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Protective Effect of Nutmeg (*Myristica Fragrans*) Against Pathogenic Bacteria and in Vitro Radical Scavenging, Ferric Iron Chelating Activities and its Profile of Oleoresin Compounds

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Abstract **Original Research Article**

Spice are widely used as a foundation for many applications such as food preservation, pharmaceuticals, medicine and natural therapies. The aim of this study was to determine the antibacterial and antioxidant activity of methanol extract of different parts (kernel, pericarp, leaf and mace) of nutmeg *Myristica fragrans* and its oleoresin compounds. Antibacterial activity was determined by agar well diffusion method against pathogenic bacterial strains of *Salmonella enteritica, Listeria monocytogenes, Shigella dysentery, Staphylococcus aureus, Escherichia coli and P. aeruginosa.* Antioxidant activity was determined using DPPH, TPC, FRAP and ABTS assays. The highest antibacterial activity was shown by pericarp against each bacterial strain and the highest inhibition was reported as 3.9 mm of diameter against *S. enteritica*. Inhibition shown by the plant extracts was significantly difference (P values=0.000). Pericarp showed the lowest activity in every antioxidant assays. Leaf extract showed the lowest IC_{50} in DPPH (0.11 µg/mL) and $ABTS^{+}$ (7.8 µg/mL), highest TPC (220.62 mg GAE/g extract) and highest ability to reduce ferric (183.41 mg Trolox /g extract). GC-MS analysis revealed the presence of 95 compounds of extracts representing 99.6%. Propanoic acid and butanoic acid Maleic anhydride were detected collectively (8.109%) showing the highest occurrence in the pericarp extract. Propanoic acid (6.846%), 5-Hydroxymethylfurfural (6.251%), Furfural (1.035, Myristicin (1.874%), Elemicin (2.406%) and Myristic acid (0.82%) were reported as possible contributors for the highest antibacterial activity of pericarp methanol extract. The results showed, potential use of nutmeg, specially the pericarp to develop drugs against the disease course bacteria.
Keywords: antibacterial activity, antioxidant activity, pathogenic bacteria, GC-MS analysis, *Myristica fragrans*, oleoresins.

INTRODUCTION

Plant derived products have been used for medicinal purpose for centuries. Antimicrobial activities of plant extracts have formed the foundation for many applications such as food preservation, pharmaceuticals, medicine and natural therapies [1]. Many spice plants were reported for their many therapeutic and pharmaceutical qualities, due to the higher content of bioactive compounds [2]. Spices have been a part of man's history for centuries. Since earliest times, spices have been used to flavour many foods and beverages. Spices are important ingredients in food industry to manufacture a variety of processed foods. In food industry spices are used to flavour many kinds of baked foods, confections, meat and meat products like sausages, different sauces, vegetables, and beverages [3]. Apart from giving flavors for many foods and beverages, like many fruits and vegetables, a number of spices are sources rich in antioxidants good for prevention and treatment of diseases such as cancer and cardiovascular diseases [4]. Nutmeg (*Myristica fragrans*), whose seed is widely used as a spice, is a tropical, dioeciously evergreen tree native to the Moluccas or Indonesia [4]. Nutmeg is the seed kernel of inside the fruit and mace is the lacy covering (aril) on the kernel [5]. It has been reported that nutmeg having anthelmintic, anti-inflammatory, insecticidal properties and as a treatment for rheumatism, diarrhea, asthma and atherosclerosis and mace having strong antifungal and antibacterial activity [6]. Hydroxyl radical ($OH^{\cdot}$), superoxide anion (O^{2-}), singlet oxygen and hydrogen peroxide (H_2O_2) like free radicals and other reactive oxygen species can cause oxidative damages to biological macromolecules which can lead to initiation and/or progression of various diseases such as cellular and metabolic injury, cancer, atherosclerosis, inflammation, aging, diabetes, ischemic etc [7]. Spices are rich in compounds such as: vitamins, carotenoids,

terpenoids, alkaloids, flavonoids, lignin, simple phenols and phenolic acids and these compound can prevent human beings from various diseases resulting due to oxidative stress [4]. Nutmeg has also been supported with its good antioxidant activity attributed to the phytochemicals present naturally [8].

