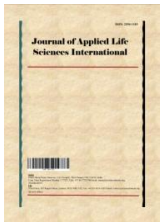

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Raw Material from Nutmeg (*Myristica fragrans*) as Effective Fungicide against *Fusarium oxysporum* and the Oleoresin Profile of Nutmeg

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Authors' contributions

This work was carried out in collaboration between both authors. Author AYLF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WSMS and AYLF managed the analyses of the study. Author AYLF managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Present study tested the antifungal activity of methanol, ethanol, acetone, chloroform and hot water extracts of nutmeg leaf, mace, kernel and pericarp at three concentration levels (5, 10 and 20%) against *Fusarium oxysporum*.
Methodology: The agar dilution technique was used and the effect of different concentration of plant extracts on radial growth of reference fungi was evaluated. GC-MS analysis was used for compound analysis.
Results: Complete inhibition of *F. oxyporum* was found at 10% and 20% concentrations of acetone, ethanol and methanol extracts of leaf, mace, and kernel and, chloroform leaf extract. Mace showed the highest inhibition among sample extracts under every solvent. Among the detected compounds, 3-acridinamine was detected from the genus *Myristica* for the first time in this study.
Conclusion: The results showed possible use of *M. fragrance* against *F. oxysporum*. Since the

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mace part was rich with many antifungal compounds such as phthalide, palmitic and oleic acid, myristicin, safrol, α-cubebene and aromandendrene, mace can be used as raw material to develop fungicide against *F. oxysporum*.

Keywords: Antifungal activity; Fusarium oxyporum; GC-MS; nutmeg.

1. INTRODUCTION

Over 20,000 species of fungi are parasites and number of economically important crops and plant are attached and cause many diseases [1]. Since fungi are the most abundant group of plant pathogens, they are considered the most prevalent plant pathogenic organism. Successful cultivation of crops is hindered by various diseases caused by fungal plant pathogens [2]. In our environment, thousands of plant pathogenic fungi are available and they are transmitted by seeds or transplants. One or many different types of plant or crop can be attacked by individual species of fungi [1]. Fungi cause wide range of diseases like powdery mildew, many leaf spot deceases, root and fruit rots etc and, fungi pathogens can spread not only from one plant to another but within the same plant [3]. Symptoms course by plant pathogenic fungi can be easily identify from other pathogenic deceases [2].

Fusarium oxysporum is one of the most wide spread and destructive plant fungal pathogen around the world [4]. According to a recent survey of international community of fungal plant pathologists, *F. oxysporum* is in the fifth place in a list of top 10 fungal plant pathogens. It survives in the soil for many years. Therefore, it has ability to make long-term restraint on crop production particularly in previously infected fields [4].

Some spices demonstrate antimicrobial activity with application in the food industry as antibacterial and antifungal agents [5]. Nutmeg (*Myristica fragrans*), whose seed is widely used as a spice, is a tropical, dioeciously evergreen tree native to the molusca or Indonesia. Nutmeg and mace are two distinctly separate spices derived from the same plant. It grows very successfully in the up country in Sri Lanka and brings a considerable income to the spice producers hence to the whole country [6]. Spices are used over centuries in food supply without any reports of deleterious effects and, they are generally recognized as safe (GRAS) [7]. In food industry, like many other spices, nutmeg is used to flavour many kinds of baked goods, confections, meat and meat products like sausages, different saucers, vegetables, and beverages [8]. The antimicrobial activity of nutmeg has been documented and the interest continues to the present because of its special fungal resistance characteristics [9]. Many spices are used to extend the storage life of foods by preventing rancidity through their antioxidant activity or through antifungal or bactericidal activity [10]. Nutmeg is rich with naturally present compounds such as vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignin, simple phenols, and phenolic acids [11].

Researchers have tested many biological properties of oleoresins extracted from many 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 [5]. Sabinene, myristicin, elemicin, α-pinene, β-pinene, limonene, terpinen-4-ol and myristic acid are reported as major compounds of the nutmeg oleoresins obtained by different techniques [9].

