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RESEARCH ARTICLE

Chemical composition and antibacterial activity of essential oils of *Lantana camara*, *Ageratum houstonianum* and *Eupatorium adenophorum*

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Abstract

Essential oils have applications in folk medicine, food preservation, and as feed additives. The essential oils of *Lantana camara* Linn. (Verbenaceae), *Ageratum houstonianum* Mill. (Asteraceae) and *Eupatorium adenophorum* Spreng. (Asteraceae) were analyzed by Gas chromatography-mass spectrometry (GCMS). In *L. camara* oil, of the total identified (83.91%) volatile constituents, five constituents [3,7,11-trimethyl-1,6,10-dodecatriene (28.86%), β-caryophyllene (12.28%), zingiberene (7.63%), γ-curcumene (7.50%) and α-humulene (3.99%)] represented the major ones. In *A. houstonianum* oil, among the total identified volatile constituents (94.51%), three [precocene-II (52.64%), precocene-I (22.45%) and β-caryophyllene (9.66%)] represented the major ones. In *E. adenophorum* oil, of the total identified volatile constituents (84.95%), six [1-napthalenol (17.50%), α-bisabolol (9.53%), bornyl acetate (8.98%), β-bisabolene (6.16%), germacrene-D (5.74%) and α- phellandrene (3.85%)] represented the major ones. The antibacterial activity expressed as Minimum Bactericidal Concentration (MBC) (μg/mL) was determined by the broth dilution method. The essential oil of *E. adenophorum* had antibacterial activity against *Arthrobacter protophormiae, Escherichia coli, Micrococcus luteus, Rhodococcus rhodochrous,* and *Staphylococcus aureus* with MBC values of 200, 100, 100, 12.5, and 200, respectively. The essential oil of *A. houstonianum* showed antibacterial activity against *M. luteus* and *R. rhodochrous* with MBC of 100 and 12.5, but not against *A. protophormiae, E. coli,* and *S. aureus.* The essential oil of *L. camara* showed antibacterial activity against *A. protophormiae, M. luteus, R. rhodochrous and S. aureus* with MBC of 50, 25, 12.5, and 200, respectively, but not against *E. coli.* MBC was lowest for *R. rhodochrous* for all the three essential oils.

Keywords: Lantana camara*;* Ageratum houstonianum*;* Eupatorium adenophorum*; essential oil; chemical composition; antibacterial activity*

Introduction

Recently, plant secondary metabolites (PSM), particularly essential oils, have evoked considerable interest as food and feed additives and as an alternative to antibiotics and synthetic antioxidants (Wallace, 2004; Acamovic & Brooker, 2005; Greathead, 2003; Burt, 2004; Kalema & Kunicka, 2003). Studies on PSM have become imperative in view of the legislation in the European Union to prohibit the use of growth promoting antibiotics in animal feeds as of January 2006 (EU 1831/2003). Plants and their extracts are compatible with the current thinking on the future of health care, agriculture, and food and consumer opinion that most "things natural" are better and safer (Greathead, 2003). This has spurred research on PSM as antimicrobial agents and nutraceuticals. Essential oils, the odorous and volatile plant secondary metabolites, have a wide application in folk medicine, food flavoring, and preservation, and in the fragrance industry (Greathead, 2003; Kalema & Kunicka, 2003). Essential oils are known to exhibit antimicrobial,