Many research have been reported antimicrobial and antioxidant activity of *M. fragrance*. Mahady et al. [9] reported methanol extract of nutmeg seed inhibited *Helicobacter pylori*, the Gram-negative bacterium associated with the development of gastritis and peptic ulcer diseases. Further, nutmeg extract possessed strong antibacterial activity against non-pathogenic and pathogenic *E. coli*, but the strain O157 showed more sensitivity to β-pinene than non-pathogenic *E. coli* strains [10]. Narasimhan and Dhake [11] reported that chloroform extract of seeds showed potent antibacterial activity against both gram-positive and gram-negative bacteria. Antioxidant and antibacterial properties of different extracts of nutmeg seed were evaluated by Gupta *et al*., [7]. Seeds were extracted with acetone, ethanol, methanol, butanol and water. Significant antifungal and antimicrobial activities were shown by all the extracts against *Staphylococcus aureus* and *Aspergillus niger*. Antioxidant and anti food-borne bacterial activities of extracts from leaf and different fruit parts of *Myristica fragrans* were analyzed by Sulaiman and Ooi [12]. The experiment concluded that only mace and seed-kernel extracts inhibited potential activities against *Staphylococcus aureus* and *Bacillus cereus*. Therefore, they suggested using mace and kernel to preserve food as natural preservatives.

Oleoresin has characteristic flavour and aroma of spices, which are the same as the original. Oleoresins have ability to resist high temperature processing to a greater extent than comparable essential oils [2]. Oleoresins are mixture of essential oils and resins the non volatile part that determine the characteristics flavour of spices. Oleoresin can be extracted by using different organic solvents and through different extraction methods. However the choice of solvent greatly affects the quality and quantity of oleoresin obtained through the extraction [8]. In food industry, ethanol extracted oleoresins are used as a safer solvent extracted oleoresin [5]. It was found that gradual extraction result much more quantity of oleoresin than direct extractions. In gradual extraction, essential oils from plant materials are extracted through steam or water distillation and then the residue is dried well and used to extract oleoresin [12]. Researchers have tested many biological properties of different types spices and herbs as they are rich source of important chemical constituent that are beneficial in medicinal, pharmaceutical and food industry. Pharmacological research revealed various activities of nutmeg such as antioxidant, antibacterial, antidiabetic, hypolipidemic, hepatoprotective and analgesic [7].

As a rich source of many biological properties, spice can be used for many product developments. Screening of these biological properties would be very useful to new product developments such as natural medicines, cosmetics etc. Consumers are well aware on antioxidant and antimicrobial properties of foods, and there is a particular attention to include spices with foods. Therefore, the objective of this research was to evaluate antibacterial properties of nutmeg kernel, leaf, pericarp and mace against 6 pathogenic bacterial strains which cause different illness for human and antioxidant activity of the extracts while analysing bio active compounds of the sample extracts through GC-MS analysis.

MATERIAL AND METHODS

MATERIAL

Reference Bacterial Strains

Salmonella enteritica, Listeria monocytogenes, Shigella dysentery, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.

Samples

Machine dried mature nutmeg kernel and mace. Sun dried fully mature pericarp and air dried leaves.

Chemicals

Ascorbic acid, gallic acid, Dimethyl sulfoxide (DMSO), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-*s*-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS+), Folin-Ciocalteu reagent and analytical grade methanol were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). All the other chemicals used were in an analytical grade. Nutrient agar medium from HiMedia, India.

Sample collection and preparation of extracts

Samples were collected from Matale district, Sri Lanka in April 2019. All the plant specimens were identified in central research station-spices, Matale, Sri Lanka. The dried sample was ground using a grinder (Panasonic, MX-AC 3000, India) to reduce the particle size. Soxhlet apparatus was used to extract oleoresin using methanol as the solvent (65^0C for 4 hours). From the each powdered samples, 15 g was extracted with 250 mL of methanol.

Collection and Preparation of Microbial Cultures

Reference bacterial strains, *Salmonella enteritica, Listeria monocytogenes, Shigella dysentery, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the bacteria collection of the Medical Research Institute (MRI), Colombo, Sri Lanka. They were separately

enriched by culturing for 24 hours at 37^0C in nutrient broth media, following refrigeration storage at 4^0C.

