### **2.3. Antibacterial activity**

*Klebsiella*, *Proteus*, *Shigella*, *Escherichia coli* and *Salmonella* were used for the study. Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard [0.5%] as recommended by WHO(2009). Antibacterial activity of the different extracts was determined by cup diffusion method on Muller Hinton agar medium [2]. Plates were kept for some time till the extract diffuse in the medium and incubated at 37°C for 24 h. After incubation, the plates were observed for the zone of inhibition [ZI], the diameter of the inhibition zone were measured and recorded.

#### **2.4. Minimum Inhibitory Concentration (MIC) – Dilution Method**

To determine Minimum Inhibitory Concentration (MIC) 1ml of nutrient broth was taken in 10 test tubes. Different sets were prepared for each bacterium. Different concentrations of plant extracts ranging from 1mg to 10mg/ml were added into test tubes. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

#### **2.5. Protein Determination**

Protein content in the plant extracts were estimated [10]. 0.2ml and 0.4ml of extract was used to determine the protein content.

#### **2.6. Estimation of total Antioxidant (Phosphomolybdenum method [14])**

Aliquot of 0.1ml sample was obtained with 1 ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling waterbath at 95°C for 90 mins. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each tube was measured at 695 nm against blank, a typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same as rest of the sample. For sample of unknown composition, water soluble antioxidant capacity was expressed as equivalents of ascorbic acid (1 ml of extracts).

#### **2.7. Phenolic content as tannic acids [8]**

The extracts were dissolved in a mixture of methanol and water (6:4 v/v). sample (0.2ml) were mixed with 1 ml of tenfold diluted folin-ciocalteus reagents and 0.8 ml of 7.5% sodium carbonate solution, after standing for 30 mins at room temperature, the absorbance was measured at 765 nm. The estimation of phenolic compound in the fractions was carried out in triplicate and the results were averaged.

#### **2.8. Separation of Active Compound from *Myristicafragrans* Extracts by Thin Layer Chromatography (TLC)**

The glass slides were cleaned and dried in hot air oven. Slurry was prepared by mixing silica gel with double the volume of distilled water in a clean beaker. One drop of slurry was placed on the slide by using another slide edge, the drop of slurry was scattered all over to make thin film. The slides were kept as such for few minutes. Then the chromo plates were activated by heating in hot air oven at 120°C for 30 min. The slides were allowed to cool at room temperature and marked about 2 cm from the bottom as the origin. The working suspensions were loaded at the center of the each slide above from the edge.

The development tank was saturated with suitable solvent [5].

Alkaloids	: Benzene/ Methanol-80:20
Flavonoids	: Chloroform/Methanol-70:30
Lipid	: Chloroform/Methanol/water-10:10:3
Terpenoids	: Acetic acid/water-1:3

The slides were kept in the tank without touching baseline by solvent. The final solvent front was marked and the slides were dried.

##### **2.8.1. Spot visualization**

For visualization of Flavonoids 1% ethanolic solution of Aluminium chloride was used and viewed under 560nm UV light. Alkaloids and Terpenoids were visualized under UV light and they were visible as yellow and orange fluorescent spots. Few pieces of iodine crystals were kept in the tank and covered with glass plate to saturate the tank with iodine vapor for detecting lipids. The plate was then kept in iodine vapor saturated tank and left for few hours and brown colored spots were visualized.

### 2.8.2. Retrieval of the active compound

Spots on the preparative silica gel slides were scratched with the help of clean and dry spatula and collected in beaker containing appropriate solvents [3] and left overnight. The content in the beaker was stirred and filtrated through Whattsman no. 1 filter paper. The filtrate was collected in clean and dry beaker. The filtrate containing active compound was used for the determination of antimicrobial effect against *Salmonella* and *Klebsiella* by cup diffusion method.