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anticancer, antioxidant activity, and sedative effects (Kalema & Kunicka, 2003; Yoo et al., 2005; Sylvestre et al., 2005; Ricci et al., 2005; Kordali et al., 2005; Carvalho-Freitas & Costa, 2002; Warnke et al., 2004). Antibacterial activity of essential oils has been successfully used to control malodor in cancer patients (Warnke et al., 2004, 2005). Essential oils such as tea tree and eucalyptus oil have gained acceptance as safe and effective antiseptics (Warnke et al., 2005; Cox et al., 2000). There is a search for plants with more potent PSM for applications in the feed and food industry and for medical applications (Greathead, 2003; Burt, 2004; Messager et al., 2005; de Sousa et al., 2004; Allahverdiyev et al., 2004). *Lantana camara* Linn. (Verbenaceae) extracts are considered antiseptic, antispasmodic, carminative, and diaphoretic. In Ayurvedic medical practice the leaves are used to treat hemorrhage and diarrhea (Parrotta, 2001; Sastri, 1962). The plants of the various species of *Ageratum* are used in indigenous systems of medicine (Sharma & Sharma, 1995). Decoction or infusion of the plant is given in various stomach diseases such as diarrhea, dysentery, and intestinal colic and also in rheumatism and fever (Sharma & Sharma, 1995). Various species of *Eupatorium* have been used in the traditional system of medicine in different parts of the world (Sharma et al., 1998). *E. adenophorum* Spreng. (Asteraceae) is used in India as an antiseptic and blood coagulant. A decoction of the plant has been recommended to treat jaundice and ulcers (Sharma et al., 1998).

L. camara, A. houstonianum, and *E. adenophorum* grow as weeds on vast expanses in many parts of the world and are an attractive source of bioactive natural products (Sharma & Sharma, 1989, 1995; Sharma et al., 1998; James et al., 2004). There are some previous reports on the antimicrobial activity of the oils of some species of *Lantana*, *Ageratum*, and *Eupatorium* (Hernandez et al., 2005; Juliani et al., 2002; Deena & Thoppil, 2000; Pattnaik et al., 1996; Habtemariam & MacPherson, 2000; Gupta et al., 2002; Sasikumar et al., 2005). However, the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants (Burt, 2004). The bioactivity of the essential oils of *L. camara*, *A. houstonianum*, and *E. adenophorum* (syn: *Ageratina adenophora* (Spreng.) King and H.E. Robins) in the sub-Himalayan region of India has not been investigated. We report here the chemical composition and antibacterial activity of the essential oils extracted from *L. camara, A. houstonianum*, and *E. adenophorum* samples collected from Kangra Valley, Himachal Pradesh, India.

Materials and methods

Plant material

L. camara, *A. houstonianum*, and *E. adenophorum* samples were collected in September 2007 from Palampur (latitude 32° 07′, longitude 76° 31′, elevation 1275 m) H.P. India, and identified by Brij Lal, Division of Biodiversity, Institute of Himalayan Bioresource Technology (IHBT), and Palampur. Voucher specimens were deposited in the Herbarium of IHBT: *Lantana camara* var. *aculeata* no. PLP 3491; *Eupatorium adenophorum* no. PLP 3492; *Ageratum houstonianum* no. PLP-3493.

Extraction of essential oil

The samples were fresh leaves of *L. camara* and *E. adenophorum* and fresh aerial parts of *A. houstonianum*. Essential oil was extracted by hydrodistillation using Clevenger-type apparatus, collected, dried using sodium sulfate and stored at 4°C. The yield (%) of oil (on fresh weight) was 0.05, 0.1, and 0.15 for the samples of *L. camara*, *A. houstonianum* and *E. adenophorum,* respectively.

Chemical composition

The analysis of volatile oil of *L. camara*, *A. houstonianum*, and *E. adenophorum* was carried by GCMS analysis recorded on Shimadzu QP2010 GCMS fitted with BP-20 polar fused and silica capillary column 30 m**,** 0.25 mm i.d., film thickness 0.25 μm. The operating conditions were: injector and detector temperatures as 250°C, carrier gas He, flow rate 1.1 mL/min, oven temperature isothermal at 70°C increase at 4°C/m up to 220°C, finally held isothermal for 5 min, sample split ratio 1:50, injection volume 0.2 μL.

