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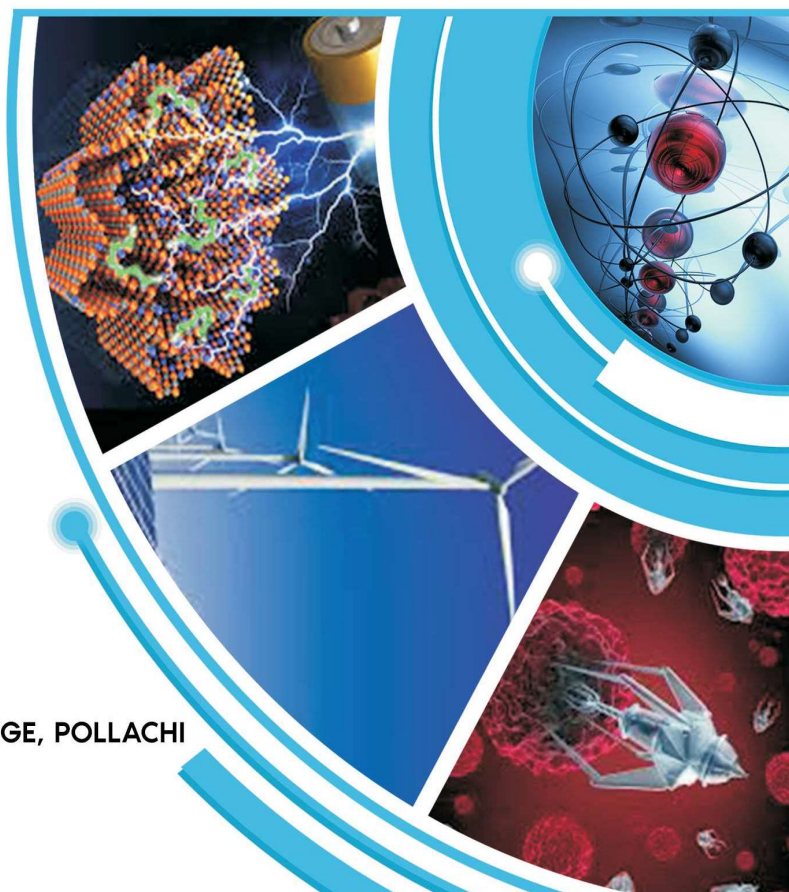
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CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *MYRISTICA FRAGRANS* HOUTT LEAVES AND ITS BIOLOGICAL ACTIVITIES

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ABSTRACT

In modern days, huge attention has been directed towards recognition of plants with anti-bacterial ability that may use effectively for human consumption. There has been rapid development of different classes of anti-bacterial drugs with distinctive pharmacological mechanism action. There are numerous anti-bacterial drugs derived from natural products are now commercially available and increase of modern synthetic drugs with more side effect and high cost are induced to search every possible natural products for anti-bacterial activity. It may useful for developing countries. Therefore, based on the exhaustive literature survey, anti-microbial activity of essential oil of *M. fragrans* has not been experimentally much studied.

Keywords: *M. Fragrans*, anti-bacterial activity

INTRODUCTION

Natural products are a source of synthetic and traditional herbal medicine and are still the primary health care system.¹ Natural products chemistry has attracted the attention of organic chemists over the ages for several well-known reasons: basic interest in organic chemical structures of immense diversity found in nature. Natural products are not just accidents or products of convenience of nature. More than likely they are a natural expression of the increase in complexity of organisms.² Plant medicines are in wide use around the world. In most of the developing world, especially in rural areas, local traditional medicine, including herbalism, is the only source of health care for people, while in the developed world, alternative medicine including use of dietary supplements is marketed aggressively using the claims of traditional medicine.³ Drugs derived from plants including opiates, cocaine and cannabis have both medical and recreational uses. Different countries have at various times made use of illegal drugs, partly on the basis of the risks involved in taking psychoactive drugs.⁴

Essential oils are often used for aromatherapy, a form of alternative medicine in which healing effects is ascribed to aromatic compounds. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of medical treatments, and treatment of cancers in particular, are now subject to regulation in most countries.⁵