Preparation of Plant Extracts and Medium

A 20 mg/mL of stock solutions of the extracted plant materials were prepared by dissolving 20 mg of the crude extracts in 1 mL of methanol.

One liter of nutrient agar was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000 mL distilled water. The dissolved medium was autoclaved (Gemmy SA-300H Sturdy Autoclave, Taiwan) at 15 lbs pressure at 121^0C.

Well Diffusion Method

Agar well diffusion method was used to evaluate the antimicrobial activity of prepared bacterial strains. A 25 mL of autoclaved medium was poured into 100 mm petriplates while still molten in a laminar flow cabinet. After about 1 minute, petriplates containing nutrient agar were seeded with 100 µL reference bacterial strains, well mixed and allowed to solidify. The agar plates were punched with sterile cork borer of 5 mm size and 200 µL of stock solutions of plant extract was added into the wells. The plates were then incubated at 37^0C and readings were taken after every 24 hours for 3 days.

The antimicrobials present in plant extract was allowed to diffuse out into the medium and interact in a plate freshly seeded with the reference organisms. The diameter of the clear inhibition zones formed around the wells was measured in millimeters. Wells treated only with methanol were used as negative control. Thereby antimicrobial activity of different extracts of *Myristica fragrans* kernel against the reference pathogenic bacteria were evaluated using those measurements. Three replicated were done for each experiment.

Antioxidant Activity of Nutmeg Extracts

Determination of Total Phenolic Compounds

Phenolic content of the extract was determined using the method described by Singleton, Orthofer and Lamuela-Raventon [13] with slight modifications using 96-well micro plate. The results were expressed as milligram of galic acid equivalents/ g of plant extract.

From the each extract, 1 mg was initially dissolved in 20 µL DMSO and then diluted in 980 µL of distilled water. A 110 µL of Folin-Ciocalteu, 20 µL of sample/ galic acid and 70 µL of 10% sodium carbonate solution were mixed and after 30 minutes of incubation period at room temperature absorbance were taken at 765 nm.

DPPH radical scavenging assay

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the extract were measured using the method of Blois [14] with modifications. The results were expressed as milligram of ascorbic acid equivalents/ milligram of plant extract.

From the each extract, 1g were initially dissolved in 20 µL DMSO and then diluted in 980 µL of methanol. For the assay, 90 µL of methanol reagent, 50 µL of sample/ standard/ methanol as the control and 60 µL of DPPH solution were mixed and after 10 minutes of incubation period at room temperature absorbance readings were taken at 517 nm.

% Radical scavenging activity of samples and standard were calculated.

A- Absorbance of sample at concentration N

B- Absorbance of control

$$\% \text{ Radical scavenging activity } = \frac{(B - A)}{B} X\ 100$$

IC_{50} (concentration of sample/ standard at 50% inhibition) were calculated.

Ferric Reducing Antioxidant Power (FRAP) Assay

Reducing power of extracts were determined using the method of Benzine and Szeto [15] with slight modifications. The results were expressed as milligram of Trolox equivalents/ g of plant extract.

From the each extract, 1 g were initially dissolved in 20 µL DMSO and then diluted in 980 µL of distilled water. For a assay in a plate, 150 µL of FRAP reagent, 20 µL of sample/ Trolox/ buffer and 30 µL of acetate buffer solution were mixed and after 8 minutes of incubation period at room temperature absorbance readings were taken at 600 nm.

$ABTS^+$ Radical Scavenging Assay

Reducing power of extracts were determined using the method of Re et al, [16] with slight modifications. The results were expressed as milligram of Trolox equivalents/ g of plant extract.

From the each extract, 1g were initially dissolved in 20 µL DMSO and then diluted in 980 µL of distilled water. For the assay in a plate, 110 µL of 50 mM phosphate buffer saline (PBS) and 50 µL of sample/ water as the control were mixed. PBS and water were used as the blank. Pre plate read was taken at 734 nm. From the $ABTS^+$ reagent 40 µL was added and after 10 minutes of incubation period at room temperature absorbance readings were taken at 734 nm.

