ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(3):604-609

ORIGINAL RESEARCH ARTICLE

In vitro Antimicrobial Activity of *Camellia sinensis* and *Myristica fragrans* Against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*

M. Kulandhaivel* and M. Palaniswamy

Department of Microbiology, Karpagam University, Coimbatore-641021, Tamil Nadu, India

Received 10 Mar 2012; Revised 11 Jun 2012; Accepted 18 Jun 2012

ABSTRACT

The antimicrobial activity of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg), against the pyogenic microbes plays a vital role in evaluation of the ethanomedicinal property of plants. Hydroethanolic extraction, phytochemical screening of medicinal plants, FT-IR spectral analysis and GC-MS Qualitative analysis enables identification of essential components present which was compared with Dr.Dukes Phyto ethanobotanical Databases. This provides and supports the therapeutical information of the plants. The medicinal value of *Camellia sinensis* and *Myristica fragrans* were investigated through agar well diffusion method. The hydroethanolic extracts of plants to be tested were prepared at a concentration of 10mg/mL, 5mg/mL, and 2.5mg/mL dissolved in dimethyl sulphoxide (Dimethyl Sulphoxide (DMSO).The zone of inhibition gives the degree antimicrobial property. It was compared with standard antimicrobial agents like Bacitracin, Neomycin and Ketaconazole. These are the common antibiotics found in the commercial topical ointment to act against skin infections (Pyogenic infection).

Key words: *Camellia sinensis* (Green tea), *Myristica fragrans* (Nutmeg), Hydroethanolic extraction, phytochemical screening, FT-IR spectral analysis**,** GC-MS Qualitative analysis antimicrobial activity *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*.

INTRODUCTION

Humans are natural hosts for many bacterial species that colonize the skin as normal flora. Skin infections are common and may be caused by bacteria, fungi or viruses. Pyogenes are infrequent resident flora, but they account for a wide variety of microbial pyodermas. Breaks in the skin integrity, particularly those that inoculate pathogens into the dermis, frequently cause or exacerbate skin infections. Predisposing factors to skin infection include minor trauma, preexisting skin disease, poor hygiene, and rarely, impaired host immunity [1]. *Staphylococcus aureus* present in the nose, throat, skin and hair of healthy humans, animals and birds. It produces skin diseases like superficial skin lesions such as boils, styes, carbuncles, and furnculosis, wound infection, breast abscess [2]. *Pseudomonas aeruginosa* is a highly relevant opportunistic pathogen. It is found in soil, water, skin flora most manmade environments throughout the world. It causes blue pus and dominates in burnt infection[3] *Candida albicans* is a diploid fungus (a form of yeast) and a causative agent of opportunistic oral and genital infections in humans.[4]

Camellia sinensis belongs to the family Theaceae. Green tea is known mainly as a stimulant and refreshing drink, The traditional preparation as an infusion contains the broad spectrum of active ingredients present in the native plants, polyphenols, methylxanthins, flavanoles, aminoacids etc., The polyphenols are potent free radical scavengers due to the hydroxyl groups in their chemical structure.This plant has been traditionally useful in treating inflammations, asthma, and heart diseases, lowering blood sugar and fighting cancer. It is also useful in treating wound ulcers, and various skin diseases [5].

Myristica fragrans (Nutmeg) an aromatic tree is said to have properties like stimulant, carminative and astringent.

The therapeutic properties are analgesic, antirheumatic, antiseptic, antispasmodic, carminative, and digestive, laxative, parturient, stimulant and tonic. It helps in treating skin disorders, encourages appetite and averts

***Corresponding Author:** M.Kulandhaivel**, Email:** kulandhai_vel@rediffmail.com

constipation, fights gallstones and is a tonic for the reproductive system, while regulating scanty periods, relieving frigidity and impotence. The red pigment in mace is due to lycopene, nutmeg and mace are used extensively as flavour ingredients in many food products.

In order to identify the traditional medicinal role of the plants, the present study was carried out to compare and evaluate the antimicrobial activity of *Camellia sinensis* and *Myristica fragrans* hydroethanolic extracts.

MATERIALS AND METHODS

The microorganism for the present investigation was responsible for the pyogenic infection (Skin infection) predominantly seen as a causative agent in nosocomial infection. The leaves of *Camellia sinensis*, and mace of *Myristica fragrans* were subjected for evaluation of bioactive compounds.

