Effects of essential oils from medicinal plants used in Brazil against epec and etec *Escherichia coli*

Duarte, M. C. T.; Leme;Delarmelina, C.; Figueira, G. M.; Sartoratto, A.; Rehder, V. L. G.
CPQBA/UNICAMP (Research Center for Chemistry, Biology and Agriculture), CP 6171, CEP 13083-970, Campinas (SP), Brazil.E-mail: mduarte@cpqba.unicamp.br

ABSTRACT: Effects of essential oils from medicinal plants used in brazil against epec and etec *escherichia coli* . Essential oils obtained from leaves of 28 medicinal plants commonly used in Brazil were screened against anti-enteropathogenic (EPEC) and anti-enterotoxigenic (ETEC) *Escherichia coli*. The oils were obtained by water-distillation using a Clevenger-type system and their Minimal Inhibitory Concentration (MIC) was determined. Among the plants studied, *Cymbopogon martinii* exhibited a more reach inhibition spectrum, presenting strong activity (MIC between 0.1-0.5 mg/mL) against three ETEC and one EPEC serotypes, while *C. winterianus* inhibited strongly two ETEC and one EPEC serotypes. *Aloysia triphylla* also shows good potential to kill *E. coli* with moderate to strong inhibition. Other essential oils showed antimicrobial properties, however with restrict action against serotypes ETEC 5041-1 and EPEC 0031-2. Chemical analyses performed by Gas chromatographic (GC) and mass spectrometry (GC-MS) analyses showed the presence of compounds with known antimicrobial activity, including limonene, geranial, geraniol, trans-geranial, trans-cariophyllene and geranil acetate. The results indicate significant antibacterial activity from these oils and suggest that they may serve as sources for compounds with therapeutic potential.

Key words: *Escherichia coli,* essential oil, antimicrobial activity, medicinal plants, minimal inhibitory concentration.

INTRODUCTION

Diarrhea caused by *Escherichia coli* infection is a emergent problem in both developing and developed world and is responsible for high rates of mortality in newborn child and animals. Although commensal representatives founded in the intestinal flora of man and animals are apathogenic, certain strains are highly pathogenic.

All diarrheagenic strains of *E. coli* were initially termed enteropathogenic *E. coli* (EPEC) but as was learnt about their pathogenic mechanisms they were grouped accordingly (Clarke, 2001).

According to Schmidt et al. (1997), EPEC and ETEC are the most important of these in terms of total diarrhea episodes on a global scale although EHEC has become more significant in developed countries in recent years due to the occurrence of numerous outbreaks. ETEC are nowadays considered a major cause of *E. coli*-associated diarrhea worldwide, producing one or both of two types of enterotoxins namely heat stable enterotoxins (ST) and heat labile enterotoxins (LT) (Clarke, 2001).

The diarrhea is often treated with antibacterial drugs, but this treatment is generally ineffective, due in part to the presence of drug resistant strains and failure to identify drug sensitivity (Cid et al., 1996). This fact has underscored the need of a quick development of antibacterial drugs that are more effective than those currently in use. Since medicinal plants play a fundamental role in traditional medicine, the use of herbal remedies has been increased in Brazil as well as in another countries. In the present study, essential oils from 28 native and exotic medicinal plants traditionally used in Brazil were screened for antimicrobial activity against EPEC and ETEC *E. coli* serotypes. The essential oils from active plants were also characterized by gas-chromatography/ mass spectrometry analyses in order to identify their major compounds.

Recebido para publicação em agosto/2004
Aceito para publicação em julho/2006

MATERIAL AND METHOD

Medicinal plants

A list of the plants studied are listed in Table 1. The plants were grown in the experimental field of the Research Center for Chemistry, Biology and Agriculture (CPQBA, State University of Campinas, São Paulo, Brazil). The plants were collected from November/2001 to February/2003. Voucher specimens were deposited at the State University of Campinas Herbarium (UEC) or at the herbarium at the CPQBA.

Essential oil extraction

The essential oils were obtained from 40 g of fresh plant parts by water-distillation using a Clevenger-type system for 3 h. The aqueous phase was extracted three times with 50 mL dichloromethane. The pooled organic phases were dried with sodium sulphate, filtered and the solvent evaporated until dryness. Dried samples were stored at -25 ^{o}C in sealed glass vials.

TABLE 1. Identification, voucher specimen and data on traditional use of the plants studied.

