CSIRO Publishing The South Pacific Journal of Natural and Applied Sciences, **35**(1), 10-19, 2017
www.publish.csiro.au/journals/spjnas 10.1071/SP17002

Antibacterial and Antifungal Activities of Essential Oils from Medicinal Plants Found in the South Pacific

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Abstract

Natural products such as essential oils have been studied since ancient times to understand their biological properties. Essential oils are noted for their antimicrobial activity. Thus, the focus of this study was to evaluate the antimicrobial effect of five essential oils (EOs) from selected medicinal plants found in the South Pacific, on selected human pathogenic bacteria and fungi affecting agricultural industries. The disc diffusion method was carried out and the diameter of inhibition zones (mm) (DZI) using 0.25, 0.5, 5, 25, 50 and 100% (v/v) of essential oil concentrations were reported. The activity of Cananga odorata essential oils against Thermus thermophiles and Pseudomonas aeruginosa were among the selected bacteria that only showed the susceptibility at the lowest concentration (0.25% v/v). The diameter inhibition zones were 1.60 mm and 4.20 mm, respectively. The inhibitory effect of Ocimum tenuiflorum L essential oils at the highest concentration (100%) showed DZI ranging above 14 mm for all the selected bacteria and above 25 mm for the all selected fungi. The inhibitory effect of selected bacteria and fungi increased with stronger concentrations of essential oils. Hence, the essential oils from medicinal plants found in the South Pacific hold great potential for the antibacterial and antifungal properties.

Keywords: *Medicinal plants, Essential oils, Antibacterial, Antifungal activities*

1. Introduction

According to the World Health Organisation (WHO), more than 65% of the world population have incorporated medicinal plants for the modality of treating diseases in general healthcare (Miller *et al.*, 2015). In the Pacific Island Countries (PICs), traditional medicines are frequently used. A survey revealed that about 80% of Fijians regularly use medicinal plants (World Health Orgainization, 2001). The active compounds from these medicinal plants are antimicrobial agents that have the ability to fight bacteria and fungi (Cowan, 1999; Hintz *et al.*, 2015; Pandey and Kumar, 2013). Bacterial infections are widespread and cause much discomfort and sickness. These bacterial pathogens continue to be the threat to human health and welfare as a result of new or resistant pathogens (Phillips *et al.*, 2004; Søborg *et al.*, 2013). Likewise, most of the fungi are destructive agents that affect agricultural commodities around the globe (Palm, 2001). This is mainly due to fungi producing biologically active compounds such as mycotoxins that are particularly toxic to several plants and animals (Souza *et al.*, 2010; Wareing, 2014).

The selected bacteria and fungi in the present study are pathogenic to humans and animals, particularly in the agriculture and food processing industry. The synthetic antimicrobial agents and chemical food preservatives have been considered an effective method since ancient times for controlling pathogens. However, today natural antimicrobial agents such as essential oils have become very popular for eliminating pathogenic microorganisms (Bevilacqua, 2014; Hayek *et al.*, 2013). Essential oil is preferred over the synthetic chemicals due to consumer awareness of increasing microbial resistance (Ahmed, 2013; Dubey *et al.*, 2008; Fernández *et al.*, 2015; Lucera *et al.*, 2011; Moreira *et al.*, 2005; Raybaudi-Massilia *et al.*, 2009; Srivastava and Sharma, 2003). In the present study, the essential oils from five medicinal plants found in the South Pacific (Fiji) were investigated for antibacterial and antifungal activities.

2. Materials and Method

2.1. Extraction and Preparation of Essential Oil Solutions

The plant materials from *Cananga odorata* (Lam.) Hook F. and Thoms (Makosoi flowers), *Cymbopogon citratus* (DC.) Stapf. (Lemongrass leaves), *Murraya koenigii* (L.) Spreng. (Curry leaves), *Ocimum tenuiflorum* L. (Tulsi leaves) and *Eudioa hortensis forma hortensis* (Uci leaves) were collected from Fiji islands in April to November, 2015 (Chand *et al.*, 2016). The collected fresh plant samples were washed to remove dirt from the surface of selected samples. The selected plant materials were then blended separately in

distilled water and the resulting mixtures were hydro-distilled using Clevenger apparatus for 5-7 h. A meniscus layer (essential oils) was formed in the collecting tube which was then collected in a vial (Chand *et al.*, 2016). The samples were dried over anhydrous sodium sulphate (Na_2SO_4) stored at 4°C.

