PHYTOTHERAPY RESEARCH
Phytother. Res. **21**, 501–506 (2007)
Published online 23 March 2007 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2124

REVIEW ARTICLE

The Chemical Composition and Biological Activity of Clove Essential Oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): A Short Review

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The essential oil extracted from the dried flower buds of clove, *Eugenia caryophyllata* L. Merr. & Perry (Myrtaceae), is used as a topical application to relieve pain and to promote healing and also finds use in the fragrance and flavouring industries. The main constituents of the essential oil are phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde. The biological activity of *Eugenia caryophyllata* has been investigated on several microorganisms and parasites, including pathogenic bacteria, *Herpes simplex* and hepatitis C viruses. In addition to its antimicrobial, antioxidant, antifungal and antiviral activity, clove essential oil possesses antiinflammatory, cytotoxic, insect repellent and anaesthetic properties. This short review addresses the chemical composition and biological effects of clove essential oil, and includes new results from GC/MS analysis and a study of its antimicrobial activity against a large number of multi-resistant *Staphylococcus epidermidis* isolated from dialysis biomaterials. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: Eugenia caryophyllata; clove essential oil; GC/MS; antimicrobial activity; *Staphylococcus epidermidis*; biological effect.

INTRODUCTION

The essential oil isolated from the buds of *Eugenia caryophyllata* L. Merr. & Perry (Myrtaceae) is widely used and well known for its medicinal properties. Traditional uses of clove oil include use in dental care, as an antiseptic and analgesic, where the undiluted oil may be rubbed on the gums to treat toothache. It is active against oral bacteria associated with dental caries and periodontal disease (Cai and Wu, 1996) and effective against a large number of other bacteria: *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica* (Friedman *et al.*, 2002), *Campylobacter jejuni*, *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus* (Beuchat, 2000; Cressy *et al.*, 2003, Kalemba and Kunicka, 2003). Previous studies have reported antifungal (Chami *et al.*, 2005), anticarcinogenic (Zheng *et al.*, 1992), antiallergic (Kim *et al.*, 1998) and antimutagenic activity (Miyazawa and Hisama, 2001). Eugenol, the primary component of clove oil displays antioxidant (Ogata *et al.*, 2000) and insecticidal (Park *et al.*, 2000) properties.

This is a review of the medicinal use of clove oil, and in order to further clarify the spectrum of antimicrobial activity and its relation to chemical composition, its constitution was examined using GC/MS analysis. This characterized oil was subjected to antimicrobial testing against a large number of multi-resistant *Staphylococcus epidermidis* isolated from dialysis biomaterials. Coagulase-negative Staphylococci (CoNS) are a part of the normal skin microflora and are the most frequently isolated bacteria in hospitals (Agvald-Ohman *et al.*, 2003). These species have the ability to survive a long time on medical devices (Neely and Maley, 2000). Although CoNS do possess some intrinsic pathogenic properties, they are usually regarded as opportunistic agents (Huebner and Goldmann, 1999). The poor health status of dialysed patients renders them particularly vulnerable.

CHEMICAL COMPOSITION OF CLOVE ESSENTIAL OIL

The major component of clove oil is usually considered to be eugenol, with β-caryophyllene and lesser amounts of other components such as benzyl alcohol, but the proportions vary widely. For example, Prashar *et al.* (2006) found the content of eugenol to be 78%, with 13% β-caryophyllene, whereas Pawar and Thaker (2006) found that the content of eugenol was 47.64%, with the concentration of benzyl alcohol at 34.10%. We have characterized the composition of a sample of clove

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Received 17 November 2006
Revised 12 December 2006
Accepted 24 January 2007

Table 1. Composition and percentage of clove essential oil (*Eugenia caryophyllata*) obtained with GS-MS analysis