Antibacterial assay

Arthrobacter protophormiae, MTCC no. 2682, *Escherichia coli* MTCC no. 739, *Micrococcus luteus,* MTCC no. 106, *Rhodococcus rhodochrous* MTCC no. 265 and *Staphylococcus aureus* MTCC no. 26 procured from the Institute of Microbial Technology, Chandigarh, were kindly provided by M.K. Gupta Department of Microbiology, CSK HP Agricultural University, Palampur, India. Essential oils are volatile, insoluble in water, viscous and complex mixtures. So, the common tests such as disc/well diffusion do not give reproducible results for determination of antibacterial activity of essential oils (Hood et al., 2003). Therefore, the validated protocol of broth dilution method for assay of antibacterial activity of essential oils was used in this study (Hood et al., 2003). The data are presented as minimum bactericidal concentration (MBC).

Results and discussion

The chemical composition of the essential oils of *L. camara*, *A. houstonianum* and *E. adenophorum* is given in Table 1. GCMS analysis of essential oil of

Lantana camara revealed the presence of 3,7,11-trimethyl-1,6,10-dodecatriene (28.86%), β-caryophyllene (12.28%), zingiberene (7.63%), γ-curcumene (7.50%) and α-humulene (3.99) as major constituents. The oil was represented with lone monoterpene hydrocarbon β-phellandrene (0.34%) but was mainly dominated by sesquiterpene hydrocarbons. The earlier workers also observed that essential oil of *L. camara* leaves is dominated by sesquiterpenes (Misra & Laatsch, 2000; Weyerstahl et al., 1999). However, there is marked difference in the nature and relative content of different constituents in the previous reports (Misra & Laatsch, 2000; Weyerstahl et al., 1999) and the data on Table 1. The sample used in this study and those in the previous studies might be representing different chemotypes. Recently, Marongiu et al. (2007) reported the composition and antibacterial activity of the essential oil extracted from *L. camara* leaves by supercritical carbon dioxide. The major constituents were: ar-curcumene (38.7%), α-humulene (9.6%), α-zingiberene (7.8%), (*E*)-caryophyllene (7.8%), γ-curcumene (7.6%), allo-aromadendrene (4.8%), β-curcumene (3.2%), (*E*)-farnesene (2.5%), 7-epi-selinene (2.4%) and β-sesquiphellandrene (2.4%).

GCMS analysis of *A. houstonianum* essential oil (Table 1) revealed that the oil was dominated by chromenes, precocene-I (22.45%) and precocene-II (52.64%). In addition two more chromene derivatives, desmethoxyencecalin (0.78%) and androencecalinol (0.3%) were also identified. The oil was devoid of

Table 1. Chemical composition (% amount in parenthesis) of essential oils of *Lantana camara*, *Ageratum houstonianum* and *Eupatorium adenophorum.*

Lantana camara	*Ageratum houstonianum*	*Eupatorium adenophorum*
β-phellandrene (0.34)	α-copaene (0.1)	α-phellandrene (3.85)
sylvestrene (0.44)	β-cubebene (1.37)	limonene (0.56)
sabinene (0.24)	linalool (0.12)	bornyl acetate (8.98)
α-ocimene (0.4)	linalyl acetate (0.11)	*t*-α-bergamotene (1)
cis-3-hexenol (0.5)	isobornyl formate (0.17)	β-caryophyllene (2.38)
3-octanol (0.17)	sesquithujene (0.08)	(*Z,Z*)-β-farnesene (0.61)
1-octene-3-ol (2.19)	β-caryophyllene (9.66)	epizonaren (2.11)
α-copaene (0.37)	(*Z*)-β- farnesene (0.09)	(*Z,E*)-β-farnesene (2.77)
linalool (1.81)	α-humulene (0.8)	γ-curcumene (1.67)
linalyl acetate (0.19)	germacrene-D (1.23)	germacrene-D (5.74)
β-caryophyllene (12.28)	*epi*-bicyclosesquiphellandrene (0.52)	alloaromadendrene (1.06)
α-humulene (3.99)	cyclosativene (0.16)	camphene (1.01)
7,11-dimethyl-3-methylene-	*cis*-α-bisabolene (0.23)	β-bisabolene (6.16)
1,6,10-dodecatriene (1.11)	β-sesquiphellandrene (1.57)	β-sesquiphellandrene (1.7)
γ-curcumene (7.5)	α-murrolol (0.24)	*cis*-α-bisabolene (0.79)
germacrene-D (1.44)	caryophyllene oxide (0.33)	caryophyllene oxide (0.57)
zingiberene (7.63	cubenol (0.14)	(*E,E*)-β-farnesene (1.08)
bicyclogermacrene (1.4)	precocene-I (22.45)	elemol (1.03)
β-curcumene (2.42)	thujopsan-2-ol (0.06)	1-napthalenol (17.5)
epi-cubebol (0.29)	longipinanol (1.18)	guaiol (0.93)
murrolol (0.8)	androencecalinol (0.3)	*t*-cadinol (1.54)
caryophyllene oxide (0.54)	precocene-II (52.64)	β-bisabolol (0.72)
longipinanol (0.3)	desmethoxyencecalin (0.78)	cedrene-13-ol acetate (2.58)
α-bisabolol (0.24)	kessane (0.28)	α-bisabolol (9.53)
humulene epoxide II (0.21)	phytol (0.2)	α-cadinol (0.59)
3, 7, 11-trimethyl-1,6,		
10-dodecatriene (28.86)		carotol (1.17)
t-sesquisabinene hydrate (1.23)		lynestrenol (0.51)
valerianol (0.3)		acorenone (2.88)
T-cadinol (0.41)		di-*epi*-α-cedrene (2.98)
cis-sesquisabinene hydrate (0.53)		
ipsdienol (0.35)		
α-cadinol (0.35)		
phytol (1.47)		
dihydrolinalyl acetate (0.13)		
Total identification: 83.91%	Total identification: 94.51%	Total identification: 84.95%