The hydroethanolic extract was prepared by simple maceration technique. About 50 g of the *Camellia sinensis* powdered plant materials was extracted with 200 mL of hydro-ethanol (1:1) with occasional shaking for about 48 hours at room temperature 22-24 ^{0}C, and filtered. Similarly about 50 g of the *Myristica fragrans* mace powdered plant materials was extracted with 200 mL of hydro-ethanol (1:1) with occasional shaking for about 48 hours at room temperature 22-24 ^{0}C, and filtered. Both the filtrate of the plant material was evaporated to dryness [7, 8].

Phytochemical Screening

The phytochemical screening was carried out using standard protocols for detecting the presence of different phyto constituents in the hydroethanolic plant extract [9].The tests for alkaloids, tannin, saponins, glycosides, carbohydrates, flavanoids and phenols were carried out. Phytochemical screening of plants was carried out to detect the bioactive compounds using qualitative test [10].

(FT-IR) spectral analysis

FTIR analysis of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) hydroethanolic extracts was analysed using Shimadzu FTIR-8400S Fourier Transform Infrared Spectrometer instrument and spectra obtained for each sample was interpreted with a chart for interpreted with a chart for Characteristics IR absorption frequencies of organic functional groups and carbonyl containing functional group.

GC-MS Qualitative analysis

GCMS analysis of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) hydroethanolic extracts was qualitatively analyzed separately using the Thermo GC-Trace Ultra Ver: 5.0 Instrument and Thermo MS DSQ II with Quarter Polsource Detector. Retention indices of separated compounds were compared and determined using standard compound indices and by matching them with similar compounds in Mass Library.

Antimicrobial Activity

In vitro antimicrobial activity of *Camellia sinensis* and *Myristica fragrans* plant extracts against the pyogens by agar well diffusion method was carried out. The hydroethanolic extracts of plants to be tested were prepared at a concentration of 10mg/mL, 5mg/mL, and 2.5mg/mL dissolved in dimethyl sulphoxide (Dimethyl Sulphoxide (DMSO).The sterile Muller Hinton Agar plates were prepared and with the help of a sterile well cutter, the punctured 6mm diameter wells with uniform spacing for various concentrations of each extracts. The log phase culture broth was taken and swabbed over the plate using sterile cotton swab to obtain a uniform lawn of culture. The wells were filled with 20 µL of the plant extracts respectively. The plates were then incubated at 37^o C for 24 hours for bacteria and at room temperature for yeast for 24 hours. After incubation, the sizes of the zone of inhibition were measured [11]. The results were observed and recorded. It was compared with standard antimicrobial agents like Bacitracin, Neomycin and Ketaconazole. These are the common antibiotics found in the commercial topical ointment to act against skin infections (Pyogenic infection) [12].

RESULTS AND DISCUSSION

Screening of Phytochemical Constituents

The screening and analysis of preliminary phytochemical constituents present in the hydro ethanolic extracts of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) were performed to identify the presence of alkaloids, terpenoids, phenolics compounds, flavonoids, amino acids, proteins, glycosides, saponins and tannins. The results were tabulated in (**Table 1**).

Table 1: Results showing the Screening of Phytochemical constituents in extracts of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg)

S.No	Phytochemical constituents	*Camellia sinensis* (Green tea) extracts	*Myristica fragrans* (Nutmeg) extracts
1.	Alkaloids	-	+
2.	Saponins	-	+
3.	Tannins and phenolic compounds	+	+

4.	Flavanoids	+	+
5.	Terpenoids	+	-
6.	Carbohydrates	+	-
7.	Glycosides	+	+
8.	Amino acids and proteins	+	+

FTIR spectral analysis

FTIR spectral analysis of *Camellia sinensis* (Green tea) extracts and *Myristica fragrans* (Nutmeg) extracts were provides the active functional groups with wave number indication present along with their type of vibrational mode. The FTIR spectrum of each plants were analysed and compared with Charateristic IR absorption frequencies of organic functional groups and frequencies of functional groups containing a Carbonyl(C=O). The results were tabulated in (**Table 2 & 3**) and their spectral representations were shown in (**Fig 1&2**).

