Malagasy Aromatic Plants: Essential Oils, Antioxidant and Antimicrobial Activities

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Keywords: Antibacterial, antifungal, eugenol, medicinal plants, monoterpenes, natural products

Abstract

This work examined the chemical profile of nine volatile oils from Madagascar and evaluated their antimicrobial and antioxidant activity. Essential oils with the highest levels of eugenol exhibited the highest antioxidant activity. Clove bud (*Syzygium aromaticum*) oil appeared the most promising for potential applications as a food preservative because showed the highest antioxidant and antimicrobial against bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*) and fungi (*Aspergillus niger, Saccharomyces cerevisiae*). Cinnamon leaf (*Cinnamomum zeylanicum*) oils with a very high antioxidant activities were also effective though less than the clove bud oil against the growth of the microorganisms tested. Cinnamon bark (*C. zeylanicum*) oil exhibited a low antioxidant activity yet had a high antimicrobial activity against the five tested microorganisms. Linalool basil oil (*Ocimum basilicum*) showed an intermediate antioxidant activity and was most active against *A. niger* and *E. coli*. *P. nigrum* essential oil was most active against fungi (*A. niger, S. cerevisiae*), while *Tagetes minuta* was most active against *S. aureus*. The bark essential oil of *Ravensara aromatica* showed low antioxidant and antimicrobial activity.

INTRODUCTION

As part of the international development program Agribusiness in Sustainable Natural African Plant Products (ASNAPP, *www.asnapp.org*) we are seeking to identify new uses and applications of African natural products including essential oils and botanicals in order to both enhance their industrial and commercial uses and to ascertain potentially new human health applications for these products.

Essential oils from aromatic plants have been known since antiquity to possess biological activity, mainly antimicrobial, antifungal and antioxidant properties (Barata, et al., 1998; Griffin, et al., 1999; Zygadlo and Juliani, et al., 2000). With growing interest in the use of essential oils in both the food and the pharmaceutical industries research has shifted into examining new applications for essential oils (Juliani and Simon, 2002). Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases (Scalbert and Williamson, 2000). The essential oils are complex mixtures of different components, some of which are rich in phenolic constituents (Williams, 1997).

This current work sought to analyze the chemical profile of commercially derived essential oils from selected Malagasy plants and to evaluate their antimicrobial and antioxidant activity.

MATERIAL AND METHODS

The oils were obtained from Biosave Madagascar SARL (BS, *Ocimum basilicum, Tagetes minuta, Pelargonium graveolans, Piper nigrum, Ravensara aromatica*, bark), EPAM Tombohobe Sud (EPAM, *Cinnamomum zeylanicum* leaf oil), Phaels Flor (PF, *C. zeylanicum* leaf and bark oils) and Parapharma (PP, *Syzygium aromaticum*).

The diluted essential oils (2-500 μl oil / 1 ml of dimethyl sulfoxide) were screened against fungi (*Aspergillus niger, Saccharomyces cerevisiae*), bacteria (*Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*) and the standard method used to determine in vitro antibacterial and antifungal activity of essential oils consisted of utilizing suspensions of microorganisms and monitoring for growth inhibition. The results were expressed as the minimal concentration of essential oil (μl essential oil/ml) that caused visual inhibition of growth. The same essential oils were screened for in vitro antioxidant activity using the ABTS screen. The antioxidant activity was related to Trolox (a water soluble analogue of Vitamin E) and expressed as mg of Trolox per ml of oil (TEAC) (Re et al., 1999).

The volatile oils were analyzed by a gas chromatograph (GC) coupled to a mass spectrometer (MS) and Flame ionization detectors (FID) (Agilent GC System 6890 Series, Mass Selective Detector, Agilent 5973 Network, FID detector). Samples were injected with an autosampler (Agilent 7683 Series), the Inlet temperature was 220 °C., in a HP5- MS (30 m, 0.25 ID, 0.25 μm) column, temperature program, 60 °C 1 min, 4 °C/min, 200 °C 15 min. Helium constant flow was set at 1 ml/min. Individual identifications were made by matching their spectra with those from mass spectral libraries (Wiley275.L). Components were listed in order of elution in HP5 (DB5 equivalent) column.