The following concentrations (0.25, 0.5, 5, 25 and 50% v/v) of essential oils were prepared using distilled water and an emulsifying agent - Tween 20 (Yang *et al.*, 2010).

2.2. Antimicrobial Activities of Essential Oils

The antimicrobial activities of different concentration of essential oils were assessed using disc diffusion method (Rajendran *et al.*, 2014). The antimicrobial activities of essential oils were evaluated against five bacteria [*Salmonella typhi* (Gram-negative), *Streptococcus pneumoniae* (Gram-positive), *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative) and *Thermus thermophiles* (Gram-negative] and five fungi [*Rhizopus stolonifer, Penicillium chrysogenum*, *Aspergillus aureus, Sodaria wild and Sodaria gray*]. The cultures were obtained from the microbiology laboratory located at the University of the South Pacific, Suva, Fiji. The bacterial culture was prepared with 20 g of nutrient agar in 1000 mL of distilled water. The solution was left in autoclave at 121°C for 15 min, after which it was stabilized in water bath at 45°C for 35 min. The nutrient broth culture (8 g/L) with selected bacteria was inoculated for 18 h at 35°C (Ewnetu *et al.*, 2014). Likewise, the Potato Dextrose Agar (PDA) (39.5 g/L solution) was used for the fungi *R. stolonifer, P. chrysogenum* and *A. aureus* culture (Breinholt *et al.*, 1996). Corn Meal Agar (CMA) (8.5 g), yeast (0.5 g), glucose (1 g) in 500 mL distilled water solution) was used to culture the fungi; *S. wild* and *S. gray*.

The fungi were first cultured in nutrient agar in petri dishes, and then bacterial cultures from the broth were streaked on the nutrient agar using sterile cotton swabs. The growth of hyphae indicated that a fungal culture was ready for streak plating using a sterile cotton swab. The prepared filter paper discs (~6 mm) were dipped in varying concentrations of essential oils and placed on the nutrient agar where the bacterial and fungal cultures were already growing. Ampicillin discs (standard control) were used for the bacteria test and Nistat discs were used for the fungi (Table S1 available as supplementary material to this paper). The petri dishes were then left in the incubator at 37°C for the bacterial culture (18-24 h) and 27°C for the fungus culture (1-2 days). After the incubation period, inhibition zones for bacteria and fungi were determined by measuring the diameter (mm) of the inhibition zones using a 15 cm ruler. The inhibition zones measured included the filter paper on which the essential oils were transferred. There were a total of 5 replicates for each bacteria and fungi with its respective concentrations (0.25%, 0.5%, 5%, 25% and 50% v/v).

2.3. Statistical Data Analysis

The software (SPSS) version 21 was used to calculate the Mean and Standard Error (SE) for both the bacteria and the fungi. To statistically evaluate the difference in the mean diameter (mm) of inhibitory zones between the same species of the bacteria and fungi using specific concentration, an ANOVA using tukey's test was performed (reported in Figures). Prior to using ANOVA, the raw data was transformed using square root (Kim *et al.*, 2000). The transformation step was considered due to the data being not normally distributed**.**

3. Results and Discussion

3.1. Physical Properties

The average yield of essential oils obtained from each plant materials is reported in the Table 1. The yield of essential oils obtained from extractions were highest for *C. citratus* (1.17%) followed by *C. odorata* (1.21%), *O. tenuiflorum* (0.68%), *E. hortensis* (0.64%) and lastly, *M. koenigii* (0.17%).

Table 1. Physical properties of selected essential oils.