### 2.9. Human Osteosarcoma (MG63 )cell line study

The Human Osteosarcoma (MG-63) was carried out in KMCH College of Pharmacy, Coimbatore. The cell line was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37<sup>0</sup>C, 5% CO<sub>2</sub>, 95% air, and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

#### 2.9.1. Cell line treatment procedure [12]

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a haemocytometer and diluted with medium containing 5% FBS to give final density of 1x10<sup>5</sup> cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37<sup>0</sup>C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the ethanol extract of *Myristicafragrans*(seed). They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the methanol extract of *Myristicafragrans*(seed) was diluted to twice the desired final maximum test concentration with serum free medium. Additional four 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37<sup>0</sup>C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

#### 2.9.2. MTT assay [13]

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37<sup>0</sup>C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

$$\% \text{ Cell viability} = [\text{A}] \text{ Test} / [\text{A}] \text{ control} \times 100$$

## III. RESULTS

### 3.1. Phytochemical analysis

Qualitative phytochemical analyses were performed for the detection of alkaloids, saponin, steroids, flavonoids, Cynogenic glycosides, Cardiac glycosides, Phlobatannins and phenols. The observed results were tabulated (Table 1).

### 3.2. Protein Determination

The total protein content in the *Myristicafragrans* was 6.7 mg/g and 12.4 mg/g of aqueous and methanol extracts of seed. The total protein content in the *Myristicafragrans* was 1.5 mg/g and 3.8 mg/g of aqueous and methanol extracts of mace.

### 3.3. Total anti oxidant and Phenolic content

The absorbance of the aqueous solution of each tube was measured for total anti oxidant content at 695nm against blank it was observed that seed contain 7.62 mg/g and 4.58 mg/g of anti

oxidant in methanol and aqueous extract. Mace contained 13.44 mg/g and 4.75 mg/g of anti oxidant content in both methanol and aqueous extract. The absorbance was measured at 765nm for Phenolic content. The estimation of phenolic compound in the fractions was carried out in triplicate and the results were averaged. Seed contain 7.47 mg/g and 1.39 mg/g of phenolic content in methanol and aqueous extract. Mace contained 15.91 mg/g and 3.99 mg/g of phenolic content in both methanol and aqueous extract.

**Table 1. Phytochemical analysis of Myristicafragrans**

Phytochemical constituents	Aqueous		Methanol	
	Seed	Mace	Seed	Mace
Alkaloids	+	+	+	+
Steroids	+	+	+	+
Saponin	+	+	+	+
Cynogenic glycosides	+	+	+	+
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phlobatannins	+	+	+	+
Phenols	+	+	+	+

“+” Present “-“ Absent

**Table 2. Total antioxidant and phenol content of Myristicafragrans**

Sample extraction	Anti oxidant content OD at 695 nm		Phenolic content OD at 765 nm	
	Seed	Mace	Seed	Mace
Methanol	7.62	13.44	7.47	15.91
Water	4.58	4.75	1.39	3.99

**3.4. Antibacterial Property**

Minimal inhibitory concentration assay was carried out for methanolic extraction *Catharanthusroseus*, *Shigella*, *Proteus*, *Klebsiella*, *Escherichia coli* and *Salmonella*. Table 2 illustrated the minimal concentration level which required to kill the pathogen. Antibacterial properties of the *Myristicafragrans* extracts were carried out by well diffusion. The pathogens *Salmonella typhi*, *Proteus*, *Shigella*, *Escherichia coli* and *Klebsiella* were tested for antimicrobial assay. The maximum zone of inhibition in *Salmonella typhi* and mace showed 1.9 cm the maximum zone of inhibition in *Proteus* sp followed by *Salmonella typhi* and minimum zone of inhibition found in aqueous extract in *Klebsiella* which is tabulated in table 3.

**Table 3. Minimal Inhibitory Concentration (Methanol extraction) of Myristicafragrans**

Test organisms	Seed OD at 540 nm			Mace OD at 540 nm		
	60µl	80µl	100µl	60µl	80µl	100µl
<i>Salmonella typhi</i>	0.60	0.18	0.09	1.53	1.30	0.93
<i>Proteus</i>	0.52	0.40	0.10	1.03	0.93	0.39
<i>Escherichia coli</i>	1.01	0.70	0.10	1.37	1.19	0.66
<i>Klebsiella</i>	1.11	1.03	0.82	1.34	1.28	0.87
<i>Shigella</i>	0.89	0.14	0.13	0.87	0.57	0.25

**3.5. Separation of Active Compound from Thin Layer Chromatography (TLC)**

Thin layer chromatography techniques were carried out using respective solvents as mentioned in the materials and methods in order to detect the presence of alkaloid, flavonoids, lipids and triterpenoids. Table 4 represented the methanol extract contain alkaloids, flavonoids, lipids and triterpenoids.