% Radical scavenging activity of samples and standard and IC_{50} (concentration of sample/ standard at 50% inhibition) were calculated as described DPPH assay.

GCMS Analysis

Model of GCMS -7890B, 5977AMSD (Agilent Technology).

Column – Agilent, HP-5MS-ULTRA INERT (30 m x 250 µm x 0.25 µm)
Injection volume was 1 µL. (Split ratio- 1:1).

Extract was dissolved in methanol (GCMS grade). Injector and transfer line temperatures were set at 280°C and 300°C respectively. The oven temperature was programmed from 50°C to 300°C. Helium was employed as a carrier gas (4.8 mL/ minutes). Initial temperature of the oven was set at 50°C and hold time was 2 minutes. Then the temperature was increased up to 240°C with a heating rate of 5°C/ minutes, with the hold time of 2 minutes at 240°C. Again the temperature was increased up to 300°C with a heating rate of 10°C/ minutes. The hold time was 2 minutes at 300°C. Total run time was 50 minutes.

MSD detector system operated at 70 eV, MS Source 230°C and MS Quad 150°C. Compound identification was carried out partly using correlations between retention times and m/z. Data library systems provided by Agilent Technologies were used (NIST14L and W9N11L).

ANALYSIS OF DATA

Data were analyzed by one-way ANOVA, inhibition was analyzed by considering bacterial separately. Least significance were tested at $P \leq 0.05$ to determine the difference of inhibition by plant extracts in each reference bacteria. Turkey test was used to determine the interaction between plant extracts.

RESULTS AND DISCUSSION

Methanol extract of dried plant samples were analyzed for its antimicrobial and antioxidant activity as many researchers stated that methanol extracts showed successful biological properties among many extracts. As shown in Table-1 and Fig-1, methanol extract of nutmeg pericarp possessed excellent antibacterial activity against the tested pathogenic bacteria.

Table-1: Antibacterial activity of nutmeg methanolic extracts.

Sample Bacteria	Pericarp	Leaf	Kernel	Mace
S. enteritica	3.90±0.12* A**	1.67±0.11 B	1.13 ± 0.08 C	1.63±0.11 B
L. monocytogenes	2.63±0.03 A	1.77±0.03 B	0.70 ± 0.06 C	0.73±0.03 C
S. dysentery	3.50±0.07 A	1.93±0.08 B	1.00 ± 0.07 C	0.57±0.04 D
E. coli	1.80± 0.07 A	1.57±0.04 B	0.77 ±0.04 C	0.60±0.07 C
P. pneumonia	3.10 ± 0.06 A	1.67 ± 0.03 B	1.03 ± 0.03 C	0.63±0.03 D
S. aureus	1.60± 0.07 A	1.4± 0.07 B	0.5± 0.07 C	0.43± 0.04 D

Note: Read the table horizontally
*Values represent mean diameter of Inhibition (cm±SEM, n=3)
**Means of the values that do not share a letter are significantly different

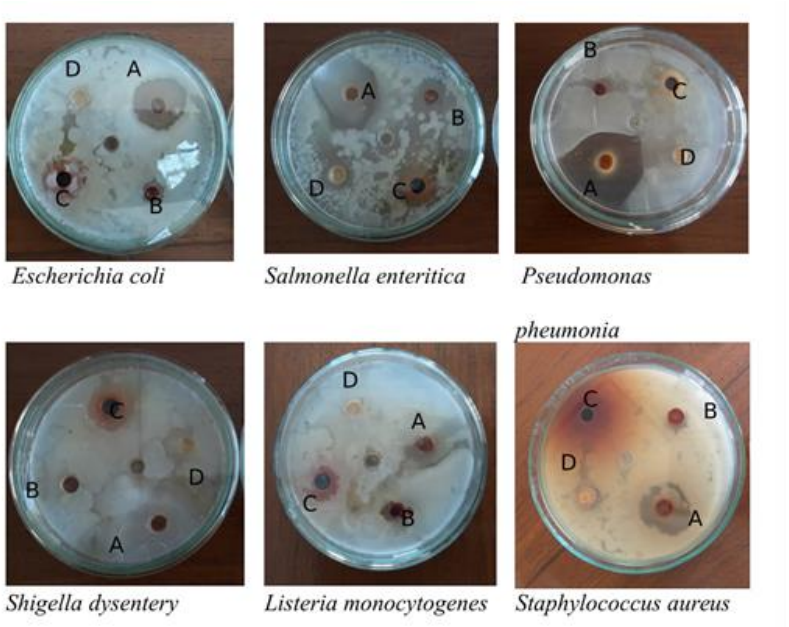

Fig-1: Inhibitory activity of different parts of nutmeg against different bacteria