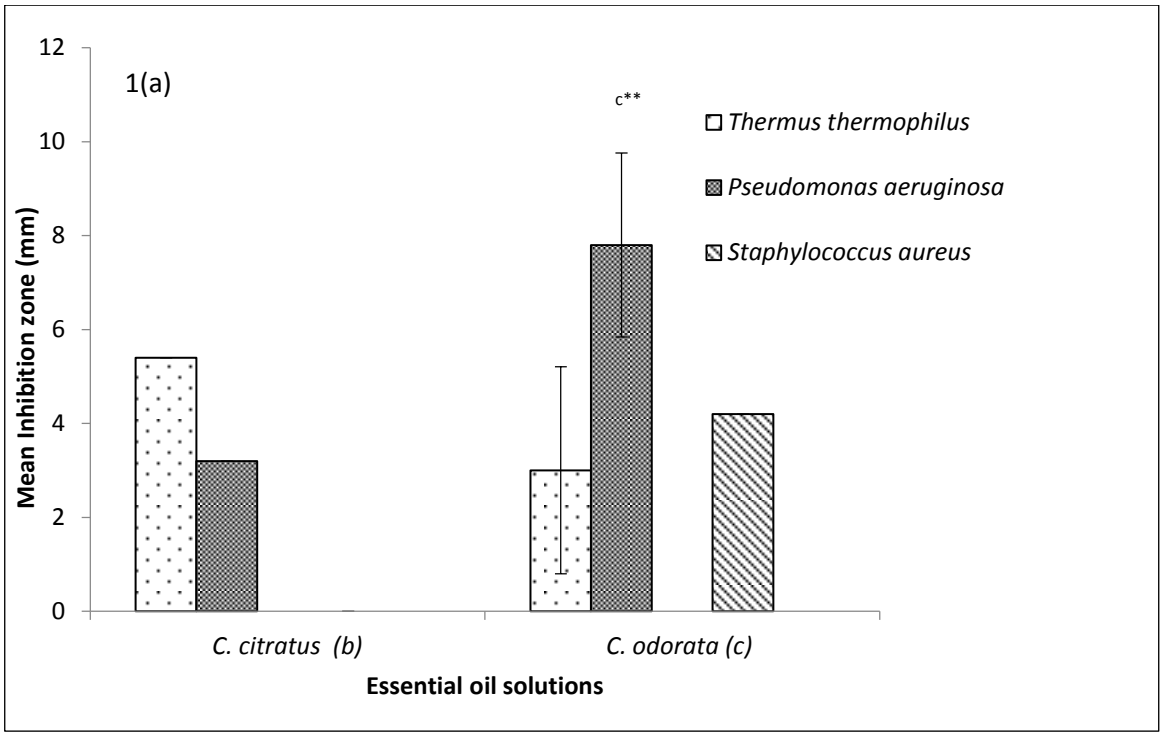
Fijian medicinal plants	Location	Plant material used	Average mass (g) taken for extraction *	Average essential oil content (mL) *	Average percentage yield (%)	Essential oil colour
Cananga odorata (Makosoi)	Suva	Flowers	215.65	2.60	1.21	light to deep yellow liquid
Cymbopogon citratus (Lemon grass)	Suva	Leaves	212.41	2.50	1.17	colourless
Murraya Koenigii (L) (Curry Leaves)	Ba	Leaves	300.42	0.50	0.17	yellowish
Ocimum tenuiflorum (L) (Tulsi)	Sigatoka	Leaves	314.35	2.15	0.68	colourless
Euodia hortensis forma hortensis (Uci)	Suva	Leaves	219.60	1.40	0.64	pale greenish to colourless

