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Antimicrobial Effect of Essential Oils of Some Fijian Medicinal Plant Leaves On Pathogenic Bacteria

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Abstract

Fiji is highly populated with plants containing essential oils (EO). The essential oils extracted from the leaves of the selected Fijian leafy plants were screened against two Gram-negative bacteria (Salmonella typhimurium, Pseudomonas aeruginosa) and three Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis and Bacillus subtilis). The agar diffusion method was used to examine the antimicrobial activities of the extracted EO. All the EO tested showed antibacterial properties against one or more strains while none of the EO was active against Pseudomonas aeruginosa. Viburnum lantana (Wayfaring tree), Annona muricata (Soursop), Coleus amboinicus (Spanish thyme) and Cinnamomum zeylancium (Cinnamon) showed good inhibition against both Gram-positive and Gram-negative bacteria and proved as worthy source of antimicrobial agent. These findings will help the Pacific population to use the studied plants leaves as antimicrobial agent.
Keywords: *Essential oils; Fiji medicinal plants; Leafy plants; Antimicrobial activity*

1. Introduction

The antimicrobial activity of different essential oils (EO) is known for many centuries around the globe. The extracts of EO from many plant species have become widespread in recent years and attempts to characterize their bioactive principles have lately gained thrust in many foods processing and pharmaceutical application (Reynolds, 1998; Sokmen *et al.,* 1999). The EO has been highly marketable in the cosmetic industries. They have also been used as alternative remedy for the treatment of many infectious diseases, pharmaceutical alternative (medicine) and natural therapies since plant origin herbal medicines are considered as safe alternatives to synthetic drugs (Reynolds, 1998). However, according to Bent (2008), natural therapies can be used for patients with conditions where there is no known effective treatment but this should be closely monitored by a clinical provider.

The necessities for the search of new antibacterial agents have become quite desirable due to their resistivity of antibiotics on certain microorganisms (Bhattacharjee *et al.,* 2005). The EO as compared to antibiotics has been proved to be highly potent antimicrobial agent (Yap *et al.,* 2014). Continued researches have demonstrated different applications of EO against a wide range of bacteria (Gram-negative and Gram-positive) including antibiotic resistant species, fungal species and yeast (Hammer *et al.,* 1998, 1999; Jimanez-Arellanes *et al.,* 2003; Nelson, 1997; Yap *et al*., 2014). There are many plants in the South Pacific which have a great potential for antimicrobial activities and can be studied for research purposes. Thus, the present study reports antimicrobial potential of EO, extracted from the leaves of nine plants, investigated against five pathogenic bacterial strains.

2. Materials and Method

2.1. Plant Material Isolation of the Essential Oil

Nine leafy plants which are available throughout the year in Fiji were selected for the study. The common as well as scientific names of these plants are presented in Table 1. The plants were collected from the central parts of Viti Levu, the main island in Fiji. The fresh 100 g leaves in 200 mL of water were subjected to 4 h distillation using a Clevenger-type apparatus. The EO obtained was dried over anhydrous sodium sulphate (Na_2SO_4) filtered and stored at -20 °C until analysed (Moghaddam *et al.***,** 2011).

2.2. Antimicrobial Activity

The evaluation of antibacterial activities was achieved with the agar diffusion method and growth of inhibition zone diameters were determined (Bădiceanu and Larion, 2009; Kalemba and Kunicka, 2003). The antimicrobial activities of extracted EO were evaluated against two Gram-negative bacteria [*Salmonella typhimurium*, (NCTC 9394), *Pseudomonas aeruginosa* (NZRM 918)] and three Gram-positive bacteria namely; *Staphylococcus aureus* (NZRM 917), *Enterococcus*

faecalis (NZRM 1106) and *Bacillus subtilis* (NZRM 143). The cultures were obtained from the culture collections of the School of Biological and Chemical Sciences, the University of the South Pacific, Suva, Fiji. The agar and broth were purchased from Mercks KGaA, 64271 Darmstadt, Germany as used as such. A loop-full of each of the microorganisms were suspended into 10 mL of the nutrient agar broth (APHA-105443). Bacterial strains were then cultured overnight at 37 °C for 18 h. The agar plates were prepared using nutrient agar from powdered formula (105450).

The freshly prepared inoculum was swabbed over the surface of the nutrient agar plate using sterile cotton swab and the plants extracts were tested using well diffusion method (Bauer *et al*., 1996; Joshi *et al.*, 2009; Vadlapudi and Naidu, 2009). Three wells of 6 mm diameter each were bored in the medium with the aid of a sterile cork-borer (Bauer *et al*., 1996). The plates were labelled properly. The EO (neat sample) extracted from the nine different plant leaves were dispensed (200 µL) into the wells with the help of a micropipette. The plates were left for 2 h till the extract diffused in the media with the lid closed after which they were incubated at 37 °C for 24 h. At the end of the incubation period, inhibition zones formed on the media were evaluated in mm. To have quality data, all the experiments were carried out in triplicates.

Table 1. List of leafy plants with their scientific names and their medicinal properties from which essential oils extracted.