N°	Compound[a,b]	Kovats index[c] (HP-20M)	Percentage (%)
1	2-Heptanone	1172	0.93232
2	Ethyl hexanoate	1232	0.66098
3	2-Heptanol	1304	tr
4	Menthyl octanoate	1384	tr
5	2-Nonanone	1392	tr
6	Ethyl octanoate	1429	tr
7	α-Cubebene	1459	tr
8	Copaene	1491	tr
9	2-Nonanol	1499	tr
10	Linalool	1548	tr
11	2-Undecanone	1588	tr
12	β-Caryophyllene	1595	1.38830
13	Menthyl benzoate	1619	tr
14	Ethyl benzoate	1647	tr
15	α-Humulene	1668	0.19985
16	Menthyl chavicol	1669	tr
17	α-Amorphene	1675	tr
18	α-Terpinyl acetate	1695	tr
19	α-Muurolene	1711	tr
20	Benzyl acetate	1714	tr
21	Carvone	1731	tr
22	γ-Cadinene	1756	tr
23	2-Phenyiethyl acetate	1826	tr
24	(E)-Anethole	1827	tr
25	Calamenene	1828	0.10538
26	Benzyl alcohol	1861	tr
27	Calacorene	1918	0.11437
28	Caryophyllene oxide	1976	tr
29	Menthyl eugenol	1985	tr
30	Humulene oxide	1986	tr
31	Cinnamic aldehyde	2018	tr
32	Ethyl cinnamate	2072	tr
33	Benzyl tiglate	2103	tr
34	Eugenol	**2151**	**88.58535**
35	Eugenyl acetate	**2263**	**5.62086**
36	Humulenol	2265	0.27527
Total identified		98.2769	98.2769

[a] Order of elution on HP-20M capillary.
[b] Identified by comparison of the mass spectral and Kovats index data.
[c] Kovats indices on HP-20M column, tr: trace (<0.1%).

essential oil isolated by hydro-distillation using GC-MS analysis. As shown in Table 1, the chemical analysis resulted in the identification of 36 components, with a high concentration of eugenol (88.58%), eugenyl acetate (5.62%), β-caryophyllene (1.39%), 2-heptanone (0.93%), ethyl hexanoate (0.66%), humulenol (0.27%), α-humulene (0.19%), calacorene (0.11%) and calamenene (0.10%). These data are in agreement with the results from other studies whilst still demonstrating the variability of the natural oil (Prashar *et al.*, 2006; Pawar and Thaker, 2006; Lee and Shibamoto, 2002).

IN VITRO STUDIES

Antibacterial activity of clove essential oil

The antibacterial activity of different *E. caryophyllata* extracts has been demonstrated against pathogenic bacteria including *Campylobacter jejuni*, *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus* (Burt and Reinders, 2003; Feres *et al.*, 2005; Larhsini *et al.*, 2001; Cressy *et al.*, 2003; Friedman *et al.*, 2002). A recent study reported that the growth rates of *Listeria monocytogenes* strains observed at 15 °C and at 5 °C were significantly reduced by treatment with 1% and 2% clove oil (Mytle *et al.*, 2006) and furthermore, Ogunwande *et al.* (2005) found that the essential oil of the fruit exhibited strong antibacterial activity against *Staphylococcus aureus*, while the leaf oil strongly inhibited the growth of *Bacillus cereus*, with an MIC of 39 μg/mL.

In view of the fact that coagulase-negative Staphylococci (CoNS) represent a hazard to hospital patients with poor immune responses and have the ability to survive a long time on medical devices (Neely and Maley, 2000), it was decided to investigate the activity on a large number of multi-resistant *Staphylococcus epidermidis*. Characterized clove oil was therefore screened against 32 multi-resistant *Staphylococcus epidermidis* strains (Table 2) isolated from dialysis biomaterial using an agar disk diffusion assay as described previously (Perez *et al.*, 1999; Bagamboula *et al.*, 2004). Each bacterial strain demonstrated a significant degree of sensitivity to the clove essential oil, and extensive activity against

DOI: 10.1002/ptr

Table 2. Antibacterial activity of essential oil of clove bunds against 32 *S. epidermidis* strains and eight human pathogenic bacteria