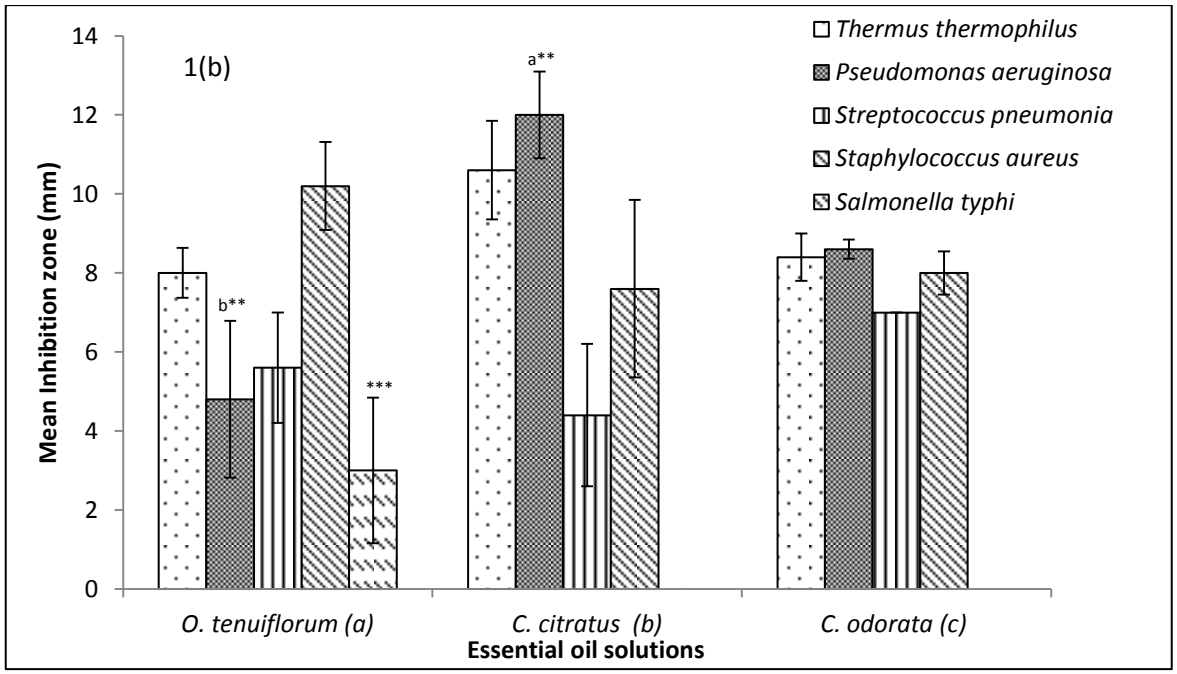
Note: * indicates an estimate of the content of essential oils extracted using a hydro-distillation apparatus for 5-7 h.

3.2. Antibacterial Activities of Selected Essential Oils

There is a clear linear correlation between the concentration of essential oils and antibacterial activity. As the concentration of essential oils increased, the diameter (mm) zone of inhibition also increased for specific bacteria (Figure 1, Table S2 available as supplementary material to this paper). Essential oils from *O. tenuiflorum* showed strong inhibition against all the tested Gram-positive and Gram-negative bacteria from 25% (v/v) concentration. Similar trends of antibacterial activities of *O. tenuiflorum* essential oils were reported in the literature where the diameter of zones of inhibition (mm) were dose dependent (Janssen *et al.*, 1989; khan *et al.*, 2015; Pandey *et al.*, 2014). The Gas chromatography – Mass spectrometry (GC-MS) analysis revealed the presence of alcohols and phenols (63%) as the major active groups (Chand *et al.*, 2016). In previous studies, alcohol and phenolic compounds were major groups to cause inhibition to Gram-positive and Gram-negative bacteria (Kalemba and Kunicka, 2003; Ng *et al.*, 2014; Puupponen-Pimiä *et al.*, 2001; Vaquero *et al.*, 2007). In this study, *S. typhi* (Gram-negative bacteria) was very resistant to all the selected essential oils except in *O. tenuiflorum*. Gram-negative bacteria have lipopolysaccharides (about 90–95% of peptidoglycan) in their outer membrane. As a result, these bacteria have the ability to tolerate components of essential oils that have antimicrobial properties (Nazzaro *et al.*, 2013; Nikaido, 2003). While Gram-positive bacteria have cell walls that easily allow hydrophobic molecules to easily pass through the cells (Navarre and Schneewind, 1999).