Note: 20 mg /mL of methanolic extract was introduced to each well. A- pericarp, B- mace, C- leaf, D- kernel

Leaf extract showed significant higher inhibition against every tested bacteria, than that of kernel and mace extract but lower than that of pericarp extract. All the P values were 0.000. Therefore it can be concluded that inhibition by the plant extracts against each bacterial strains are significantly difference. The results showed the potential use of nutmeg specially the pericarp, to develop drugs against the disease course by tested bacteria. The highest inhibition was showed by the pericarp against *S. enterica*, which is a medicinally important pathogen for both humans and animals. It is a Gram-negative, food-borne pathogen that causes human diseases ranging from mild gastroenteritis to severe systemic infections [17].

Pericarp showed a considerable inhibition against *P. pneumonia*, which is life-threatening microorganism gives lung infection that can affect people with weakened immune systems. Through the Turkey test, it was identified that the inhibition by four plant extracts are significantly different against *S. dysentery* and *P. pneumonia*. However, there was no significant difference between the inhibition of leaf and mace extracts against *S. enterica* and kernel and mace extracts against *E. coli*.

Antibacterial activity of these extracts could be attributed to the occurrence and concentration of chemical substances present in nutmeg extracts. Even though the pericarp showed the highest inhibition against every tested bacteria, it showed the lowest results for every antioxidant assays carried out in this study (Table-2, Figs 2 & 3).

Table-2: Total phenolic content and ferric reducing ability of nutmeg extracts.

Sample	TPC (mg GAE /g extract)	FRAP (mg Trolox /g extract)
Pericarp	12.51±1.43	31.40±1.34
Leaf	220.62±2.38	183.41 ± 6.29
Mace	83.56 ± 3.55	178.53 ±1.82
Kernel	114.95 ± 4.3	158.91 ± 6.99

Values represent mean ± SE (n=3)

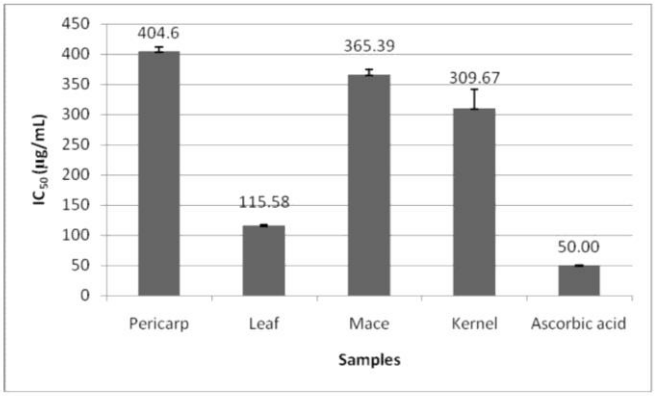

Fig-2: DPPH radical scavenging activity of nutmeg

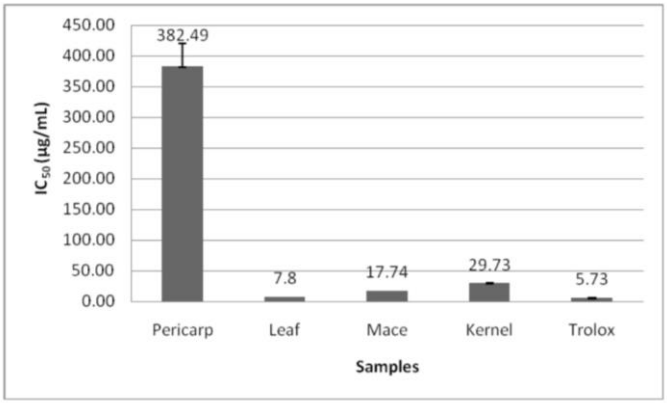

Fig-3: ABTS radical decolourization activity of nutmeg

Leaf extract showed the highest DPPH radical scavenging ability (IC_{50}; 0.12 mg/mL) (Fig-2). Ascorbic acid was used as the positive control for the DPPH assay and its scavenging ability to 50% DPPH radical was 0.05 mg/mL. Among the tested sample pericarp showed the lowest scavenging ability of 0.4 mg/mL.