Bacterial strains	Oxa[a]	Number of resistance	Diameter of inhibition (mm)	SD[b]	Antibacterial activity[c]	Gent[d]
S. epidermidis S15	R	10	13.33	0.57	(++)	17
S9	S	5	13.33	0.57	(++)	29
E5	S	1	13.66	0.57	(++)	30
S43	S	4	12.33	0.57	(++)	28
S16	S	3	12	0	(++)	31
S26	R	13	11	0	(++)	20
S40	R	8	11.66	0.57	(++)	23
E21	S	2	12	0	(++)	25
S48	R	8	12.66	0.57	(++)	22
E24	S	4	12	0	(++)	24
S22	S	4	12.66	0.57	(++)	26
E15	R	12	12.33	0.57	(++)	22
E11	R	6	11.33	0.57	(++)	23
E7	S	8	11.66	0.57	(++)	28
S33	S	5	13.66	0.57	(++)	33
E6	S	3	11.66	0.57	(++)	25
E4	R	9	12.66	0.57	(++)	27
S35	S	1	11.33	0.57	(++)	21
S2	S	3	10.33	0.57	(+)	25
E18	S	5	11.66	0.57	(++)	30
S59	S	4	14.66	0.57	(++)	24
S56	R	2	10	0	(+)	23
E9	S	6	13	1	(++)	23
S21	R	4	13.33	0.57	(++)	29
E10	S	1	14	1	(++)	29
E13	R	8	15.66	0.57	(+++)	31
E20	R	6	15	1	(++)	30
S25	S	5	13.33	0.57	(++)	31
S27	R	14	16.66	0.57	(+++)	33
S23	S	6	19.66	0.57	(+++)	36
S38	S	2	15.33	0.57	(+++)	34
S12	S	8	14.33	0.57	(++)	29
S. epidermidis CIP106510	[e]ND	ND	16.33	0.57	(+++)	22
S. aureus ATCC25923	ND	ND	14.66	1.15	(++)	33
E. coli ATCC 35218	ND	ND	13.66	0.57	(++)	26
L. monocytogenes ATCC19115	ND	ND	15	0	(++)	38
E. faecalis ATCC29212	ND	ND	11	0	(++)	26
P. aeruginosa ATCC 27853	ND	ND	9	0	(+)	30
Micrococus luteus NCIMB 8166	ND	ND	12.33	0.57	(++)	26
Salmonella typhimurium LT2	ND	ND	15.66	0.57	(++)	20

[a] Oxacilline; [b] SD, standard deviation; [c] (–) <8 mm; (+) 8–10 mm; (++) 11–15 mm; (+++) >16 mm; [d] Gent, gentamicin (500 μg); [e] ND, not determined.

Gram-positive bacteria, producing a clear zone of inhibition against the majority of the tested strains (Fig. 1). Furthermore, the highest level of activity was observed against five strains of *S. epidermidis* (reference strains *S. epidermidis* CIP106510, E13, S27, S23 and S38), with an inhibition zone of >16 mm (Table 2). The oil was also active against 26 strains of *S. epidermidis* isolated from dialysis fluids, three human pathogenic Grampositive cocci, two Gramnegative bacilli and one Grampositive bacillus (diameter of inhibition zone: 11–15 mm). In contrast, the oil was ineffective against *P. aeruginosa* ATCC 27853 (diameter of inhibition zone: 9 mm). These results are in agreement with those of another study reporting that clove essential oil exhibited antibacterial activity against a large number of methicillin-resistant *S. epidermidis* and *S. aureus* (Enzo and Susan, 2002). However, the oil also appears to be effective against both Gram-positive and Gram-negative microorganisms, contrasting with results found in other studies (Zaika, 1988; Smith-Palmer *et al.*, 1998).

Fungicidal activity

Natural compounds remain an interesting source of new antifungal metabolites. The phenolic components, carvacrol and eugenol, are known to possess fungicidal characteristics (Manohar *et al.*, 2001), including activity against fungi isolated from onychomycosis (Gayoso *et al.*, 2005). The main antifungal action appears to be exerted on the cellular membrane (Cox *et al.*, 2001). Eugenol has shown antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* (Tampieri *et al.*, 2005) and Núñez *et al.* (2001) demonstrated that the mixture of clove oleoresin with concentrated sugar solution produced a strong fungicidal effect by reducing fungi inoculum size. In a recent report, SEM micrographs showed significant morphological damage with cellular deformity to *Saccharomyces cerevisiae* cells by clove oil (Chami *et al.*, 2005). The fungicidal activity of *E. caryophyllata* essential oil has also been reported on several food-borne fungal species (Velluti *et al.*, 2004;