Interestingly, the essential oil activity of *C. odorata* showed the evident zones of inhibition at the lowest concentrations (0.25 and 0.5% v/v), which was not observed for the other tested essential oils. Essential oils from *C. odorata* showed better antibacterial activities against Gram-negative bacteria than Gram-positive bacteria in this study. However, literature has shown that the *C. odorata* essential oils are more active in Gram-positive bacteria than Gram-negative bacteria (Thompson *et al.*, 2013). Gram-negative bacteria are generally very resistant to the antibacterial properties of the essential oils. The hydrophobic components of essential oils are able to affect Gram-negative bacteria by gaining the access through the periplasm of the porin protein in the outer membrane which eventually allows essential oils to travel inside the cells of the bacterium (Helander *et al.*, 1998; O'bryan *et al.*, 2015; Plésiat and Nikaido, 1992). According to Deans and Ritchie (1987) and Deans *et al.* (1995), the inhibitory effect of essential oil is weakly correlated to whether the bacteria is Gram-positive or Gram-negative, this observation is supported by present study. This statement was further supported by Oussalah *et al.* (2007), where *Listeria monocytogenes* (Gram-positive bacteria) was reported to be slightly more resistant than other tested bacteria.
Generally, a similar trend of higher antibacterial activity with increasing concentration of the essential oil was noted in the literature for *C. citratus* (Onawunmi and Ogunlana, 1986) , *E. hortensis* and *M. koenigii* (Bisht and Negi, 2014). However, antibacterial properties of essential oils from selected medicinal plants are not only dependent on the Gram-reaction, as other factors may influence the inhibitory activity including temperature, pH, incubation period, differences in media, and different nitrogen and carbon sources which certainly needs further investigation (Noaman *et al.*, 2004).