Botanical name	Family	Voucher	Plant part[a]	Traditional use[b]
Achyrocline satureoides (DC.)Lam.	Asteraceae	UEC 127.116	lv	antinflamatory, analgesic
Allium schoenoprasum L.	Liliaceae	UEC 121.397	lv, rt	digestive, antibiotic
A. triphylla (L'hér.) Britten.	Verbenaceae	UEC 121.412	lv	spice, digestive, sedative
Anthemis nobilis L.	Asteraceae	UEC 121.411	lv	antispasmodic, aromatic, digestive
Artemisia annua L.	Asteraceae	CPQBA 1246	lv	disinfectant, antimalaric
Artemisia camphorata L.	Asteraceae	CPQBA 63	lv	diuretic, aperitive
B. trimera (Less.) Dc.	Asteraceae	CPQBA 1	lv	digestive, antihelminthic
Cordia curassavica (Jacq.) Roem.	Boraginaceae	UEC112744	lv	antinflamatory
Cymbopogon martinii Motia.	Poaceae	UEC 127.115	lv	antiseptic, repellent
C. winterianus L.	Poaceae	UEC 121.414	lv	antiseptic, repellent
Cyperus articulatus L.	Cyperaceae	UEC 121.396	rt	antibiotic, antinflamatory
C. rotundus L.	Cyperaceae	CPQBA 1252	rt	antibiotic, antinflamatory
Lippia alba (Mill) N.E. Br.	Verbenaceae	UEC121413	lv	soothing, analgesic
Mentha piperita L.	Lamiaceae	UEC 127.110	lv	antiseptic, vermifug
Mikania glomerata Sprengel	Asteraceae	UEC 102047	lv	expectorant, anticold
M. laevigata Sch. Bip. ex Baker	Asteraceae	UEC 102044	lv	expectorant, anticold
Ocimum basilicum L.	Lamiaceae	UEC 121.408	lv	digestive, vermifug
O. gratissimum L.	Lamiaceae	UEC 121.407	lv	anticold, diuretic
Origanum applii (Domin) Boros	Lamiaceae	UEC 121.410	lv	analgesic, expectorant
O. vulgare subsp. *virens* L.	Lamiaceae	UEC 121.409	lv	analgesic, expectorant
Piper abutiloides	Piperaceae	UEC 127122	lv	tonic, antispasmodic
P. aduncum L.	Piperaceae	UEC 127.118	lv	tonic, antispasmodic
P. marginatum Jacq.	Piperaceae	UEC 121.395	lv	tonic, antispasmodic
P. mollicomum Kunth	Piperaceae	UEC 127121	lv	tonic antispasmodic
P. regnellii (Miq.) C.DC.	Piperaceae	UEC 127.120	lv	tonic, antispasmodic
Stachys byzantina C.Koch.	Lamiaceae	UEC 121.404	lv	antinflamatory
Stachytarphetta cayenensis (L.C.)	Verbenaceae	UEC 121.394	lv	tonic, diuretic, stimulant
Thymus vulgaris L.	Lamiaceae	UEC 121.405	lv	antiseptic, antispasmodic
Urena lobata L.	Malvaceae	CPQBA 1251	lv	artritis

[a]lv, leaves; rt, roots. [b]Data from Lorenzi and Matos (2002).

TABLE 2. Essential oil Yield (% w/w) and MIC results (mg.mL^{-1}) from the plants tested.

Plant species[a]	Essential oil Yield	ETEC TR 441/4	ETEC 6/81 H5J	ETEC O63	ETEC5041-1 5041-1	EPEC O551	EPEC O119	EPEC E2348/69	EPEC 0031-2
Achyrocline satureoides	0.08	*	*	*	*	*	*	*	*
A. schoenoprazum	0.04	*	n.d.	*	1.0	*	0.5	*	0.9
A. triphyla	1.60	0.6	1.0	*	0.5	0.8	0.4	0.8	0.5
Anthemis nobilis	0.26	*	*	*	*	*	*	*	*
Artemisia annua	0.35	*	*	*	0.8	*	*	*	0.5
A. camphorata	1.00	*	*	*	0.5	*	0.6	*	0.6
B. trimera	0.27	*	*	*	0.6	*	*	*	*
Cordia curassavica	0.10	*	*	*	*	*	*	*	*
Cymbopogon martinii	1.77	0.4	0.5	*	0.1	*	0.2	0.9	0.2
C. winterianus	0.70	0.8	0.5	*	0.5	0.6	*	0.8	0.2
Cyperus articulatus	0.15	1.0	*	*	*	*	0.7	*	0.8
C. rotundus	n.d.	1.0	*	*	*	*	*	*	*
Lipia alba	0.26	1.0	*	*	*	*	*	*	0.4
Mentha piperita	0.42	*	*	*	*	*	*	*	*
Mikania glomerata	0.29	*	*	*	*	*	*	*	*
M. laevigata	0.02	*	*	*	0.5	*	*	*	0.5
Ocimum basilicum	0.10	*	*	*	*	*	*	*	*
O. gratissimum	0.74	0.9	*	*	1.0	*	0.7	*	0.6
Origanum applii	0.20	*	*	*	*	*	*	*	*
Piper abutiloides	1.10	*	*	*	*	*	*	*	*
P. aduncum	0.19	*	*	*	*	*	*	*	0.9
P. marginatum	0.30	*	*	*	*	*	*	*	0.9
P. mollicomum	2.98	*	*	*	*	*	*	*	*
P. regnelli	2.70	*	*	*	*	*	*	*	0.3
Stachys bizantina	0.10	*	*	*	*	*	*	*	*
Stachytarpetta cayenensis	0.15	*	*	*	*	*	*	*	*
Thymus vulgaris	0.56	*	*	*	*	*	*	*	1.0
Urena lobata	0.20	*	*	*	*	*	*	*	*
Control: Chloranphenicol		0.008	0.004	0.004	0.006	0.004	0.004	0.004	0.004