DOI: 10.1002/ptr

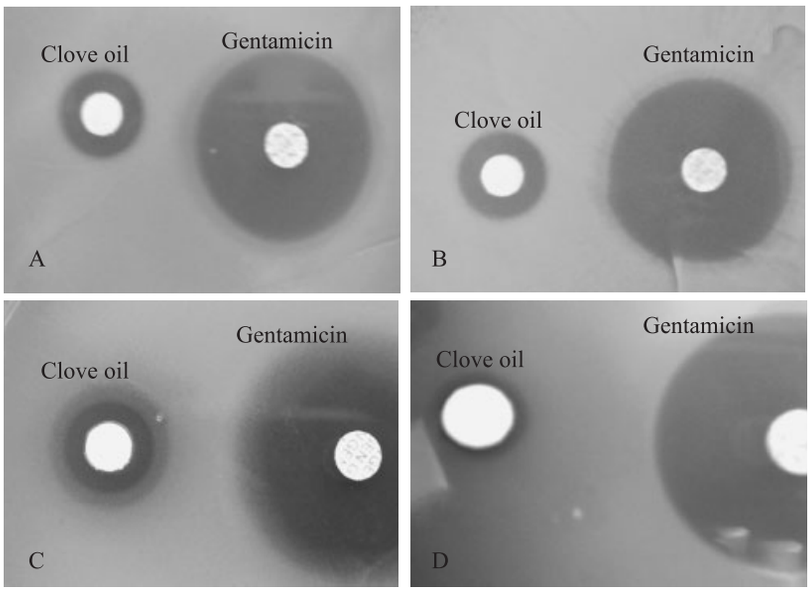

Figure 1. Effect of clove oil on bacterial growth. Bacterial strains were seeded on Muller Hinton infusion agar. Paper disks containing 10 mg of clove oil or gentamicin (500 μg) were placed onto the seeded agar. Disks without clove oil or antibiotics were used as negative controls. The cultures were incubated for 24 h at 37 °C, and the inhibition zone diameter was measured. (A) *S. epidermidis* CIP106510; (B) *S. aureus* ATCC 25923; (C) *E. coli* ATCC 35218; (D) *P. aeruginosa* ATCC 27853.

Lopez *et al.*, 2005) and it was observed in a recent study that the essential oil of clove even inhibited the growth of *Aspergillus niger* (Pawar and Thaker, 2006).

Antiviral activity

Generally speaking, viruses are highly sensitive to the components of essential oils, and phenylpropanoids, monoterpenols and monoterpenals have shown *in vitro* antiviral activity. Hussein *et al.* (2000) found that *Syzygium aromaticum* extract was highly active at inhibiting replication of the hepatitis C virus (≥90% inhibition at 100 μg/mL). Kurokawa *et al.* (1998) isolated and identified an anti-HSV compound, eugeniin, from the extracts of *Syzygium aromaticum*, which showed specificity in inhibiting HSV-1 DNA polymerase activity.

Antioxidant capacity

Many natural antioxidants are plant phenolics found in every part of the plant, including the fruit, seeds and leaves (Kim *et al.*, 1997). The major constituent of clove oil is eugenol, to which are attributed many of the antioxidant properties (Ogata *et al.*, 2000). The antioxidant activity may occur via various mechanisms such as scavenging the radicals and chelating metal ions. Eugenol reportedly participates in photochemical reactions (Mihara and Shibamoto, 1982), and displays strong antioxidant activity (Ogata *et al.*, 2000) and photocytotoxicity (Atsumi *et al.*, 2001). Jirovetz *et al.* (2006) found that the antioxidant action of 0.005% clove oil was identical to that of standard butylated hydroxytoluene at a concentration of 0.01%. It manifests considerable chelating potential against Fe^{+3}, resulting in the prevention of the initiation of hydroxyl radicals (Jirovetz *et al.*, 2006). Clove oil thus shows powerful antioxidant activity; moreover, it can be used as an easily accessible source of natural antioxidants and in pharmaceutical applications (Gülçin *et al.*, 2004).