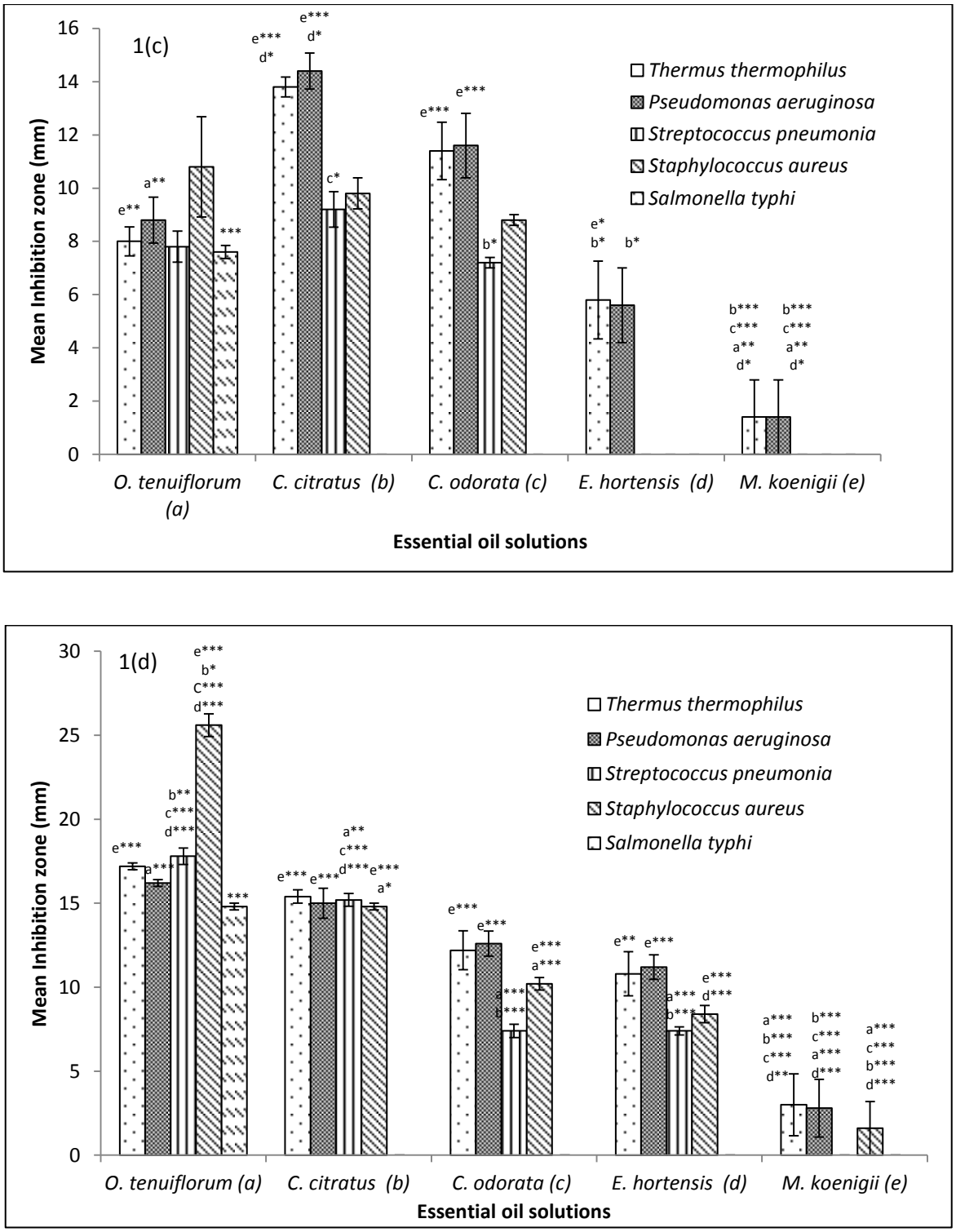


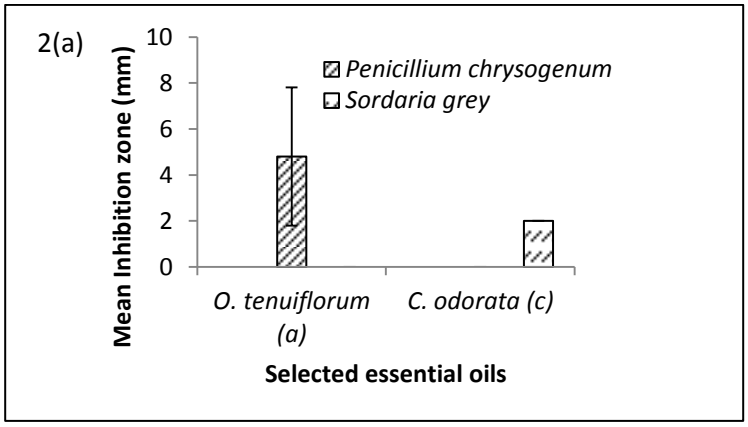
Figure 1. Antibacterial effect of essential oils at 5% (1a), 25% (1b), 50% (1c) and 100% (1d) (v/v) solutions. The alphabetical letters and the asterisks on different bars of the bacteria indicate statistical difference at 5% level of significance of mean diameter (mm) of the inhibition zones of specific bacteria at same concentration of essential oils. For example, at a concentration of 5% (v/v) of essential oils, the inhibitory activities of *P. aeruginosa* in five tested essential oils were statistically compared with each other, that is, $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) using Tukey's test.

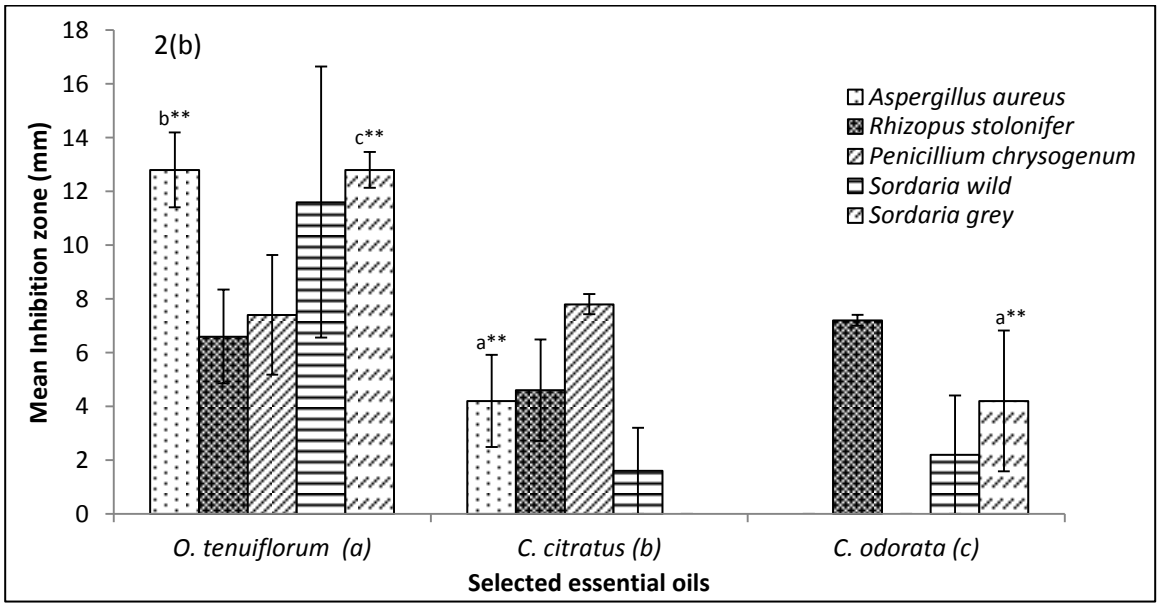
3.3. Antifungal Activities of Selected Essential Oils