Common name	Botanical name	Medicinal properties
Wayfaring tree	*Viburnum lantana*	Used in the treatment of antispasmodic, diuretic, sedative properties and uterine excitability.[a]
Fiji Christmas bush	*Decasperum vitiense*	Used in cancer treatment of the womb, wounds (knife, spear, axe), and loss of appetite in children.[b]
Uci	*Euodia hortensis*	Leaves are used as a laxative, to ease fevers, for treatment of swellings and to treat headaches.[b]
Cinimoni/ Bay rum	*Pimenta racemosa*	Used in cosmetics perfumes, aftershaves, lotions, enhancing hair growth and strength and commercial food flavoring.[c]
Goat weed	*Ageratum conyzoide.L*	Leaves are directly applied to aid healing of wounds.[b]
Tulsi	*Ocimum sanctum*	Used to treat cough, chest pains and painful breathing.[b]
Soursop/Custard apple	*Annona muricata*	An infusion of the leaves is used for treatment of stomach ailments.[b]
Spanish thyme	*Coleus amboinicus*	Used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsions and epilepsy.[d]
Cinnamon	*Cinnamomum zeylancium*	Applied in the biocontrol of spoiled and pathogenic bacteria in foodstuffs.[e]

[a]Cometa *et al.*, 1998; [b]Sotheeswaran *et al.*, 1998; [c]Jirovetz *et al.*, 2007; [d]Hullatti and Bhattacharjee, 2011; [e]Trajano *et al.*, 2010.

3. Results and Discussion

To progress towards excellence in healthcare, especially in developing Pacific Island countries, it is essential to investigate those plants which have continuously been used as traditional medicines (Muhammad and Awaisu, 2008). Essential oils are one of the potential sources of novel antimicrobial compounds especially against bacterial pathogens. The antibacterial properties of the nine extracted EO were studied within 24 h of their extraction. The results presented in Table 2 suggest that the EO have good antibacterial activities. *In vitro* studies clearly showed that the studied EO inhibited pathogenic growth but their effectiveness varied (Table 2). The antimicrobial activities of many EO have been reviewed and classified by previous researchers as strong, medium and weak or resistant, intermediate and sensitive (Parveen *et al*., 2013; Zaika, 1988). Zaika (1988) also proposed that Gram-positive bacteria are more resistant than Gram-negative bacteria to the antibacterial properties of plant volatile oils which is in contrast to the hypothesis proposed by other researchers where the susceptibility of bacteria to plant volatile oils appears to have little influence on growth inhibition (Deans and Ritchie, 1987; Deans *et al.*, 1995).

The results presented in Table 2 clearly demonstrate that *Decasperum vitiense* (Fiji Christmas bush), *Euodia hortensis* (Uci), and *Cinnamomum zeylancium* (Cinnamon) showed good inhibition against Gram-positive bacteria but was ineffective towards two Gram-negative bacteria: *Salmonella typhimurium* and *Pseudomonas aeruginosa.* Conversely, the antibacterial activites of the extracted EO of *Cinnamomum zeylancium* (Cinnamon), *Viburnum lantana* (Wayfaring tree), *Annona muricata* (Soursop) and *Coleus amboinicus* (Spanish thyme) were highly significant towards both Gram-positive bacteria as well as one of the Gram-negative; *Salmonella typhimurium*. All extracted EO showed antibacterial activity against one or more strains while none of the EO analysed were active against the Gram-negative bacteria: *Pseudomonas aeruginosa*. This could be attributed to the differences in the cell wall structure between the two types of bacteria, since the outer membrane of certain Gram-negative bacteria acts as an obstruction to various environmental materials including antibiotics (Burt, 2004).

Table 2. Observation of antimicrobial property of the different medicinal leafy plants extracted essential oils against different pathogenic microorganisms.

Plant species	*Bacillus subtilis* (Gram-positive)	*Enterococcus faecalis* (Gram-positive)	*Staphlococcus aureus* (Gram-positive)	*Salmonella typhimurium* (Gram-negative)	*Pseudomonas aeruginosa* (Gram-negative)
Viburnum lantana	+++	+++	+++	+++	+
Decasperum vitiense	+++	+++	+++	+	+
Euodia hortensis	+++	+++	+++	+	+
Pimenta racemosa	++	++	++	++	+
Ageratum conyzoide	++	++	++	++	+
Ocimum sanctum	++	++	++	+	+
Annona muricata	+++	++	+++	+++	+
Coleus amboinicus	+++	+++	+++	+++	+
Cinnamomum zeylancium	+++	+++	+++	+++	+

+: Resistant, ++: Intermediate, +++: Sensitive (Krempels, 2008)

As observed, the strength of the antibacterial activity of the studied EO varies between the various plants. This could be due to the hydrophobicity of the plant extracts which is a significant characteristic of plant extracts and their components that permits them to partition the lipids of the bacterial cell membrane and mitochondria (Rastogi and Mehrotra, 2002). The results of the present study exhibited that traditional medicinal plant leaves possess compounds with antimicrobial properties that can be further explored. The findings validate the use of the studied plants for antibacterial activity. Thus, use of such natural resources should be emphasised among the local communities incorporating the traditional knowledge with the scientific findings. The results demonstrated that traditional medicinal leafy plants' extracts has potential to be used as an effective treatment to fight pathogenic microorganisms and treat infectious diseases.

4. Conclusion

The EO extracted from of the nine different plant leaves studied showed sensitivity against all the Gram-positive bacteria. Six of the EO were sensitive against both Gram-positive and Gram-negative bacteria while none were sensitive against Gram-negative bacteria; *Pseudomonas aeruginosa*. The antibacterial activities of EO of the plants; such as *Cinnamomum zeylancium* (Cinnamon), *Viburnum lantana* (Wayfaring tree) and *Coleus amboinicus* (Spanish thyme) were highly significant. The oils of these plants can thus be utilized in supplementation to modern medicine and hence their proper conservation and sustainable use needs to be promoted.

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