RESULTS AND DISCUSSION

Essential oils with the highest levels of eugenol (Table 1) exhibited also the highest antioxidant activity (Table 2). Accordingly, the highest antioxidant activity was found in *Syzygium aromaticum* (clove bud) oil (eugenol 75 %, TEAC 600), follow by the *C. zeylanicum* (cinnamon) leaf oils (eugenol 59-64 %, TEAC 560-580). A lower activity was found in *O. basilicum* oil (linalool basil) (linalool 60 %, eugenol 9 %, TEAC 192), while the *C. zeylanicum* bark was even lower (traces of eugenol, cinnamaldehyde 38 %, cinnamylacetate 14 %, TEAC 22.9). The lowest antioxidant activity was observed in *R. aromatica*, richest in methychavicol (1.7 TEAC) (Table 1, 2). All essential oils exhibited a relatively high antifungal activity (Table 2), with most oils being effective inhibiting *A. niger* more than *S. cerevisiae*. Cinnamon bark and leaf, and *P. nigrum* oils were the most active against *A. niger* and *S. cerevisiae*. Other authors, observed that cinnamaldehyde, the main component of cinnamon bark, inhibited the growth of bacteria and yeast (Barata, et al., 1998; Zygadlo and Juliani, 2000).

The essential oils showed a large variation in the antibacterial activity (Table 2). The most active oils against *E. coli* were geranium (*P. graveolans*) and basil (*O. basilicum*). The eugenol containing oils (cinnamon leaf and clove bud) inhibited the growth of *S. aureus*, as did the tagetes (*T. minuta*) oil. In the eugenol-rich containing essential oils, there are a close relationship between eugenol content and antimicrobial activity against *P. aeruginosa*, with clove bud oil the most active. Eugenol has been shown to be more active against microorganisms than methyleugenol and methylchavicol (Griffin, et al, 1999; Zygadlo and Juliani, 2000). This is in accordance with the relative inactivity of *R. aromatica*, composed mainly of methylchavicol.

In conclusion, there was a close relationship between antioxidant activity and eugenol levels. Of all the organisms tested, only in *P. aeruginosa* appeared to be an association between antioxidant and antimicrobial activity. Within this range of nine essential oils, the clove bud oil appeared the most promising for potential applications as a food preservative because showed the highest antioxidant and antibacterial activities. Cinnamon leaf oils with very high antioxidant activities were also effective, though less than the clove bud oil, against the growth of the microorganisms tested. The cinnamon bark although with a very low antioxidant activity showed a high antimicrobial activity against the tested microorganisms. Further studies are needed to identify the minimum concentration required to achieve this inhibitory action and then to evaluate the flavor/odor impact of those oils at that minimum level of concentration needed.

While many African essential oils are now available on regional and world markets, studies that can identify new applications and uses of both traditional and exotic oils can ultimately assist growers and rural communities by increasing interest in their products. The food industry is actively seeking natural preservatives and other agents that can replace the synthetic compounds now used in fresh and processed food. Our results show that some essential oils from Madagascar have a potential role to serve as natural antioxidants and antimicrobials.

ACKNOWLEDGEMENTS

We wish to express our appreciation to US-AID for their funding of Agri-Business in African Sustainable Natural African Plant Products (ASNAPP, www.asnapp.org), Chemonics International, LDI; and to Biosave Madagascar SARL, Societe Phael-Flor, EPAM Tombohobe Sud, Parapharma, and C.B.D, who graciously provided samples of their essential oils to us to examine for quality and oil composition. We also acknowledge the New Jersey Agricultural Experiment Station for their support and the financial support from "Consejo Nacional de Investigaciones Científicas y Tecnológicas" of Argentina for their partially support of HR Juliani.

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Tables

Table 1. Chemical constituents (% of total oil) of selected Malagasy essential oils (*Cinnamomum zeylanicum, Ocimum basilicum, Pelargonium graveolens, Piper nigrum, Ravensara aromatica, Syzygium aromaticum, Tagetes minuta*).