However, the use of plant products to control plant pathogenic fungi is limited. Use of synthetic fungicide is the most common practice for fungal disease management [2]. Even though those practices may cause adverse effects on the environment and human health, synthetic fungicides are used by farmers to ensure crop quality and production. The residue of synthetic fungicides remaining in fruits and vegetables may decrease the quality of the products [4]. However, there is an increasing public awareness on environmental impact associated with use of synthetic fungicides for agricultural practices. Therefore, this makes a pressure, an interest and also a challenge on many researchers around the world to discover safer eco-friendly fungicides. In this study, effectiveness of different extracts of different parts from nutmeg tree against *F. oxysporum* was evaluated with the aim of suggesting a new raw material to develop new fungicide.

The antifungal activities of essential oils and extracts of nutmeg seed and mace against many

plant pathogens have been evaluated by many researchers. Excellent antifungal activities of nutmeg spice powders have been observed against the plant pathogens such as *Colletotrichum acutatum, Colletotrichum fragariae, and Colletotrichum gloeosporioides* [12]. Although the chemical composition of nutmeg was extensively investigated over the past century, its antifungal potential has not been extensively investigated. The growth of *Colletotrichum gloeosporoides*, *C. musa, F. oxysporum, F.semitectum, A. niger* and *A. glaucus* has been inhibited by the crude nutmeg essential oil [12]. It has been detected successful antibacterial activity against *S. aureus* and *E. coli* and weak antifungal power against *A. flavus* and *F. moniliforme* by the essential oil derived from nutmeg flesh [13].

2. MATERIALS AND METHODS

2.1 Materials

Reference fungal strains, *F. oxysporum* was obtained from the fungal collection of the Postharvest Research Institute, Anuradhapura, Sri Lanka.

Acetone, methanol, chloroform and ethanol were obtained from Sigma Aldrich chemicals, St. Louis, Missouri, USA. All the chemicals used in this study were in analytical grade.

Freshly harvested, mature, healthy leaves, fruit pericarps, kernel of the seeds and mace of plant nutmeg *(M. fragrans*) were used.

2.2 Methods

2.2.1 Sample collection and preparation

All the samples were collected from Matale, Sri Lanka in June, 2018 and all the plant specimens were identified at Central Spice Research Centre, Matale, Sri Lanka. Nutmeg kernels and mace that fulfil the first grade specification by the Department of Export Agriculture, Sri Lanka were selected for the study. Plant materials were washed using tap water, then chlorinated water and finally with deionised water. Leaves were dried in air until crushable. Fresh pericarps, mace and seed kernels were dried in an oven 50ºC for 8 hours. Then they were ground (Panasonic, MX-AC 3000, India) to fine powders and stored at 4ºC until use.

A 30 g sample of each finely ground plant materials was mixed with 100 mL of distilled water and kept in a water bath at 80ºC for 30 min. Another 30 g of each sample was mixed with 100 mL of other solvents separately (acetone, methanol, chloroform and ethanol) and kept for 48 hrs at 25ºC ± 1 in the dark. All the samples were filtered using filter paper (Whatman No.1). After filtration, the samples were directly used for the experiments.

2.2.2 Preparation of fungal cultures

Reference fungal strain, was cultivated in potato dextrose agar (PDA: HiMedia Laboratories, India) medium and incubated at 25^0C± 1 for 7 days.

2.2.3 Evaluation of antifungal activity of extracts

In this study, the agar dilution technique [14] was used and the effect of different concentration of plant extracts on radial growth of reference fungi was evaluated. One litre of PDA was prepared by dissolving 39 g of commercially available PDA (HiMedia) in 1000 mL distilled water. The dissolved medium was autoclaved (Gemmy SA-300H Sturdy Autoclave, Taiwan) at 15 lbs pressure at 121ºC. To avoid bacterial contamination 0.5 g of antibacterial streptomycin was added to 1 L of PDA medium.