Table 2: FTIR spectral analysis results of *Camellia sinensis* (Green tea) Hydroethanolic extract

S. No	Wave number (absorptions) (cm^{-1})	Functional groups	Type of vibration
1	729.09	=C-H (Alkene)	Bending
2	748.38	=C-H (Alkene)	Bending
3	821.68	=C-H (Alkene)	Bending
4	1033.86	(C-O) Ester	Stretch
5	1082.07	(C-N) amine	Stretch
6	1145.72	(C-N) amine	Stretch
7	1203.58	(C-O) Ester, (C-N) amine	Stretch
8	1234.44	(C-N) amine, (C=O) acid	Stretch
9	1354.03	(C-N) amine, (N-O) Nitro compounds	Stretch
10	1456.26	(C=C) aromatic, (-C-H) alkane	Stretch
11	1521.84	(N-O) Nitro compounds	Stretch
12	1620.21	(C=C) alkene, (N-H)) Amide	Stretch
13	2862.43	(-C-H) alkane, (C=O) acid	stretch
14	2924.09	(-C-H) alkane, (C=O) acid	stretch
15	3350.35	(O-H) hydroxyl group	Stretch
16	1697.36	Ketone	Stretch

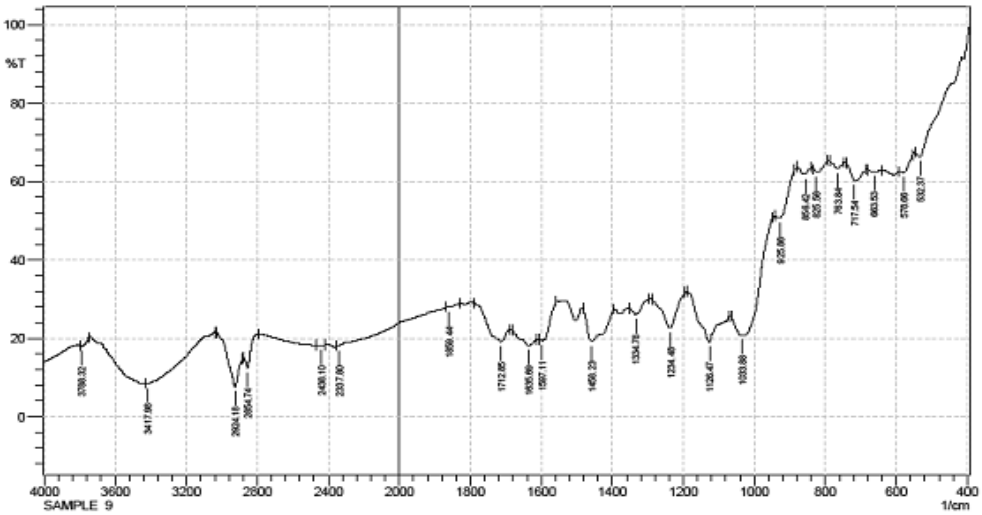
Figure 1: FTIR spectral representation of *Camellia sinensis* (Green tea) Hydroethanolic extract

Table 3: FTIR spectral analysis results of *Myristica fragrans* (Nutmeg) extracts

S.No	Wave number (absorptions) (cm^{-1})	Functional groups	Type of vibration
1	532.37 663.53 717.54 763.84 825.56 856.42 925.66	=C-H (Alkene)	Bending
2	1033.86	(C-O) Ester, (C-O) Alcohol	Stretch
3	1126.47 1334.78	(C-N) amine	Stretch
4	1234.48	(C-N) amine, (C=O) acid	Stretch

5	1458.23	(C=C) aromatic (-C-H) alkane	Stretch Bending
6	2924.18	(-C-H) alkane	Stretch
7	1597.11	(C=C) aromatic, (N-H)) Amide	Stretch
8	2854.74	(C=O) acid	stretch
9	1635.69	(C=C) alkene	Stretch
10	1712.85	Acyclic Ketone, (C=O) carbonyl, (O-H) acid	Stretch
11	3417.98	(O-H) alcohol, (N-H)) Amide, (N- H)amine	Stretch

Figure 2: FTIR spectral representation of *Myristica fragrans* (Nutmeg) extracts



GC-MS Qualitative analysis

The chromatogram for GC-MS qualitative analysis of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) hydroethanolic extracts was done, and the separated compounds are screened with direct and reverse search for similar standard compounds in Mass Library. A GCMS normalized ion absorbance verses mass to charge (m/z) ratio for each separated compounds, produced bar peak of electron impact spectra at specific retention times. Compounds with search indices higher than 650 was considered for *Camellia sinensis* and search indices higher than 450 was considered for *Myristica fragrans*. The results were tabulated in (**Table 4 & 5**).