The separated active compounds alkaloid, flavonoids, lipids, and triterpenoids were found that effective against *Salmonella* and *Escherichia coli* and the zone of inhibition tabulated in table 5 which represented the maximum zone of inhibition found in *Escherichia coli* in the active compound of lipid and minimum zone of inhibition found in active compound of alkaloids figure 1 represented the zone of inhibition of active compounds of alkaloids and lipids.

Table 4. Antimicrobial activity of Methanolic extract of *Myristicafragrans*

Test organisms	Zone of inhibition(in diameter)				Standard drug (ampicillin 1mg/ml)
	Seed (in cm)		Mace (in cm)		
	40µl	80µl	40µl	80µl	20 µl
<i>Salmonella typhi</i>	0.9	1.4	0.1	0.6	1.2
<i>Shigella</i>	-	-	-	-	2
<i>Escherichia coli</i>	-	0.6	-	1.2	2
<i>Proteus</i>	-	-	1.1	1.9	1
<i>Klebsiella</i>	-	0.3	-	-	-



*Salmonella*

*Klebsiella*

Figure 1 Antimicrobial activity of active compounds of *M. fragrans*

### 3.6. MG-63 Cell line study (tumorolytic activity)

After treatment with various concentrations of *Myristicafragrans* seed (18.75 µg/ml, 37.5 µg/ml, 75 µg/ml, 150 µg/ml, 300 µg/ml) parameters like cell viability, growth of growth and morphological changes of the cell line were noted and compared with control (untreated) cell sample. From the results, it is observed that the cell viability was decreased and increased in inhibition of growth by the *Myristicafragrans*.

With respect to different concentrations of the extracts, significant decreases in cell viability were observed in the concentrations 18.75 µg/ml, 37.5 µg/ml, 75 µg/ml and 150 µg/ml, (Fig.2). The growth inhibitory activity was more significant than the percentage viability. IC<sub>50</sub> was found at 95.74 µg/ml. The percentage of growth inhibition of the treated cells with different doses of *Myristicafragrans* was seen in Fig3a, Fig 3b and Table 6 and Table 9.

Table 5. Rf values of metabolic compounds of *Myristicafragrans* by TLC

Methanol extraction of <i>Catharanthus roseus</i>	Flavonoids	Alkaloids	Lipids	Terpenoids
Seed	0.75	0.45	0.9	0.9
Mace	0.56	0.45	0.67	0.67

Table 6. MTT effect on MG-63 cell line- % of viability

Conc (µg/ml)	% Cell viability
18.75	1.880878
37.5	3.500522
75	37.04284
150	73.71996
300	100

IC<sub>50</sub> 95.74 µg/ml

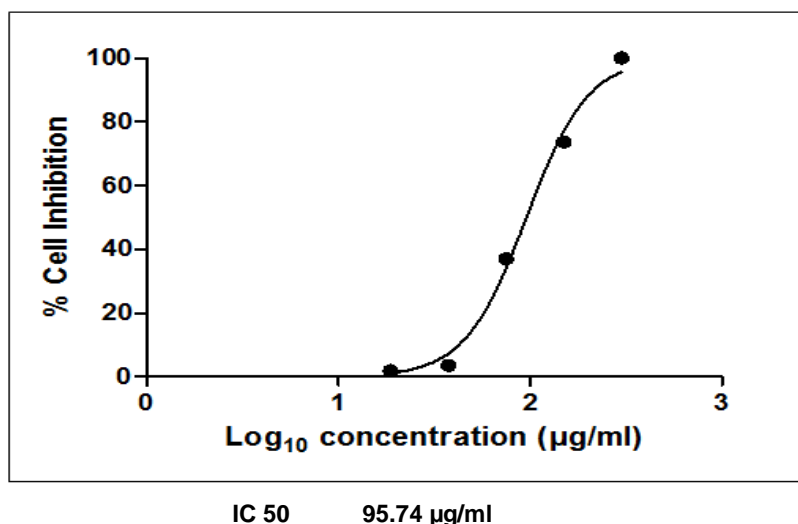


Figure 2 Inhibitory level of *M. fragrans* at different concentration in MG-63 cell line