In $ABTS^+$ assay, leaf extract showed the highest scavenging ability to 50% $ABTS^+$ radical (7.8±0.16 µg/mL) Trolox was used as the positive control (5.73± 0.04 µg/mL) (Fig. 03). It is rare to find any evidence of consumption of nutmeg leaf for any kind of propose. However, the pericarp, the fruit peel is used for preparation of different types of food products such as jams, jellies, marmalades, sources, salad dressings, wines etc. Kernel and mace are used as spices to flavour the food and beverages. Leaf extract showed the highest ability to reduce ferric (183.41 ± 6.29 mg Trolox / g extract) compared to other extracts (Table 2). The mace showed 178.53 ±1.82 mg Trolox / g extract and kernel showed lower than mace, that was 158.91 ± 6.99 mg Trolox / g extract. However, the pericarp showed the lowest among the tested sample 31.40±1.34 mg Trolox / g extract, even though it showed the highest antibacterial activity among the samples. The same results were observed with the assay of TPC (Table-2). Pericarp showed the lowest polyphenol contents. Therefore, it can be concluded that the antibacterial activity of pericarp was not due to its polyphenolic content.

Narasimhan and Dhake [11] reported trimyristin and myristic acid isolated from the chloroform extract of nutmeg seeds showed good antibacterial activity. Many chemical substances which are responsible for antimicrobial properties of the pericarp were identified through GC-MS analysis. The oleoresins extracted in this study were stored at 4^0C for two weeks of time before GC-MS analysis. Methanol was used as the solvent to dissolve oleoresins for GC-MS analysis. Some of the compounds identified through GC-MS analysis are seen in Table-3.

Table-3: GC-MS analysis of pericarp oleoresin.

Compound	% content	RT (min)
Furfural	1.035%	3.904
Maleic anhydride	8.109%	4.501
2,5-Furandione, dihydro-3-methylene-	1.452%	6.126
gamma-terpinene	0.355%	8.890
Furyl hydroxymethyl ketone + methyl 2-furoate	0.748%	9.619
Linalool	0.277%	10.106
3-Acetoxy-3-hydroxypropionic acid + methyl ester dimethyl di-malate	3.624%	11.079
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	5.101%	11.488
Terpinen-4-ol	0.509%	12.262
5-Hydroxymethylfurfural	6.251%	14.263
Propanoic acid	6.846%	15.523
Propanoic acid + Butanoic acid	17.681%	16.706
Caryophyllene + Bicyclo nonane, 2-methylene-4,8,8-trimethyl-4-vinyl- trans-Isoeugenol	1.465%	18.630
trans-Isoeugenol	2.089%	19.414
1-Dodecanol + Cyclododecane +Tridecan-1-ol	1.340%	20.056
Isomethyleugenol	0.976%	20.608
Myristicin	1.874%	21.161
Elemicin	2.406%	21.670
Iso elemicin	0.719%	24.168
7-Tetradecene	0.826%	24.699
Myristic acid	0.820%	26.634
Pentadecanoic acid	0.184%	28.624
Palmitic acid	0.419 +1.080% + 1.724%	29.840
Petroselinic acid + oleic acid	2.523%	33.864
Stearic acid + Palmitic acid	1.327%	34.306
Eicosanoic acid	0.115%	37.612
Phthalic acid	0.101%	40.486
Campesterol	0.829%	48.457
Stigmasterol	1.093%	48.756
gamma.-Sitosterol	1.457%	49.275
Nalmefene + 1,3-Dimethoxypropan-2-yl oleate	2.494%	50.005

Propanoic acid was detected in 6.846% and, propanoic acid and butanoic acid were detected in the highest percentage 17.681%. Moreover, propionic acid is an organic acid with the chemical formula $C_3H_6O_2$ and is used as a food additive [18] and as a preservative and flavoring agent in packaged foods, commonly in baked goods and cheese [19] Propanoic acid has antibacterial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Bacillus subtilis* [20]. Gunes and Cosar [21] reported a number of 1H-benzimidazole-2-propanoic acid derivatives showed considerable activity against both *Candida albicans* and *Candida tropicalis*.