[a]lv, leaves; rt, root; [b]n.d. = not determined; * = MIC > 1.0 mg.mL^{-1}

E. coli serotypes were kindly supplied by Dra. Tânia A. T. G. do Amaral (UNIFESP - Brazil). The bacteria were grown overnight at 36°C in Mc Conkey Agar (Merck), and inocula for the assays were prepared by diluting scraped cell mass in 0.85% NaCl solution, adjusted to McFarland scale 0.5 and confirmed by spectrophotometrical reading at 580 nm.

Minimal Inhibitory Concentration (MIC) tests were carried out using Müller-Hinton Broth on a 96 wells culture testplate (Eloff,1998). culture testplate (96 wells) - (Eloff,1998). The stock solutions of the oils were diluted and transferred into the first well, and serial dilutions were performed so that concentrations in the range of 1.0-0.016 mg.mL^{-1} were obtained. Chloramphenicol (Merck) was used as the reference control in the range of 0.0625-0.0005 mg.mL^{-1}. The inoculum was added to wells and the plates were incubated at 36°C for 24 h. Antimicrobial activity was detected by adding 20 µL of 0.5% TTC (triphenyl tetrazolium chloride, Merck). MIC was defined as the lowest concentration of oil that inhibited visible growth, as indicated by the TTC staining.

Gas chromatographic (GC) and mass spectrometry (GC-MS) analyses

The identification of volatile constituents was performed using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a HP-5971 mass selective detector and capillary column HP-5 (25 m x 0.2 mm x 0.33 mm diam.). GC and GC-MS were done using *split/splitless* injection, with injector set at 220°C, column set at 60°C, with heating ramp of 3 °C.min^{-1}and final temperature 240°C for 7 min, and the FID detector set at 250°C. Helium was used as carrier gas at 1 mL.min^{-1}. The GC-MS electron ionization system was set at 70 eV. A sample of the essential oil was solubilized in ethyl acetate for the analyses. Retention indices (RI) were determined by co-injection of hydrocarbon standards. The oil components were identified by comparison with data from literature (Adams, 2001), the profiles from the Wiley 138 and Nist 98 libraries, and by co-injection of authentic standards, when available.

RESULT AND DISCUSSION

Oil yields of the plant expressed in relation to dry weight plant material, are presented in Table 2. Most plants had oil yield below 1% (w/w), though higher amount was obtained from *A. triphylla* (1.60% w/w), *C. martinii* (1.77% w/w), *Piper mollicomum* (2.98% w/w) and *P. regnelli* (2.7% w/w).

MIC results of the oils obtained from the plants tested are shown in Table 2. Considering the standard antibiotic used as control, the MIC from chloramphenicol varied from 0.004 to 0.008 mg.mL^{-1} for *E. coli* strains. There is not an agreement on the acceptable inhibition level for plant materials. A classification, based at MIC results as: strong, until 0.5 mg.mL^{-1}; moderate, between 0.6 and 1.5 mg.mL^{-1} and weak inhibitors, above 1.6 mg.mL^{-1} was proposed (Aligianis et al., 2001). Thus in the present work have been established 1.0 mg.mL^{-1} as highest concentration and only the oils presenting MIC below 0.5 mg.mL^{-1} were considered as potential antimicrobial.

Among the 29 medicinal species investigated, *C. martinii* exhibited a more reach inhibition spectrum, presenting strong activity (MIC between 0.1-0.5 mg.mL^{-1}) against three ETEC and one EPEC serotypes. The other *Cymbopogon* specie studied, *C. winterianus* also inhibited strongly two ETEC and one EPEC. *Aloysia triphylla* shows good potential to kill *E. coli* with moderate to strong inhibition (MIC between 0.5-0.8 mg.mL^{-1}). The most of other essential oils presented antimicrobial

TABLE 3. Identified compounds from the essential oils of *A. triphyla* (AT), *Cymbopogum martini* (CM), *Cymbopogum winterianus* (CW).