Antitumor activity

Clove essential oil has been reported to show anticarcinogenic (Zheng *et al.*, 1992) and antimutagenic potential (Miyazawa and Hisama, 2001). Volatile oils display cytotoxic action towards the human tumor cell lines PC-3 and Hep G2 (Ogunwande *et al.*, 2005; Yoo *et al.*, 2005) and in a recent study, eugenol was shown to induce apoptosis of human cancer cells (Namiki, 1994) with the major antimutagenic compound being identified as dehydrodieugenol. More recently, the antimutagenic activity of cinnamaldehyde was reported in human-derived hepatoma cells, where it suppressed the frequency of micronuclei induced by various heterocyclic amines (Sanyal *et al.*, 1997). Ohta *et al.* (1983) suggested that cinnamaldehyde reduced UV-induced mutagenesis, as well as mutagenesis induced by furylfuramide (AF-2) in *E. coli* WP2s.

Anaesthetic activity

Eugenol is used in a wide range of applications, such as a local anaesthetic in dentistry and as an ingredient in dental cement for temporary fillings (Markowitz *et al.*, 1992). It is relatively user-friendly and can be used in lower concentrations than other local anaesthetics (Keene *et al.*, 1998) and it is rapidly metabolized and excreted, thus requiring no withdrawal period (Wagner *et al.*, 2002). It has been shown to be effective in anaesthetizing fish such as rainbow trout, *Oncorhynchus mykiss* Walbaum (Anderson *et al.*, 1997; Keene *et al.*, 1998) and channel catfish (Waterstrat,

DOI: 10.1002/ptr

1999). Eugenol at 65 mg/L was shown to be safely and effectively induce all stages of anaesthesia in juvenile and sub-adult tambaqui fish within the desired time. Further research needs to focus on assessing its efficacy in other tropical species, as well as investigating its lethal dosage (Roubach *et al.*, 2005).

Insecticidal activity

The use of drugs to control parasitic arthropods presents several challenges, including drug resistance and environmental damage (O'Brien, 1999). The biological activity of *Eugenia caryophyllata* oil has been investigated against several parasites. It was shown to inhibit the emergence of *Culex pipiens* larvae (El Hag *et al.*, 1999) and to display insecticidal activity against *Pediculus capitis* (Yang *et al.*, 2003), *Anopheles dirus* mosquitoes (Trongtokit *et al.*, 2005) and some stored product insects and suppresses progeny development of *Tribolium castaneum* and *Sitophilus zeamais* with isoeugenol being particularly active (Ho *et al.*, 1994). Clove essential oil has also showed acaricidal activity towards *Dermatophagoides farinae* and *D. pteronyssinus* with eugenol being identified as the acaricidal constituent of the oil. Eugenol congeners also exhibited potent acaricidal activity against both mite species (Kim *et al.*, 2003), and Perrucci *et al.* (1995) reported its effects against the causative organism of psoroptic mange, *Psoroptes cuniculi*, by direct contact and by contact only with the vapour phase. A more recent study has confirmed that clove oil could be used as a novel fumigant against Japanese termites (Park and Shin, 2005).

CONCLUSIONS

This review describes the known effects of clove oil and additional work has clarified its chemical composition by GC-MS and enabled the identification of 36 components. The highest concentration was, as expected, of eugenol (88.58%), but the second most abundant compound was eugenyl acetate (5.62%), followed by β-caryophyllene (1.38%). In view of the known activities of the constituents and the variability of the oil, it is important that the composition is known.

It has been shown that clove oil possesses significant antioxidant properties and shows potential as a natural preservative or as a source of natural antioxidants for use in pharmaceutical applications. It induced apoptosis of human cancer cells and is considered to be an effective guard against virus infection when used as an antiseptic. The highest antibacterial activity of the essential oil was observed against five strains of *S. epidermidis* (reference strains CIP106510, E13, S27, S23 and S38) and is thought to be due mainly to the presence of eugenol. In addition, this oil was active against 26 *S. epidermidis* isolated from a dialysis fluid, three human pathogenic Grampositive cocci, two Gramnegative bacilli and one Grampositive bacillus.

In terms of a practical use of *Eugenia* oil-derived materials as novel insecticidal applications, further research is necessary to address the safety issues pertaining to human health as well as formulations for improving insecticidal potency and stability. Clove oil is non-toxic to fish, and acts as an anaesthetic for some fish species, but the toxicity to other animals is not known.

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