Calcium propionate and sodium propionate, the salt forms of propionic acid, are used in bread and tortillas to prevent mold. It is found naturally on the skin and in the gastrointestinal tract. Butanoic acid is also known as butyric acid is commonly found in esterified form in animal fats and plant oils. It is a saturated short-chain fatty acid [18].

Maleic anhydride was detected with 8.109% and it is used in the manufacture of unsaturated polyester resins (UPR). Chopped glass fibers are mixed with maleic anhydride to produce fibreglass reinforced plastics. These plastics are used in a wide range of applications such as pleasure boats, bathroom fixtures, automobiles, tanks and pipes [22].

5-Hydroxymethylfurfural (6.251%) (5-HMF) serves as building block for many applications in several industries like packaging, construction, textile, cosmetics, food and health [23]. HMF are toxic and considered as probably or potentially carcinogenic to humans or might be metabolized by humans to potentially carcinogenic compounds [24]. Furfural (1.035%), is a fungicide and weed killer [25]. Furfural is called as "the sleeping beauty bio-renewable chemical" as it is consider as a potential chemical for the production of biofuels and biochemicals. Production of furfural is very flexible and among the industrial chemicals, it is the most commonly produced. Furfural and its derivatives have been extensively used in many industries such as plastics, pharmaceutical and agrochemical industries for different purposes. Some common products include, fungicides and nematicides, transportation fuels, gasoline additives, lubricants, resins, decolorizing agents, jet fuel blend stocks, drugs, insecticides, bio-plastics, flavour enhancers for food and drinks, rapid all-weather repair system for bomb-damaged runways and pot holes and also for wood modification and book preservation [26]. Further, furfural has a good antibacterial activity than furfuryl alcohol and furoic acid [27].

Myristicin (1.874%) and elemicin (2.406%) are also in found the extracts of other parts of nutmeg as well [7]. Myristicin acts as a natural bioactive agent and it is the principal aromatic constituent of the volatile oil. Myristicin is one of the compounds in nutmeg for its insecticidal effect. Several intoxications have been reported after an ingestion of approximately 5 g of nutmeg, corresponding to 1–2 mg myristicin/kg body weight [28]. Myristic acid was detected in 0.820%. Myristic acid is rich in butter fat and an ingredient in soaps and cosmetics. Myristic acid is widely used as raw material in the production of emulsifiers, anionic and nonionic surfactants, ester and flavours. It is a very important ingredient in cosmetic industry and also has a variety of uses in the beauty industry as a fragrance Ingredient [18].

In this research, considerable amount of myristicin and elemicin were detected but, safrole or eugenol were not detected. Myristicin, elemicin, safrole and eugenol are chemical compounds which either individually or collectively have been suggested to be the active agent of nutmeg (Table-3 & Fig-4).