any monoterpene hydrocarbons but sesquiterpene hydrocarbons were dominating (15.97%) among which β-caryophyllene was the major constituent (Table 1). The oxygenated monoterpenes were represented only by linalool and isobornyl formate while the sesquiterpene alcohols were represented only by α-murrolol, cubenol, thujopsane-2-ol and longipinanol (Table 1). There is only one earlier report on the chemical composition of essential oil of *A. houstonianum* (Chandra et al., 1996). The major constituents were precocene-II (43.99%), precocene-I (23.34%) and β-caryophyllene (9.16%). The chemical composition of the solvents extracts of *A. houstonianum* from different locations has been investigated by earlier workers as well (Sharma & Sharma, 1995). The predominant constituents in these extracts also were precocene I and precocene-II. Hitherto, potential application of isolated precocenes and *Ageratum* essential oil is insecticidal activity (Sharma & Sharma, 1995).

GCMS analysis of the essential oil of *E. adenophorum* leaves revealed the presence of 1-napthalenol (17.50%), α-bisabolol (9.53), bornyl acetate (8.98), β-bisabolene (6.16), germacrene-D (5.74) and α-phellandrene (3.85) as major constituents (Table 1). This is the first report on the essential oil of the leaves of *E. adenophorum*. Weyerstahl and co-workers (1997) reported the composition of the essential oil of flowers of *E. adenophorum*. The major constituents were α-phellandrene (15.3%), camphene (12.2%), bornyl acetate (10.6%), *p*-cymene (8.5%), γ-curcumene (4.5%) and 2-carene. Pala-Paul and co-workers (2002) reported the analysis of the essential oil of the aerial parts of *E. adenophorum*. The major constituents were *p*-cymene (11.6%), α-phellandrene (5.7%), γ-curcumene (5.0%), δ-2-carene (5.0%), camphene (4.8%), and endo-bornyl acetate (4.4%).

Antibacterial activity of essential oils of *L. camara*, *A. houstonianum* and *E. adenophorum* is shown in Table 2. Essential oil of *E. adenophorum* leaves showed antibacterial activity against all five organisms tested (Table 2). The growth of *S. aureus* was not inhibited by the essential oil of *A. houstonianum* and the MBC for the oil of *L. camara* and *E. adenophorum* was rather high for this organism. *R. rhodochrous* was the most susceptible organism with an MBC value of 12.5 μL/mL for the essential oil of all three plants. The growth of *E. coli* was not inhibited by essential oil of *L. camara* and *A. houstonianum*. Bhattacharjee and co-workers (2005) also observed that essential oil of *Cestrum diurnum* (L.) did not inhibit the growth of *E. coli*. The essential oil of *Abies balsamea* was also found to be inactive against *E. coli* and active against *S. aureus* (Pichette et al., 2006). Antibacterial effect of essential oils is, generally, less against Gram-negative bacteria than against Gram-positive bacteria (Burt, 2004). The *E. coli* strain was resistant to tetracycline as well (Table 2).

