Effect of essential oil of nutmeg (*Myristica fragrans*) leaves to treat human pathogenic bacteria and to manage plant pathogenic fungi

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Introduction

Essential oils (EOs) are complex natural mixtures that can be extracted from plant raw materials such as flowers, leaves, stems, roots, fruits and fruit-peels. Essential oils are being studied for many decades, but a growing interest has emerged in the recent decades due to a desire for a rediscovery of natural remedies [1]. EOs are highly interesting for pharmaceutical uses and many activities such as antioxidant, anti-inflammatory, antimicrobial, wound-healing, and anxiolytic activities have been discovered by many researchers. The oils extracted from different parts exhibit different biological and medicinal properties even though they have been extracted from the same plant [2].

EOs from spices and herbs are used in traditional medicine in many Asian countries since ancient times. Nutmeg tree gives two spices as nutmeg and mace. Nutmeg and mace have been used traditionally for thousands of years by many cultures as flavouring agents. Nutmeg volatile oils exhibit antibacterial activity against different types of animal and plant pathogens, food poisoning and spoilage bacteria [3]. EOs extracted from nutmeg and mace have been tested for different bioactivities by many researchers, but of the leaves of nutmeg very rarely. However, leaves of some spice trees are also used for cooking and medicinal purposes in their fresh or dried form [4]. For example, both fresh and dried leaves of cinnamon are used for culinary purposes. Further, some spice leaves are used as homemade remedies for diarrhea, fever, cough, etc. in traditional medicine in Asian countries. Many researchers have recommended different parts of trees of spices including leaves for the development of functional foods, nutraceuticals and drug recovery as herbal drugs have fewer side effects or toxicity compared to the synthetic drugs [5]. Many studies have been conducted to discover antimicrobial activities of the spice nutmeg and mace, but the leaves have been tested very rarely. Therefore, in this study, leaves of nutmeg were tested to discover its antibacterial and antifungal activities.

Methodology

Extraction of essential oils. Nutmeg leaves (fresh and mature) were air dried until crushable and ground. EOs were extracted with modified Clevenger method and water distillation method. The oil part, hexane and ether mixture were separated from water.

Preparation of referred bacteria and fungi. Bacteria were collected from the Medical Research Institute, Colombo. The average number of viable bacteria (*Salmonella enterica, Listeria monocytogenes, Shigella dysenteriae, Escherichia coli* and *Pseudomonas aeruginosa)* per mL of the stock suspensions was determined by means of the surface viable counting method. Inoculums of 10^8 - 10^9 colony-forming units per mL were prepared.

Aspergillus niger and *Fusarium oxysporum* were isolated from black moulds in onions and rotten potatoes with dry rot. Isolated fungi were identified at the Institute of Post-Harvest Institute, Anuradhapura.

Antibacterial assay. Agar disc diffusion method (nutrient agar medium) was used to evaluate the antibacterial activity of EOs. Amoxicillin, the antibiotic, was used as the positive control.

Antifungal assay. PDA plates with EOs impregnated discs were used for the

experiment. Mycelia blocks (4 mm diameter) were inoculated in the centre of the PDA plate and incubated at 25 ± 1°C. Captan, the commercially available fungicide, was used as the positive control.

GC-MS analysis of essential oils. Model of GC-MS-7890B, 5977AMSD (Agilent Technology) was utilized to analyze the EO samples.

Statistical Analysis. One-Way Analysis of Variance (ANOVA) is used.

Results and Discussion

Antibacterial activities of EO. The agar disc diffusion technique was used using 2 µL of EO. Nutmeg leaf EO showed the highest inhibition activity against *S. dysenteriae* and, then against *L. monocytogenes*. Statistically, there is no any difference in inhibition between *E. coli* and *P. aeruginosa*. EO showed the lowest inhibition against *S. enterica*.

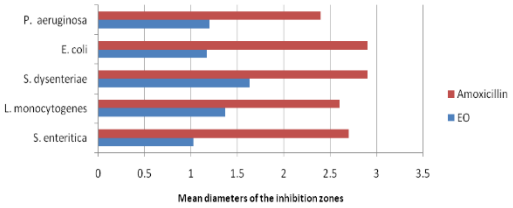

Figure 1. Antibacterial activity of nutmeg leaves EO against *S. enterica, L. monocytogenes, S. dysenteriae, E. coli* and *P. aeruginosa*.

Table 1. Antibacterial activity of nutmeg essential oil against human pathogenic bacteria. *Means of the values that do not share a letter are significantly different.

Essential oil	Mean diameters of the inhibition zones by the different essential oils against referred bacteria (cm) ± SE *				
	S. enteritica	*L. monocytogenes*	*S. dysenteriae*	*E. coli*	*P. aeruginosa*
L	1.03±0.04^{d}*	1.37±0.04^{b}	1.63±0.04^{a}	1.17±0.08^{c}	1.20±0.07^{c}

Antifungal activities of EO. As shown in Figure 2, EO showed higher inhibition activity against *F. oxysporum* than *A. niger*. However, the positive control showed more inhibition against *F. oxysporum* as three times higher than EO. Both EO and the positive control showed lower inhibition against *A. niger* than *F. oxysporum*.

Table 2. Antifungal activity of nutmeg essential oil against *F.oxysporum* and *A. niger*.

Essential oil	Mean diameter of inhibition zones ± SE (cm) *	
	F.oxysporum	*A.niger*
L	1.27±0.18	0.57±0.04

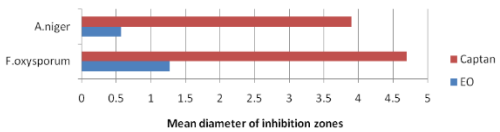

Figure 2. Antifungal activity of nutmeg leaves EO against *F.oxysporum* and *A. niger*.

GC-MS Profile of EO. According to the resuts of GC-MS, leaf EO contains conpounds from different chemical groups.

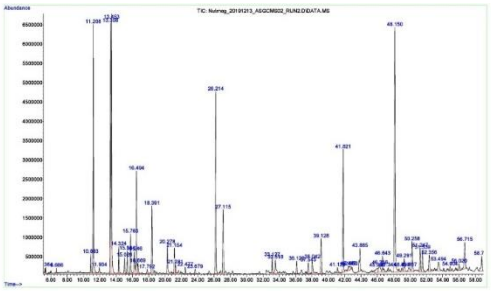

Figure 3. GC-MS chromatogram of nutmeg leaves EO.

The chromatogram (Figure 3) showed, 51 peaks representing 99.7% of compounds in the oil. Myristicin was detected as the major compounds (14.19%). Alpha-pinene, sabinene, 4-terpineol, phellandrene, caryophyllene, elemicin and beta-pinene were also detected in higher amounts among 51 of compounds. Other than the above mentioned compounds, some other compounds such as linalool, copaene, alpha-humulene, spathulenol, caryophyllene oxide, 2-pentadecanone, propane, 2-(chloromethyl)-1,3-dimethoxy-2-methyl- were detected more than 1% in the library. Some new compounds were detected in leaf

oil which had not been detected from the genus *Myristica* earlier. 2-pentadecanone was one compound among them which was detected in 2.152%.

Conclusion

The EO of nutmeg leaves possesses marked antibacterial and antifungal properties. The results of this study clearly indicated the potential use of EOs of nutmeg leaves for the treatment of diseases caused by human pathogenic bacteria and, for the management of important plant fungal diseases.

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