Table 4: Results showing the Qualitative GC MS analysis of extracts of C*amellia sinensis*

S.No	Search index	Reverse search Index	Retention time	Compound name	Molecular formula	Molecular weight	Area%
1	925	935	4.14	Thujene	$C_{10}H_{16}$	136	1.50
2	919	919	4.14	Phellandrene	$C_{10}H_{16}$	136	1.50
3	931	935	5.79	Terpinene	$C_{10}H_{16}$	136	1.50
4	913	920	6.98	Terpineol	$C_{10}H_{18}O$	154	2.50
5	905	931	11.22	3,6-Dimethyl benzo furan-4-ol	$C_{10}H_{10}O_2$	162	1.37
6	912	923	12.46	Alpha-Copaene	$C_{15}H_{24}$	204	1.44
7	897	900	13.03	Cis-methyl isoeugenol	$C_{11}H_{14}O_2$	177.9	2.38
8	914	958	16.33	3-propanoyl-2H-benzo(f) chromene	$C_{16}H_{14}O_2$	238	6.28
9	881	916	20.33	2-phenyl-4-methyl pyrido ()2,3-d) pyrimidine-5-one	$C_{14}H_{11}N_3O$	238	0.89
10	629	913	24.03	5-methyl-4,5-borazarotheniol(2,3-c) pyridine	$C_6H_7BN_2S$	150	1.25

Table5: Results showing the Qualitative GC MS analysis of extracts of *Myristica fragrans*

S.No	Search index	Reverse search Index	Retention time	Compound name	Molecular formula	Molecular weight	Area%
1	660	968	23.92	Trans-1-(1-hydroxy-3,5-di methoxyphenyl) propene	$C_{11}H_{14}O_3$	194	0.65
2	565	805	25.57	Ferulic acid	$C_{10}H_{10}O_4$	194	0.52
3	511	869	24.17	Trans-4-propenyl- syringol	$C_{11}H_{14}O_3$	194	0.52
4	634	807	26.19	1,3-difluoro azulene	$C_{10}H_6F_2$	164	0.59
5	490	801	26.19	Cis-ios-eugenol	$C_{10}h_{12}O_2$	164	0.59
6	992	999	26.58	O-Benzyl-L-serine	$C_{10}H_{13}NO_3$	193.9	0.72
7	867	999	27.41	5-iodo uracil	$C_4H_{31}N_2O_2$	236.9	19.29
8	862	999	28.17	O-Benzyl-L-serine	$C_{10}H_{13}\ NO_3$	193.9	2.91
9	924	975	31.98	Di-(2-ethyl hexyl) phthalate	$C_{24}H_{38}O_4$	390	0.79
10	505	808	33.06	3,4,5-trimethoxy-7-hydroxyl-1-allyl-3,5-dimethoxy-8-o-4-Neolignan	$C_{23}\ H_{30}\ O_7$	418	2.06

11	516	900	33.06	Ih-Benzimidazole, 2-phenyl-(CAS)	$C_{13}H_{10}N_2$	194	2.06
12	824	916	35.66	2-methyl-1, 10-phenanthroline	$C_{15}H_{14}$	194	1.12

Comparison with the Duke's Data base

Duke's Phytochemical and ethanobotanical databases of *Camellia sinensis* and *Myristica fragrans*

The component present in the medicinal plant extract was compared with the known/reported phytochemicals from Duke's Phytochemical and ethanobotanical databases of *Camellia sinensis* and *Myristica fragrans*. It provides the maximum information of the known bio active compounds in *Camellia sinensis* and *Myristica fragrans.* Dr. Duke's Phytochemical and ethanobotanical databases have screened out all phytochemicals along with their bioactive properties present in plant extracts. As per Dr. Duke's Phytochemical and Ethnobotanical Databases, the chemicals and its biological activities in whole plant parts of *Camellia sinensis* (Green Tea) and *Myristica fragrans* were compared. The components Chemicals found in plant shown to be effective for the ailment medicated and Plant itself shown to be effective for the ailment medicated.