Deena and Thoppil (2000) observed that essential oil of *L. camara* had antimicrobial activity towards both Gram-positive as well as negative bacteria. More recently, Marongiu et al. (2007) observed that essential oil of *L. camara* extracted using supercritical carbon dioxide inhibited the growth of *S. aureus* and *S. epidermis*. The assay of antibacterial activity in both these studies was done by the filter paper disc diffusion method. Hood et al. (2003) observed that disc diffusion, well diffusion and agar dilution methods are unreliable and give inconsistent results for the assay of antimicrobial activity of essential oils. The composition, organoleptic profile and antimicrobial activity of essential oils prepared by steam distillation, carbon dioxide under low pressure and solvents like hexane, vary (Burt, 2004).

Our report is the first one on the antibacterial activity of the essential oils of *A. houstonianum* and *E. adenophorum*. Earlier, *A. houstonianum* essential oil has been reported to have acaricidal activity (Tedonkeng Pamo et al., 2005). A foam soap containing essential oil of *A. houstonianum* leaves was tested on *Rhipicephalus lunulatus* collected from West African dwarf goats. The LD_{50} of the foam soap containing essential oil of this plant

Table 2. Antibacterial activity of essential oils of *Lantana camara*, *Ageratum houstonianum* and *Eupatorium adenophorum.*

	MBC (μL/mL)			MBC (μg/mL)	
Microorganism	LC	AH	EA	TR	SP
Arthrobacter protophormiae MTCC no. 2682	50	No inhibition*	200	12.5	12.5
Escherichia coli MTCC no. 739	No inhibition*	No inhibition*	100	No inhibition	25
Micrococcus luteus MTCC no. 106	25	100	100	100	100
Rhodococcus rhodochrous MTCC no. 265	12.5	12.5	12.5	100	50
Staphylococcus aureus MTCC no. 26	200	No inhibition*	200	200	200

LC, *Lantana camara*; AH, *Ageratum houstonianum*; EA, *Eupatorium adenophorum*; TR, tetracycline; SP, streptomycin. *At 200 μL/mL; MBC, minimum bactericidal concentration.

was 0.0259 and 0.0173 mL/g on day 2 after treatment, in the laboratory and on the farm, respectively (Tedonkeng Pamo et al., 2005). Essential oils comprise a large number of components and it is likely that their mode of action involves several targets in the bacterial cell (Burt, 2004). Some locations and mechanisms of the antibacterial action of the essential oil components are: degradation of the cell wall, damage to cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm and depletion of the proton motive force (Burt, 2004). Carvacrol, an important constituent of the essential oils of oregano and thyme stimulates *E. coli* O157:H7 to produce Hsp60 and prevents the synthesis of flagellin, causing cells to be aflagellate and therefore non-motile (Burt et al., 2007). The hydrophobicity of essential oils enables them to partition in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents (Burt, 2004; Cristani et al., 2007).

In general, several constituents appear to contribute to the overall antibacterial action of essential oils (Koutsoudaki et al., 2005). In view of the antibacterial activity and the abundance of the raw material, there is a potential for further investigations on the use of the essential oils of *L. camara*, *A. houstonianum* and *E. adenophorum* in various applications in food, feed, animal health, and in palliative care medicine (Acamovic & Brooker, 2005; Greathead, 2003; Burt, 2004; Kalema & Kunicka, 2003; Yoo et al., 2005).

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Declaration of interest

The authors report no conflict of interest. The authors are responsible for the content and writing of the paper.

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