The antifungal activity of *O. tenuiflorum* showed a strong inhibition activity against all the selected fungi when compared to other tested essential oils (Figure 2, Table S3 available as supplementary material to this paper). A great potential for antifungal activity was noted for *Ocimum* species in the present study, a trend that is consistent with the literature (Campaniello *et al.*, 2010; Chang *et al.*, 2008; Pandey and Kumar, 2013; Sethi *et al.*, 2013). The GC-MS of *O. tenuiflorum* revealed the presence of strong antifungal agents, such as eugenol (58.20%), linalool (0.21%) and α-cardinol (0.87%) (Chand *et al.*, 2016). *Penicillium chrysogenum* was the only fungus in the present study that was susceptible to the lowest concentration (5%) as this could possibly be due to eugenol compounds (Campaniello *et al.*, 2010).

Cymbopogon citratus essential oils also hold great potential when it comes to antifungal activity. The present study showed a dose-dependent relationship between the tested fungi and the essential oil concentrations as similarly reported in literature (Mishra *et al.*, 2015; Silva *et al.*, 2008). The GC-MS analysis showed the presence of linalool (0.21%), citronellal (45.09%) and citronellol (19.11%) as the main contributors toward the broad antifungal activities (Chand *et al.*, 2016; Lee *et al.*, 2008; Olorunnisola *et al.*, 2014; Pauli and Knobloch, 1987).

The antifungal effects of *C. odorata, E. hortensis* and *M. koenigii* resulted in zones of inhibition mostly at higher concentrations. The weak antifungal properties of these essential oils are in agreement with those reported in literature such as, *C. odorata* (Kuspradini *et al.*, 2016; Lee and Lee, 2010), *E. hortensis* (Huish *et al.*, 2014; Yazdanpanah and Mohamadi, 2014), *M. koenigii* (Bhuva and Dixit, 2015; Kumar *et al.*, 2010). The weak antifungal activity of essential oils in this study can be due to the absence of strong antifungal compounds that include linalool, eugenol and other phenolic compounds as reported in different plant extracts (Campaniello *et al.*, 2010; Tan *et al.*, 2015).