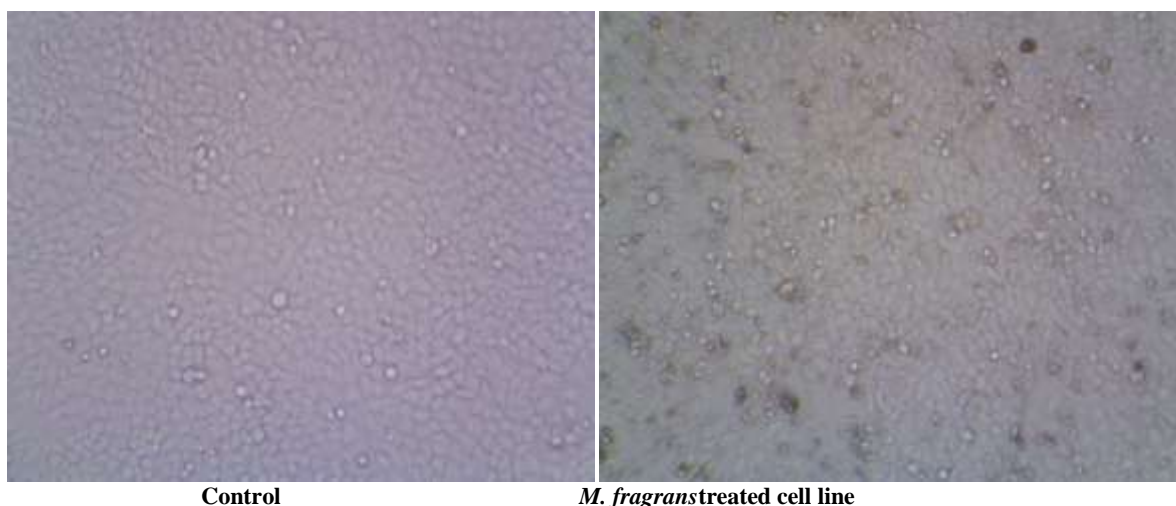


Figure 3a and 3b MG-63 Cell line- Cell viability

#### IV.DISCUSSION

Plant essential extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine, and natural therapies. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In-vitro* studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied.

The aqueous and methanol extracts of *Myristicafragrans* seed and mace contain presence of alkaloids, saponin, steroids, flavonoids, Cynogenic glycosides, Cardiac glycosides, Phlobatannins and phenols. The medicinal values of the secondary metabolites are due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include, alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, and body building [9].

The pathogens *Salmonella typhi*, *Proteus*, *Shigella*, *Escherichia coli* and *klebsiella* were tested for antimicrobial assay. The methanol extract of *Myristicafragrans* seed showed 1.4 cm the maximum zone of inhibition in *Salmonella typhi* and mace showed 1.9 cm the maximum zone of inhibition in *Proteus* followed by *Salmonella typhi* and minimum zone of inhibition found in aqueous extract in *Klebsiella*.

The antibacterial activity of aqueous, ethanol and acetone extracts of *Corriandersativum*, *Abutilon indicum*, *Boerhaviadiffusa* and *Rographispaniculata*, *Plantagoovata*, *Bacopamonni*eri,

*Bauhinia variegata*, *Flacourtiaramontchi*, *Embeliatfgerium*, *Euphorbia ligularia*, *Zinziberofficinale*, *Terminaliachebula*, *Azadirachtaindica*, *Ocimum sanctum* and *Cinnamomum cassia* was determined against 33 UTI isolates i.e. *Proteus mirabilis*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiellapneumoniae*, *Enterobacter cloacae*, *Providenciapseudomallei*, *Pseudomonas aeruginosa* and *Klebsiellaoxytoca* by disc diffusion method. The studies concluded that crude extracts of the selected plants especially the acetone and ethanol extracts exhibited significant activity against UTI pathogens [1].