2.2.4 Agar dilution technique

To obtain 5% extract to the PDA medium a 5 mL aliquot of each filtered extract was mixed with 95 mL of molten PDA. In this way, 5, 10 and 20% extracts to a PDA medium were obtained. From the medium 25 mL of aliquots were poured into petriplates while still molten in a laminar flow cabinet. After the solidification, 6 mm diameter of mycelia blocks from 7-day-old colonies of *F. oxysporum* were inoculated in the center of each plate and incubated at 25± 2^0C. The diameters of the colonies were measured after 10 days of incubation. Three replicates were done with each experiment. The media amended with acetone, methanol, chloroform, ethanol, distilled water were considered as negative controls. The recommended fungicide (TILT- 250 g/L Propiconazole) was used as the positive control. The efficacy of the tested nutmeg samples were expressed as a percentage of radial mycelia growth over the negative control. The inhibition % was calculated by using the following formula.

$$\text{Inhibition } \% = \frac{(C - T)}{C} \times 100$$

Where, C is the diameter of the control colony and T is the diameter of treated colony.

2.2.5 GCMS analysis

Acetone was evaporated from the acetone mace extract by using a rotary evaporator. Oleoresin of acetone mace extract was used for GC-MS analysis.

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

Column – Agilent, HP-5MS-ULTRA INERT (30 m x 250 µm x 0.25µm)

Injection volume was 1µL. (Split ratio- 1:1)

Extract was dissolved in acetone (GC-MS grade). Injector and transfer line temperatures were set at 280ºC and 300ºC respectively. The oven temperature was programmed from 50ºC to 300ºC. Helium was employed as a carrier gas (4.8 mL/min). Initial temperature of the oven was set at 50ºC and hold time was 2 minutes. Then the temperature was increased up to 240ºC with a heating rate of 5ºC/min, with the hold time of 2 minutes at 240ºC. Again the temperature was increased up to 300ºC with a heating rate of 10ºC/minute. The hold time was 2 minutes at 300ºC. Total run time was 50 minutes [15].

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

2.2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was used to find an interaction between the two independent variables of samples and concentration levels on the dependent variable of mean inhibition% against *F. oxysporum* under each solvent. Tukey test was used at 5% significant probability level to find mean inhibition% that are significantly different from each other. The ANOVA was computation by MINITAB 19 software.

3. RESULTS AND DISCUSSION

Spices are rich with many bioactive compounds. Three types of alcoholic solvents were used (methanol, ethanol and, acetone) in this study, as the successful extraction of biologically active compounds form plant materials is largely depends on the type of solvent used for the extraction [11].

Table 1 showed the mean inhibition% by the sample extracts at different concentration levels. The data were analyzed under each solvent used for the extractions.

As shown in Table 1, maximum inhibition of 100% against *F. oxysporum* was found by 10% and 20% concentrations of acetone, ethanol and methanol extracts of leaf, kernel and mace samples except pericarp. Also there was a 100% inhibition by 20% and 10% of chloroform leaf extracts, but not with any other chloroform

Table 1. Inhibition % of different nutmeg extracts against the growth of *F. oxysporum*

		Mean** inhibition % against *F. oxysporum*				
Sample		**Methanol**	**Ethanol**	**Acetone**	**Chloroform**	**Hot water**
Leaf	5%	21.67± 2.7^{d}*	22.22±3.77^{e}	20.45±3.21^{d}	25.76±4.91^{cde}	2.38±0.58^{g}
	10%	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	18.64±4.79^{ef}
	20%	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	37.78±4.70^{bcd}
Kernel	5%	33.33± 3.68^{cd}	49.57±2.77^{c}	47.73±1.61^{c}	22.73±6.42	24.76±2.54^{def}
	10%	100±0.00^{a}	100±0.00^{a}	84.127^{b}	48.72±11.32^{bcde}	35.59±3.17^{cd}
	20%	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	70.37±18.15^{ab}	51.11±1.36^{b}
Mace	5%	69.17±11.77^{b}	72.65 ±3.77^{b}	80.30 ±2.46^{b}	24.24±4.91^{de}	29.05±3.55^{cde}
	10%	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	51.28±12.56^{bcde}	42.37±1.2^{bc}
	20%	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	66.67±20.79^{abc}	66.67±2.36^{a}
Pericarp	5%	31.67±4.08^{cd}	26.50±2.77^{de}	33.33±1.86^{d}	10.61±1.68	12.38±2.33^{fg}
	10%	42.03±7.78^{c}	42.86±8.91^{cd}	53.97±1.94^{c}	33.33±3.14^{bcde}	18.64±3.17^{ef}
	20%	49.02±4.80^{c}	44.44±7.20^{c}	60.00±0.00^{c}	51.85±4.54^{bcd}	26.67±6.24^{de}