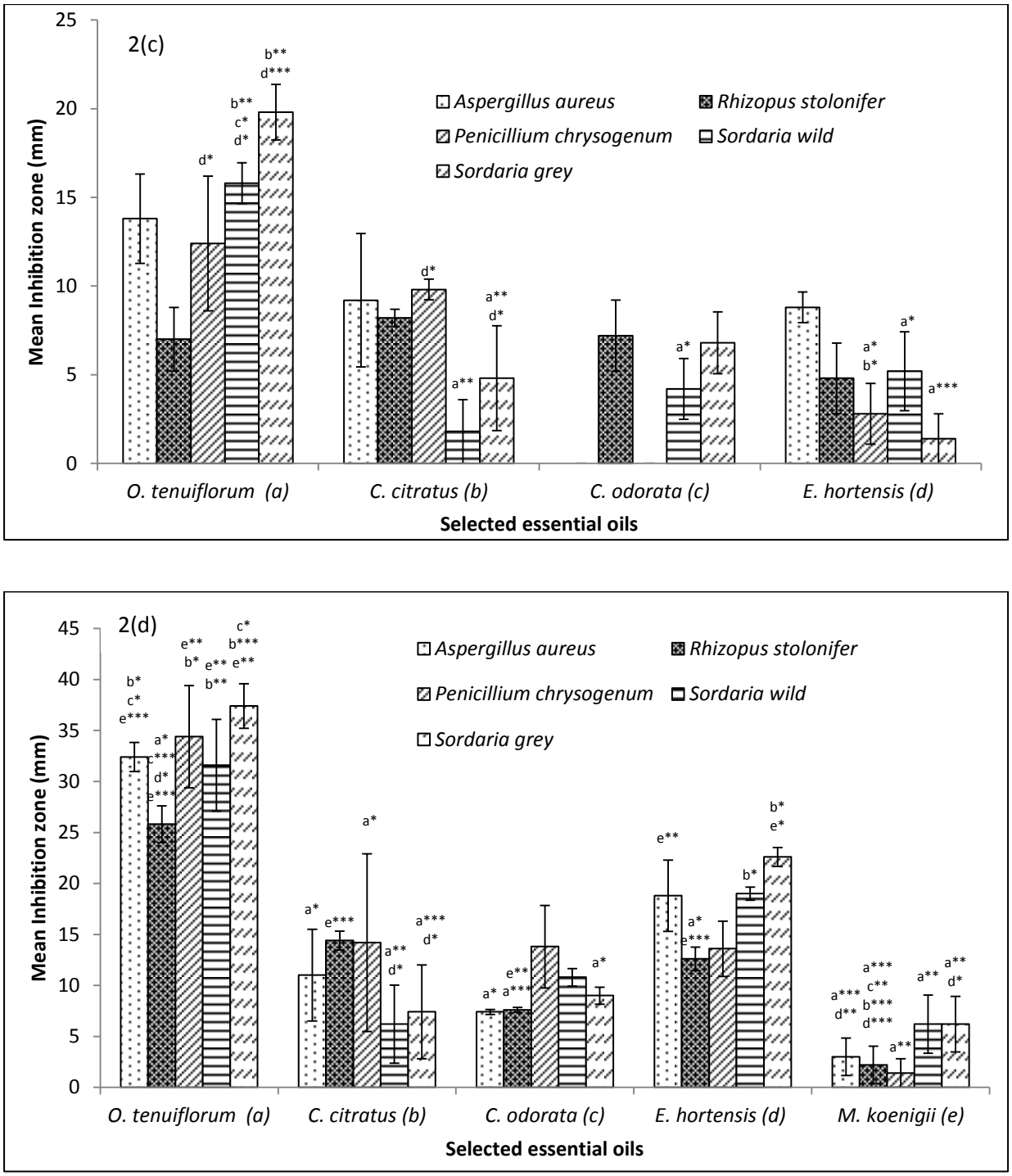


Figure 2. Antifungal effect of essential oils at 5% (2a), 25% (2b), 50% (2c) and 100% (2d) (v/v) solutions. The alphabetical letters and the asterisks on different bars of fungi indicate statistical difference at 5% level of significance of mean diameter (mm) of inhibition zones of specific fungus at same concentration of essential oils. For example, at a concentration of 25% (v/v) of essential oils, the inhibitory activities of *A. aureus* in five tested essential oils were statistically compared with each other, that is, P<0.05 (*), P<0.01 (**), P<0.001 (***) using Tukey's test.

4. Conclusion

Essential oils have great potential as antimicrobial agents. In the present study, the essential oil extracts from five medicinal plants showed inhibiting activities against all the tested bacteria (Gram-positive and Gram-negative) and fungi. *Salmonella typhi* bacteria (Gram-negative were found to be very resistant despite increasing essential oil concentration except for *O. tenuiflorum* EOs. A wide range of inhibitory activities were seen on the tested fungi mostly above 25% (v/v) concentrations. The data obtained from the present investigation indicated that the selected essential oils from medicinal plants showed effectiveness in inhibiting the growth of selected bacteria and fungi. Hence, selected essential oils (especially *O. tenuiflorum*) represent a potential alternative to eliminate microorganisms that can be harmful to human health, food and agricultural industries.

Acknowledgement

The authors are thankful to Mr Karuna Reddy (Research Data Analyst) and Mrs Reema Prakash for their support and guidance provided throughout the research journey especially with the analysis of data. The authors are also thankful to the University of the South Pacific, Fiji islands for supporting the main author with a Graduate Assistant scholarship and research funding.

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Supplementary electronic material available.