Myristica fragrans Houttis commonly has known as nutmeg is a well-known as aromatic evergreen tree. Nutmeg belongs to the family *Myristicaceae*. It produces two spices: mace and nutmeg. Nutmeg is the seed kernel inside the fruit and mace is the red lacy covering (aril) on the kernel. *M. fragrans* belong in the order Magnoliales which contains about 150 genera and more than 3000 species. Nutmeg is a powerhouse of the properties that can treat indigestion effectively. Nutmeg is said to be a diuretic in nature and promote proper urination that results in healthy kidney.⁶ It is widely used in herbal medicine to regulate high blood pressure levels. Nutritionists suggest that potassium

found in the spices can relax your blood vessels and allow proper blood circulation.⁷ Scientists have found that being enriched with antioxidant, nutmeg can help alleviate risks that contribute to the development of cancerous cells.⁸ In homoeopathy, nutmeg is used to treat anxiety and depression. In Chinese medicine, it is used to treat impotence and liver disease.⁹ Incorporation spices like nutmeg can prevent your liver from the adverse effects of environmental pollution, stress, unbalanced diet and medication.¹⁰

There is limited work on antimicrobial activity. So our main objective of the present work is to evaluate the phytoconstitutions and the antimicrobial activity of essential oil using for disc diffusion method and DPPH assay.

MATERIALS AND METHOD

Plant Material

Fresh leaves of *M. fragrans* were collected from near Pollachi between the periods of July to august. The plant material was identified and authenticated by Department of Botany, NGM College, Pollachi, Coimbatore, Tamilnadu.

Isolation of essential oil

About 500g of fresh leaves was taken in a round bottom flask and subjected to hydro distillation using Clevenger type apparatus for 4h. The essential oil was dried over anhydrous sodium sulphate (Merck) until the last traces of water were removed and then stored in a container at 4°C prior to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of the phytoconstituents of *M. fragrans* was carried out using thermo GC –trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non – polar column (30m × 0.25 mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 700C and held for 2 minutes and the temperature of the oven was raised to 2600C for 10min and raised 60C/MIN and final temperature was 3500C for 10 min. The sample of 100mL was dissolved in 1mL of acetone and injected with split less mode. Mass spectra were recorded over 50-500 amu range with electron impact ionization energy 70 eV, while injector and MS transfer line temperature were set at 2800C respectively.

Identification of phytoconstituents

The components were identified by comparison of their mass spectra with those of National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

ANTI-BACTERIAL ACTIVITY

Preparation of discs for bacterial activity:

Whatman No:1 filter paper was taken and punched in to small circular discs (6mm dia) and then wrapped in aluminium foil and sterilized using an autoclave.

Preparation of media for disc diffusion assay:

1. Muller Hinton Agar (MHA) (38 g) was dissolved in 1000 ml of sterile sea water and kept for sterilization in autoclave for 45 minutes.
2. The prepared MHA was poured in the Petri plates and then allowed to solidify under Sterile condition.
3. Pathogens were subjected to preliminary screening in order to test their antimicrobial activity against the extracts using disc-diffusion method.
4. The sterilized filter paper discs were treated with the extracts under laminar air flow chamber.
5. 200 µl of indicator strains of human pathogens were spread plated over the Petri plate to form a lawn on the agar.
6. The extract treated discs was placed in their respective place in the Petri plates using sterile forceps under aseptic conditions.
7. The plates were incubated for 24-48 hours for bacteria and fungi respectively.
8. After 24 hours of incubation the plates were observed for activity against the bacterial pathogens.
9. The zone of growth inhibition was measured in millimetre for those extract which showed activity.

RESULT AND DISCUSSION

The essential oils extracted by hydro-distillation from the leaves of *M. Fragens* were found to be colourless in colour. Its volatile composition was analyzed by GC-MS. Total of 26 components was identified and represents 99.6% of the detected oil composition. The major essential oil composition was Terpinen-4-ol (15.05%), Benzene,1,2,3,-trimethoxy-5(2-propenyl), Cyclohexene 1-methyl- 4-(1-methylethylidene) (11.1817%), D-Limonene(10.736%), Safrole (10.235%), γ -Terpinene(10.195%), 1,3Benzodioxole,4-methoxy-6-(2-propenyl) (9.883%), Bicyclo [3,1,0] hexane 4,methylene1-(1-methylethyl) (6.700%).