*Note: ** Average of triplicates. Please read the table vertically. *Means that do not share a letter are significantly different*

extracts. When increasing the extract concentrations, there was an increment of inhibition% in every solvent. Mace extracts showed the highest inhibition% in all the solvent against *F.oxysporum*. Even at 5% concentration level, considerably higher inhibition% (80.30%) was shown by the acetone mace extract. The p-values for the interaction effect of sample and concentration levels under every solvent was higher than 0.05. In methanol it was 0.309, ethanol 0.316, acetone 0.889, chloroform 0.890 and in hot water 0.795. Therefore, it can be concluded that there is no significantly difference in the interaction effect between sample and the concentration levels on inhibition% under each solvent.

Fig. 1 showed the radial growth of *F. oxysporum* in ethanol extracts of leaf, mace, seed and pericarp at 5% and 10% of concentration levels.

Same like in ethanol, the highest inhibition% was shown by the mace sample in every solvent even at 5% concentration level. As shown in the Fig. 1, inhibition by 10% ethanol extract of kernel, leaf and mace was greatly similar to the inhibition% showed by the commercial fungicide. Since ethanol is not toxic to the human consumption at recommended level, ethanol extracted oleoresins are more commonly used for food uses and for many pharmaceutical uses [16]. Hence, ethanol mace extract can be suggested as a better option for controlling the *F. oxysporum.*

Table 2 showed some compounds which were detected in more than 0.1% through GC-MS analysis of acetone mace extract. Phenols, polyphenols and fatty acids were detected in higher percentage in the chromatogram.

Fig. 2 showed the chromatogram of acetone mace extract. 44 peaks were detected representing 99% of compounds in the extract. Twenty compounds had more than 0.2% and some of them were shown in the Table 2. Phenol,2,6-dimethoxy-4-(2-propenyl), called as methoxyeugenol was detected in the highest percentage in the chromatogram collectively with 3-acridinamine and 2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(3,4 dimethoxyphenyl) propan-1-ol (20.283%). In this study phenol,2,6-dimethoxy-4-(2-propenyl) was detected twice in 20.283% at 45.008 with some other compounds as mentioned above and again in 4.581% at 23.129 minute as well. Phenol,2,6-dimethoxy-4-(2-propenyl) is used as a food additive, specially to add the scent of smoke and to preserve the food like fish and meat. It is used in food at the maximum of 5 ppm [17]. During isolation of myristicin from nutmeg essential oil, 0.39% of Phenol,2,6-dimethoxy-4-(2-propenyl), 83.45% of myristicin and 4.01% of phenol,2,6-dimethoxy-4-(2-propenyl) have been detected in the isolate [17].

Importantly, 3-acridinamine was detected from the genus *Myristica* for the first time in this study. 2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(3,4 dimethoxyphenyl)propan-1-ol has been discoverd and isolated as a chemical constituent found in nutmeg seed [18]. Further, it is a compound present in herbs and spices specialy in *M. Fragrance* [19].