Components	*C. zeylanicum* leaf (E)[1]	*C. zeylanicum* bark(F)	*C. zeylanicum* leaf (F)	*O. basilicum* (B)	*P. graveolens* (B)	*P. nigrum* (B)	*R. aromotica* bark (B)	*S. aromaticum* (P)	*T. minuta* (B)
α-Pinene					-	20.4	-		-2
Sabinene	0.03	0.4	-	0.3	-	-	0.1	0	-
β-Pinene	0.4	1.3	0.4	0.8	-	14.1	0.2	0	-
δ-3-Carene	-	-	-	-	-	7.9	-	-	-
Limonene	-	-	-	6.8	-	20.3	1.5	0	7.4
β-Phellandrene	1	9	1	-2	-	-	-	-	-
1,8-Cineole	-	-	-	0.1	-	-	-	0	-
Ocimene	0.1	0.3	-	0.5	-	-	-	-	-
Dihydrotagetone	-	-	-	-	-	-	-	-	11.6
Linalool	1	3.3	0.8	59.3	3.8	-	2.6	-	-
Tagetone	-	-	-	-	-	-	-	-	14.1
Menthone	-	-	-	-	5.1	-	-	-	-
Methylchavicol	-	-	-	0.1	-	-	81.4	-	-
Citronellol	-	-	-	-	16.9	-	-	-	-
Z-Ocimenone	-	-	-	-	-	-	-	-	6.7
E-Ocimenone	-	-	-	-	-	-	-	-	9
Geraniol	-	-	-	-	9.6	-	-	-	-
(E)-Cinnamaldehyde	2.7	37.8	2.3	-	-	-	-	-	-
Citronellyl formate	-	-	-	-	7.9	-	-	-	-
Safrole	10.7	-	2.2	-	-	-	-	-	-
Geraniol formate	-	-	-	-	3.9	-	-	-	-
Eugenol	58.6	2.6	64	8.4	-	-	-	74.7	-
(E)-Caryophyllene	1.7	5.6	2.3	0.1	1.9	13.2	0	7.5	1.1
(E)-Cinnamylacetate	3.3	13.7	7.1	-	-	-	-	-	-
3,7-Guaiadiene	-	-	-	-	10.3	-	-	-	-
Geranyl N Propanoate	-	-	-	-	1.8	-	-	-	-
Germacrene-D	-	-	-	2.4	2.3	0.2	-	-	-
β-Selinene	-	-	-	-	-	2.7	-	-	-
Bicyclogermacrene	-	-	-	-	-	-	-	-	2.2
Caryophyllene oxide	-	-	-	-	-	4.7	-	-	-
Eugenyl acetate	8	-	10.5	-	-	-	-	15.8	-
Geranyl tiglate	-	-	-	-	3.3	-	-	-	-
Benzylbenzoate	4.8	0.6	1.7	-	-	-	-	0	-

[1] F: Phaels flor, E: EPAM Tombohobe Sud, P:Parapharma, B: Biosave Madagascar.

[2] -, not detected

[3.] 0=less than 0.1 %.

Table 2. Antimicrobial and antioxidant activities of Malagasy essential oils.

Species	Fungi		Bacteria			Antioxidant activity (TEAC)[2]
	S. cerevisiae	*A. niger*	*E. coli*	*S. aureus*	*P. aeru-ginosa*	
C. zeylanicum bark (F)[4]	<2	8[1]	32	8	8	22.9
C. zeylanicum leaf (F)	32	<2	32	<2	32	578.4
C. zeylanicum leaf (E)	8	<2	32	8	32	557.1
S. aromaticum (PP)	32	32	32	<2	<2	601.1
O. basilicum Linalool (B)	32	<2	8	32	500	192.3
P. graveolans (B)	32	8	<2	32	500	NT[3]
P. nigrum (B)	<2	<2	32	32	32	NT
R. aromatica bark (B)	32	32	125	32	>500	1.7
T. minuta (B)	32	32	>500	<2	125	NT

1 Minimal concentration (μl essential oil/ml) that caused visual inhibition of growth.
2 The antioxidant activity was related to Trolox (a water soluble analogue of Vitamin E) and expressed as mg of Trolox per ml of oil (TEAC).
3 NT=Not Tested
4 F: Phaels flor, E: EPAM Tombohobe Sud, P:Parapharma, B: Biosave Madagascar.