The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. Due to variable diffusability in agar medium, Therefore Zone of inhibition value has also been computed in this study.

Antioxidant methanol extract potential of flesh, seed and mace of nutmeg (*Myristicafragrans*Houtt) were evaluated with methods of 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), ferrous ion chelating activity and antioxidant activity assay in a linoleic acid system with ferrothiocyanate reagent (FTC). Similar result was obtained tannin, flavonoid and terpenoid were also found in seed and mace extract, where as flesh extract contain flavonoid and terpenoid[7].

Thin layer chromatography techniques were carried out using respective solvents as mentioned in the materials and methods in order to detect the presence of, alkaloid, flavonoids, lipids and triterpenoids. Reproducible TLC preparations can be guaranteed only if standardized adsorption layers are used. Silica gel is an efficient adsorbent for the separation of most plant extracts [20]. The system selection entails choosing the mobile phase, the stationary phase and the detection method. While recovering the separated components from silica plates, it should be considered that polar eluants, e.g., alcohol, can dissolve not inappreciable amounts of colloidal silica in it [16]. Ethanol, methanol, and water can dissolve appreciable amounts of silica gel which appear as a residue when the eluant is concentrated [6].

The phytochemical analysis shows the presence of Phenols, Flavonoids, Quinones, Saponins, Cardinolites, Steroids, Tanins and Terpenoids in various extracts. Thin layer chromatography (TLC) have been carried out on different extracts of plant leaves, which show different R<sub>f</sub> values and possible combinations of chromatography solvents for separation of these phytochemicals [17].

Aqueous extracts of *Azadirachtaindica* (Neem) was subjected to Thin layer chromatography (TLC) by using different solvent system for the analysis of lipid, alkaloids, flavonoids present in plant extract. The active components separated through TLC were subjected to antimicrobial activity against the pathogens. The present study will be successful in identifying candidate plant with different antimicrobial activity which could be further exploited for isolation and characterization of the novel phytochemicals in the treatment of infectious diseases especially in light of the emergence to produce more effective antimicrobial agents [18].

After treatment with various concentrations of *Myristicafragrans* seed (18.75 µg/ml, 37.5 µg/ml, 75 µg/ml, 150 µg/ml, 300 µg/ml) parameters like cell viability, growth of growth and morphological changes of the cell line were noted and compared with control (untreated) cell sample. From the results, it is observed that the cell viability was decreased and increased in inhibition of growth by the *Myristicafragrans*.

*Myristicafragrans* (nutmeg) contains antibacterial, antiviral and anticancer activities. However, the mechanisms underlying those activities have not been clearly explained. To study the effect of *Myristicafragrans*Houtt. Methanolic extract on Jurkat human leukemia T cell line. Methanol extract of *Myristicafragrans*Houtt. (*Myristicaceae*) was used to study the effect on Jurkat cell metabolic activity using an MTT assay and on apoptosis using annexin V staining. Expression of SIRT1 gene was determined by RT-PCR. At the concentrations 50 and 100 µg/mL, the methanol extract of *Myristicafragrans*Houtt significantly inhibited Jurkat cell proliferation and induced apoptosis as detected by annexin V staining. Down regulation of SIRT1 mRNA expression in Jurkat cells was observed even when the amount of methanol extract was 10 µg/mL. Methanol extract of



*Myristicafragrans*Houtt induced apoptosis of Jurkat leukemia T cell line in a mechanisms involving SIRT1 mRNA down regulation [4].

## V. CONCLUSIONS

Medicinal plants have been found as important contributors to the pharmaceutical, agriculture and food industries. With the onset of the synthetic era, pharmaceutical industries are producing a lot of synthetic drugs that help to alleviate the chronic diseases. The natural pharmaceuticals are receiving extra ordinary importance and popularity as safe, efficacious and cost effective medicines with extraordinary benefits due to combination of medicinal ingredients with vitamins and minerals. The research work which is going on this direction is a proof of benefits by reducing toxicity of chemo and radio therapies and providing better and healthier life style by *Myristicafragrans*. Therefore, it was worthwhile to review its anticancer properties to give an overview of its status to scientist both modern and ancient.

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