Compounds[a]	RI[b]	AT[c]	CM	CW
6-methyl-5-hepten-2-one	984	3.63		
β-myrcene	990	0.74	0.12	
dl-limonene	1026	18.75		1.61
cis-β-ocimene	1034		0.23	
Trans-β-ocimene	1044	0.82	0.98	
isopulegol	1143			0.99
citronelal	1154			36.24
nerol	1226	6.52		
citronelol	1230			18.43
trans-geraniol	1254	8.29	63.46	11.63
geranial	1269	21.77		0.23
citronelil acetate	1352			2.51
eugenol	1355			1.04
geranil acetate	1382	3.15	28.83	1.05
β-elemene	1387			0.91
trans-cariophylene	1414	4.94	2.15	
γ-muurolene	1477			1.80
<AR> curcumene	1481	1.43		
biciclogermacrene	1492	2.63		
δ-cadinene	1520			1.66
elemol	1546			7.15
germacrene D-4-ol	1569			1.30
spatulenol	1572	2.61		
γ-eudesmol	1626			1.12
EPI?α-muurolol	1640			2.58
β-eudesmol	1644			0.77
α-cadinol	1651			4.77
(E,E) farnesol	1720		1.57	
Total		75.28	97.34	95.77

[a]MW = molecular weight. [b]RI = retention index. [c]Results expressed as % of area.

Antimicrobial activity tests

properties, however with restrict action against serotypes ETEC 5041-1 and mainly against EPEC 0031-2, which demonstrate a more susceptibility from these strains to essential oils.

The negative results obtained against the gram-negative bacteria by the rest of the plants were not surprising as, in general, these bacteria are more resistant than Gram-positive bacteria (Kudi et al., 1999).

Oils with good antimicrobial potential were subjected to GC and GC-MS analyses (Table 3). The majority constituents were identified using the data sources available. Among the identified compounds, some were previously reported to have antimicrobial activity, including 1,8-cineole, limonene and linalool (Mazzanti et al., 1998) and germacrene-D (Ngassapa et al., 2003).

The results of the present study indicate that the essential oils obtained from 3 out of 28 plants commonly used in Brazilian folk medicine had good anti-*E. coli* activity. The essential oils from *A. triphylla*, *C. martini* and *C. winterianus* presented strong activity against *E. coli* serotypes, with good oil yield. This study corroborates the importance of ethnopharmacology survey data in the selection of plants for bioactivity screening representing an expressive contribution to the characterization of the anti-*E. coli* activity of traditional Brazilian medicinal plants, as previous studies have not focused into this type of assays. Subsequently, bioguided fractionation will be conducted to the potential plants for identification of the active compounds.

ACKNOWLEDGEMENT

Research was supported by a grant from FAPESP (SP, Brazil).

REFERENCE

Adams, R.P., 2001. Identification of essential oils components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Illinois, USA.

Aligiannis, N., Kalpotzakis, E., Mitaku, S., Chinou, I.B., 2001. Composition and antimicrobial activity of the essential oils of two *Origanum* species. Journal of Agricultural and Food Chemistry 40, 4168-4170.

Clarke, S.C., 2001. Diarrhoeagenic *Escherichia coli* – an emerging problem? Diagnostic Microbiology and Infections Disease 41, 93-98.

Cid, D., Blanco, M., Blanco, J.E., Quiteira, J.A.R.S., Fuente, R., Blanco, J. 1996. Serogroups, toxins and antibiotic resistance of *Escherichia coli* strains isolated from diarrhoeic goat kids in Spain. Veterinary Microbiology 53, 349-354.

Ellof, J.N.,1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711-713.

Kudi, A.C., Umoh, J.U., Eduvie, L.O., Gefu, J., 1999. Screening of some Nigerian medicinal plants for antibacterial activity. Jounal of Ethnopharmacology 67, 225-228.

Lorenzi, H., Matos, F. J.A., 2002. Plantas medicinais do Brasil: nativas e exóticas cultivadas. Instituto Plantarum, 512 pp.

Mazzanti, G., Battinelli, L., Salvatore, G., 1998. Antimicrobial properties of the linalool-rich essential oil of *Hyssopus officinalis* L. var *decumbens* (Lamiaceae). Flavour and Fragrance Journal 13, 289-294.

Ngassapa, O., Runyoro, D.K.B., Harvala, E., Chinou, I.B., 2003. Composition and antimicrobial activity of essential oils of two populations of Tanzanian *Lippia javanica* (Burm.f.) Spreng. (Verbenaceae). Flavour and Fragrance Journal 18, 221-224.

Schmidt, H., Henkel, B., & Karch, H., 1997. A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. FEMS Microbiology Letters 148, 265-272.