The Antimicrobial activity

Agar Well Diffusion method described gives efficacy comparison between hydroethanolic extracts of *Camellia sinensis* and *Myristica fragrans*. against Pyogenic Microorganisms *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans,* and the results were observed, zone of inhibitions were measured and findings were tabulated in (**Table 6**). Even though, *Myristica fragrans* (Nutmeg) found to be less active than *Camellia sinensis*, the bioactive nature of plants rendered good Phytochemical property.

Table 6: Results showing the antimicrobial activity of C*amellia sinensis and Myristica fragrans* Extract againt Pyogenic Microorganisms

Sample	Extract type	Concentration used	Zone of Inhibition(mm)		
			Staphylococcus aureus	***Pseudomonas aeruginosa***	***Candida albicans***
Camellia sinensis	Hydroethanolic Extract	10mg/mL	26	19	23
		5mg/mL	16	17	16
		2.5mg/ml	8	12	7
Myristica fragrans	Hydroethanolic Extract	10mg/mL	20	16	22
		5mg/mL	16	14	16
		2.5mg/ml	8	No zone	9

The comparison of the antimicrobial nature of the herbal extracts with the commercially available antibiotics like Bacitracin, Neomycin and Ketaconazole, were tabulated in (**Table 7**). This shows that the plant can be used as a drug of choice to treat skin infection.

Table 7: Results showing the Antimicrobial activity of commercial Antibiotics againt Pyogenic Microorganisms

Antibiotics	Concentration	Zone of Inhibition(mm)		
		Staphylococcus aureus	***Pseudomonas aeruginosa***	***Candida albicans***
Bacitracin	15mcg	20 (Sensitive)	17(Moderate)	13(Resisiant)
Neomycin	15mcg	22(Sensitive)	19(Moderate)	15(Resisiant)
Ketoconazole	15mcg	17(Moderate)	15(Resisiant)	21(Sensitive)

CONCLUSION

The active phytochemical constituents present in *Camellia sinensis* (Green Tea) and *Myristica fragrans* (Nutmeg) imparts high therapeutic properties that can prevent, pyogenic infection, skin inflammation and reduce the number of transient microbial flora. This can be observed by the zone of inhibition and comparison with commercial antibiotics. The results of the FTIR spectral analysis and GC-MS Qualitative analysis indicate the presence of bioactive compounds. This was further compared with the Dr. Duke's Phytochemical and Ethnobotanical Databases to analyse the therapeutic roles. For further studies,

the hydroethanolic extracts were subjected to Phyto-Topical applicant preparation studies.

REFERENCES

1. Hostettmann, K., M.J, Pettei, I. Kubo and K. Nakanishi, 1977. Direct obtaining of crude plant extracts by preparative liquid chromatography Lloydia. Helv. Chim. Acta, 60: 670-672. Make references like this style.
2. Warrier, P.K., Nambiar, Mankutty, C.2002.Indian Medicinal plants, Orient Longman.Chennai, India. 33
3. Evans WC. 1989 Trease and Evans Pharmacognosy 13th Edn E.LBS with Bailliere Tindall.
4. Odebiyi, P.P., Sofaware, E.A. 1979. Phytochemical screening of Nigerian medicinal plants II. Lloydia. 41: 234-240

5. Harborne, J.B.1984. Phytochemical Methods Chapman and Hall, London, Sixth Edition, 27-34
6. Brindha, P., Saraswathi, A. (1981) Phytochemical comparison of Pentatropis, Oldenlandia and plumeria. In: Proc. Natl. Seminar on Recent Trends in Natural Products Chemistry held on March 30-31, at Bharatidhasan Univ., Tiruchirappali, India.
7. Weiddenmaier, C., Faigle M, Van Kessel, K.P., Van Strijp, J.A., Goetz, F., Neumeister, B., Peschel, A. (2002) *Staphylococcus aureus* strains lacking D alanine modifications of teichoic acids are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J.Infect.Dis.186, 214-219
8. Perez, C., Alcia Mariel., Jose Luis Cabrera. 1991. The essential oil of senecio graveolens (compositae): Chemical composition and antimicrobial activity tests. J. Ethanopharmacol. 66: 91-96
9. Nair R., T Kalariya, Sumitra Chanda., Antibacterial Activity of Some Selected Indian Medicinal Flora, 2005. *Turk J Biol*, 29: 41-47.
10. Weiss E.A, *Spice Crops*. 2002. CABI Publishing, CABI International, UK.11