Licarin A, Phenol, 4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2methoxy- and Bis pentamethylcyclopentadienyl) iron were collectively detected in 18.599% in this study. The anti-allergic effects of licarin A, isolated from various plants, on antigen-stimulated rat mast cell line have been evaluated. It has been reported that licarin A reduces TNF-α and PGD2 secretion via the inhibition of PKCα/βII and p38 MAPK pathways. Therefore, this compound may be useful for attenuating immediate hypersensitivity [20]. Licarin A has been detected and isolated from nutmeg by many researchers. Further, antioxidant activities of licarin B that isolated from nutmeg have been evaluated [21]. Many compounds including licarin A, B, C and methoxylicarin A have been isolated from nutmeg utilizing spectroscopic methods, mainly NMR, and mass spectrometry [22]. Phenol, 4-[(2S, 3S)-2,3-dihydro-7-methoxy-3-methyl-5-(1E)-1-propenyl-2-benzofuranyl]-2,6-dimethoxy has been detected from methanol crude extract of dried mace [23]. In this study, phenol, 4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy- was detected and in the literature it is mensioned as diisoeugenol and isoeugenol [19]. In this study, isoeugenol was again detected in 2.438% at 19.425 minute. Isoeugenol is one of the flavour components of nutmeg. As a fragrant, it is incorporated into numerous products like perfumes, cream lotions, soaps, and detergents. As a flavouring agent isoeugenol is added to nonalcoholic drinks, baked foods, and chewing gums in food industry. However, isoeugenol has been nominated and selected for carcinogenicity testing by the National Cancer Institute-US because of its widespread human exposure as a fragrance and flavouring agent in many household and personal hygiene products and food products [24]. Some

researches have showed succesful antifungal activity of eugenol and isoeugenol against *Cryptococcus neoformans* [25]. Clear evidence were found on carcinogenic activity of isoeugenol in male and female B6C3F1 mice in experiments [24]. In male mice the evidance was based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma and in female B6C3F1 mice based on increased incidences of histiocytic sarcoma [24].

Phthalide is used in development of many fungicides [26]. In this study phthalide was detected in 3.338%. The succesful fungicidal effect of phthalide against the production of conidial wall pigments by *Penicillium* and *Aspergillus* species has been reported [26]. Therefore, phthalide may be one compound in mace to show its fungicidal effect in this study.

Palmitic acid is the most common saturated fatty acid found in animals, plants, and microorganisms and it is found to have antimicrobial properties. Oleic, stearic and myristic acids have potential antibacterial and antifungal principle for clinical applications [27]. Palmitic acid in soap showed positive antifungal effects against *S. apiospermum* [28]. Further, antifungal activity of fatty acids against phytopathogenic fungi has been studied [29]. It has been reported that fatty acids such as butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, oleic acid, and linoleic acid exhibit successful antifungal activity against phytopathogenic fungi such as *Alternaria solani, Colletotrichum lagenarium, Fusarium oxysporum f.* sp. *Cucumerinum*, and *F. oxysporum* [29]. Palmitic acid is a saturated fatty acid and it shows stronger antifungal activity than the unsaturated fatty acid, oleic acid. Further it has been suggested that fatty acids might be applicable to exploring for alternative approaches to integrated control of phytopathogens [29]. In current study palmitic acid was detected in 3.249% and oleic acid was detected in 3.744%. Therefore, palmitic and oleic acid may also have contributed for the antifungal effect of mace extract.