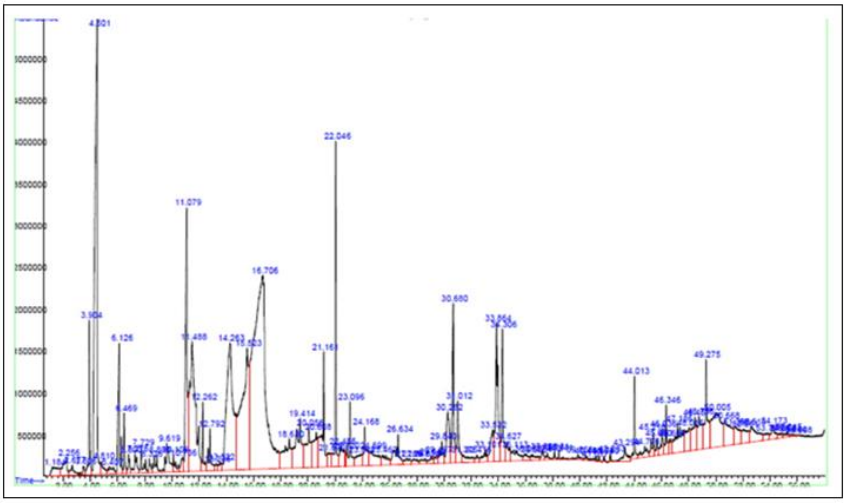

Fig-4: GC-MS Chromatogram of methanolic extract from nutmeg pericarp

Sherry, Ray and Herron [29] also not detected of myristicin, elemicin, safrole or eugenol but trimyristin with ligroin were found in nutmeg extract.

1-Dodecanol was detected as a mix with cyclododecane and tridecan-1-ol (1.340%). 1-dodecanol is a fatty alcohol and used in apple and pear orchards as a lepidopteran pheromone/sex attractant [18]. Further it is used to disrupt the mating behaviour of certain moths whose larvae destroy crops and it has a role as a cosmetic, a pheromone, an insect attractant, a pesticide and a plant metabolite.

The GC-MS result of this study showed the possible use of pericarp extract for different product developments. Further industrially valuable chemical compounds can be isolated by pericarp. Having lower amount of phenolic compounds and showing higher antibacterial properties may be a good trend to think about isolation of compound from oleoresin. The reason is that isolation of other chemical compounds are easy than isolation of phenolic compounds by a column chromatography as those are adhere to the column with the phenolic nature of polyphenols makes them relatively hydrophilic [30].

Dilika, Bremner and Meyer [31] reported antibacterial activity of petroselinic acid and oleic acid against *Staphylococcus aureus* and *Micrococcus kristinae*. Petroselinic acid and oleic acid was detected in 2.523% in GC-MS analysis in this research.

Da Silva *et al*., [32] reported the antibacterial properties of stearic acid at 10 µg/mL concentration by using disc diffusion method against gram-positive bacteria *Bacillus cereus* and *Bacillus subtilis*. Palmitic acid is the most common saturated fatty acid found in animals, plants, and microorganisms and it is found to have antibacterial properties. Both stearic acid and palmitic acid were detected in pericarp with 1.327%. Agoramoorthy *et al*., [33] reported that oleic, stearic and myristic acids has potential antibacterial and antifungal principle for clinical applications. Many research have been conducted to determine the antibacterial properties of fatty acids in different plant extracts. Yusf *et al.*, [34] reported successful antibacterial activity of stigmasterol against *Staphylococcus aureus*, vancomycin resistance enterococci, *S. aureus*, *Streptococcus faecalis*, *E. coli* and *Pseudomonas Fluorescens*. Ciprofloxacin (5 µg/mL) has been used as the standard antibacterial drug. Further, Tamokou *et al.*, [35] also reported that *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida tropicalis* and *Cryptococcus neoformans* are more sensitive to mixture of sterols which includes campesterol.

CONCLUSION

The present study has demonstrated the antibacterial and antioxidant properties of methanol extract of nutmeg leaf, pericarp, kernel and mace. It was observed that the pericarp extract has shown the highest antibacterial activity against the tested pathogenic bacteria. However, the highest antioxidant activity was observed in the leaf extract. Compound identified through the GC-MS analysis may be responsible for the antibacterial activity showed in this study. Therefore, highest antibacterial activity of pericarp could not be due to the polyphenols in the extract, but may be due to fatty acids and organic acids identified in GC-MS analysis. A 95 of chromatographic peaks were detected representing 99.6% of compounds in the pericarp extract. Propanoic acid and butanoic acid Maleic anhydride were detected collectively (8.109%) showing the highest occurrence in the pericarp extract. Propanoic acid (6.846%), 5-Hydroxymethylfurfural (6.251%), Furfural (1.035, Myristicin (1.874%), Elemicin (2.406%) and Myristic acid (0.82%) were reported as possible contributors for the highest antibacterial activity of pericarp methanol extract.

Conflict of interest: Both authors declared that there is no any conflict of interest.

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