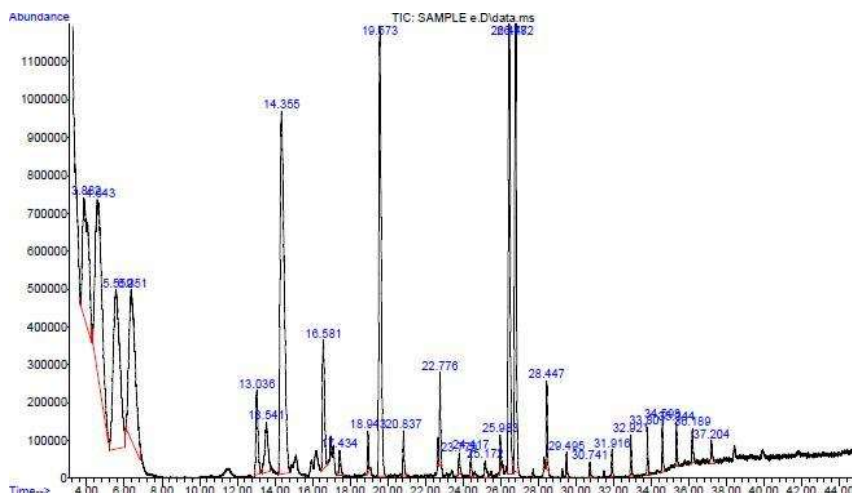


Figure 1: GC-MS chromatogram of essential oil of *M. fragrans*

Table 1: Chemical composition of essential oil of *M. fragrans*

S.No	Name of the Compound	Retention Time	Area
1	Bicyclo[3.1.0]hexane-4-methylene-1-(1-methylethyl)-	3.882	6.700
2	D-Limonene	4.643	10.736
3	γ -terpinene	5.559	10.195
4	Cyclohexene-1-methyl-4-(1-methylethylidene)-	6.351	11.187
5	1,6-octadien-3-ol,3,7- dimethyl	13.086	1.887
6	2-cyclohexen-1-ol,1- methyl-4-(1- methylethyl)	13.541	1.514
7	Terpinen-4-ol	14.355	15.053
8	α -terpineol	16.581	2.650
9	2-cyclohexen-1-ol,3- methyl-6-(1-methylethyl)-cis	17.434	0.434
10	Octadecane	18.943	0.468
11	Safrole	19.573	10.235
12	Nanadecane	20.837	0.599
13	Methyleugenol	22.776	1.035
14	Guaiol	23.779	0.305
15	Heneicosane	24.417	0.292
16	Eugenol	25.172	0.214
17	Benzene-1,2-dimethoxy-4-(1-propenyl)	25.983	0.352
18	Benzene-1,2,3-trimethoxy-5-(2-propenyl)	26.447	11.039
19	1,3-benzodioxole,4- methoxy-6-(2-propenyl)	26.782	9.883
20	Trans-isoeugenol	28.447	1.084
21	Isoelemicin	29.495	0.304
22	Octacosane	30.741	0.173
23	Nonacosane	31.916	0.276
24	Tricontane	34.598	0.510
25	Hentriacontane	35.344	0.448
26	Heptaethyleneglycol-Monododecylether	36.189	0.435

Anti-bacterial activity

The antibacterial activity for essential oil of *M. Fragrans* against three microorganism *Stathylococcusaureus*, *Streptococcus anginosus* and *Klebsiellapneumonia* was evaluated and results were given in the table 2. From the results, the bacteria *S. aureus*, *S. Anginosus* and *K.pneumonia* was sensitive to the tested essential oil of *M.fragrans* with zone of inhibition of 10mm, 12mm and 14mm respectively. In our result all the three bacteria was affected by essential oil from *M.fragrans* leaves. It posses potent antibacterial activity.

Table 2: Anti-bacterial activity of *M.fragrans* against microorganism.

S.No	Test organism	Zone of inhibition by sample	Zone of inhibition by standard (<i>tetra mycin</i>)
1	<i>S.aureus</i>	10mm	22mm
2	<i>S. anginosus</i>	12mm	26mm
3	<i>K. pneumonia</i>	14mm	26.5mm



Figure 2a

Figure 2b



Figure 2c

Figures 2a,2b,2c: Antibacterial activity of essential oil of *M. fragrans* against *Stathylococcusaureus*, *Streptococcus anginosus* and *Klebsiella pneumonia*

CONCLUSION

The chemical composition of essential oil of *M. fragrans* was analyzed by GC-MS method. The essential oil of *M. fragrans* was colourless, total of 26 components were identified. The Antimicrobial activity of essential oil was evaluated for disc diffusion method and DPPH assay. From the result *M. fragrans* essential oil has significant

Antimicrobial activity. Further biological activities and their possible mechanism will explore in future.

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