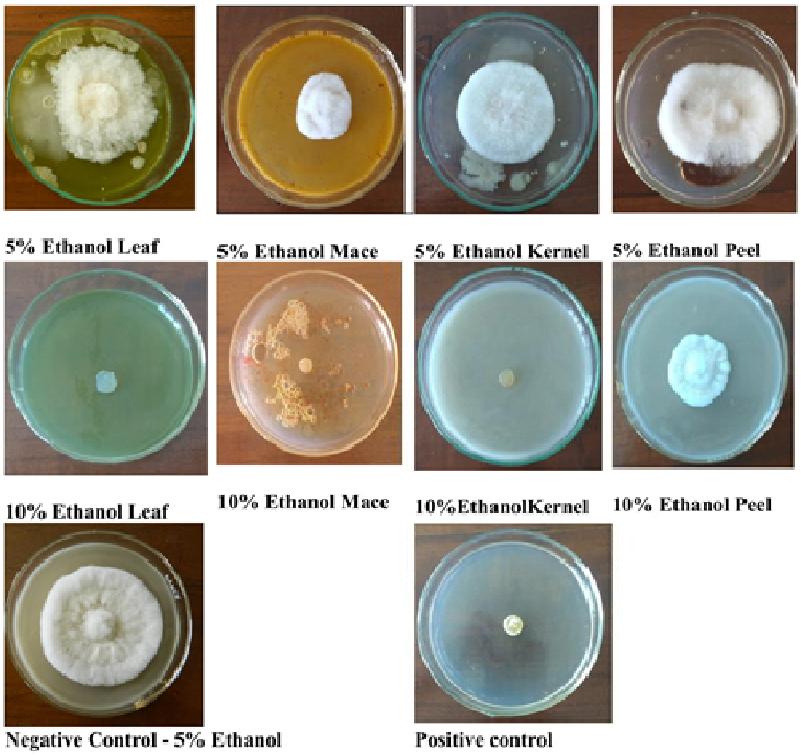

Fig. 1. Inhibition of *F. oxysporum* by ethanol extracts of leaf, mace, kernel and pericarp after 10 days

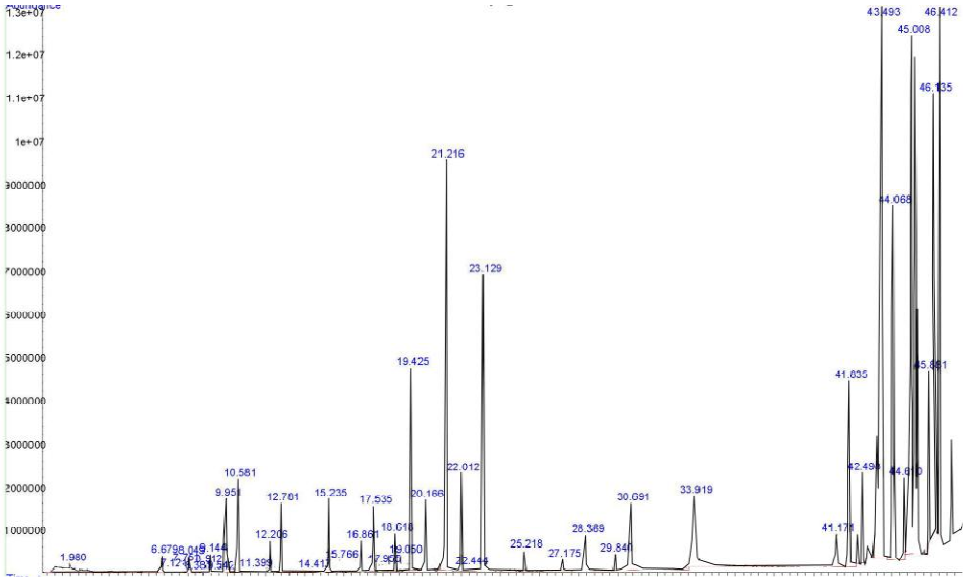

Fig. 2. GC-MS chromatogram of acetone mace extract

Table 2. Chemical compounds detected from GC-MS analysis of acetone mace extract

Compound	Content %	Retention time
methyl abietate + 4-Tosyl-1,3:2,5-dimethylene-l-rhamnitol	1.494%	1.980
Sabinene	0.241%	6.679
beta.-Myrcene + beta.-Pinene	0.051%	7.121
β-Terpinene +Cyclopropane, 1,1-dimethyl-2-(3-methyl-1,3-butadienyl)- +Cyclopentene, 3-isopropenyl-5,5-dimethyl-	0.265%	8.049
gamma terpinene (uses) + 3-Carene	0.108%	8.912
Sabinene hydrate	0.207%	9.144
cis-4-methoxy thujane + trans-4-methoxy thujane + (1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo[3.1.0]hexane	2.902%	10.581
Terpinen-4-ol	0.325%	12.206
beta phellandrene	1.016%	12.781
Linalyl acetate	0.09%	14.417
Safrole	0.906%	15.235
alpha.-Cubebene	0.668%	16.861
alfa.-Copaene + alpha.-Cubebene	0.823%	17.535
Aromandendrene	0.700%	18.618
Isoeugenol	2.438%	19.425
Germacrene D	1.217%	20.166
Myristicin	6.491%	21.216
Elemicin	1.039%	22.012
Phenol,2,6-dimethoxy-4-(2-propenyl)	4.581%	23.129
Palmitic acid	3.249%	29.840
Oleic acid	3.744%	33.919
Phthalide + 4,6-dimethoxy-1(3H)-isobenzofuranone, 5,6-dimethoxy and 2-Hydroxy-4,5-methylenedioxypropiophenone	3.338%	41.835
Licarin A + Phenol, 4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy- + Bis(pentamethylcyclopentadienyl)iron	18.599 %	43.493
(S)-5-Allyl-1,3-dimethoxy-2-((1-(3,4,5-trimethoxyphenyl)propan-2-yl)oxy)benzene + Indeno[2,1-c]pyridine, 1,4,6-trimethyl + (10H)-Acridinone	4.944%	44.068
Phenol,2,6-dimethoxy-4-(2-propenyl), 2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)propan-1-ol and 3-acridinamine	20.283%	45.008

Antifungal properties of myristicin and safrol isolated from nutmeg have been extensively studied and identified as mojor compounds in nutmeg essential oil which show higher antifungal activity against many pathogenic fungi [30]. Further, myristicin and safrol could serve as lead compounds to develop other fungicides [30]. Thefrefore, myristicin (6.491%) and safrol (0.906%) detected in study may also have contributed for the antifungal effect of mace extract.

Germacrene D was detected in 1.217% in acetone extract of mace in this study. Germacrene D has been reported to its insecticidal activity against mosquitoes , as well as repellent activity against aphids and ticks [31]. Germacrenes are a class of volatile organic hydrocarbons and are typically produced in a number of herbs. Germacrene is responsible for antimicrobial and insecticidal properties of those herbs and play a role as insect pheromones. Germacrenes D is one prominent molecules in germacrene class [31].

Further, antifungal potential of the essential oil and various extracts of *Mikania scandens* (L.) Willd has been reported and α-cubebene has been detected through GC-MS analysis as a responsible compound for its anifungal activity [32]. In this study, α-cubebene was detected in 0.668% and, 0.823% collectively with alpha.-cubebene. Aromandendrene was detected (0.700%) in this study. Aromandendrene is present in some plants and succesful antifungal effect of *Scapania verrucosa* has been reported due to presence of aromandendrene [33].

Beta phellandrene was detected in 1.016% in mace extract and it is used in production of fragrances because of its pleasing aromas. The odor of beta-phellandrene has been described as peppery-minty and slightly citrusy and commonly used in many cosmatic and pharmaceuticals. Further, beta-phellandrene is incest-resist and insecticidal compond [34].

Therefore, isoeugenol, phthalide, 3-acridinamine, palmitic acid, oleic acid, myristicin, safrol, α-cubebene, germacrene D, aromandendrene and beta phellandrene were identified as contributing compounds for the antifungal activity of acetone mace extract in this study. Then the results of this study showed the potential used of mace as a raw material to developmet a fungicide against *F. oxysporum*.

4. CONCLUSION

Among the nutmeg samples used in this study mace, kernel and leaf samples showed complete inhibition of *F. oxyporum* in methanol, ethanol and acetone solvent extracts. Among the chloroform extracts, leaf showed the complete inhibition. Mace extracts showed the highest mean inhibition% against *F. oxyporum* among all the nutmeg parts in every solvent. Through the GC-MS analysis of acetone mace extract phenolic compounds, saturated and unsaturated fatty acids were detected as possible contributors for its antifungal effect. Therefore with the findings in this study, it can be concluded that as a GRAS compound, nutmeg can be used as an raw material to develop fungicide against *F. oxyporum*. To develop fungicide, ethanol mace extract is recommended to use, as ethanol is non-toxic to the human consumption in recommended amount, and as the ethanol mace extract showed successful inhibition of *F. oxyporum* in this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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