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In vitro antibacterial activities of the essential oils of aromatic plants against *Erwinia herbicola* (Lohnis) and *Pseudomonas putida* (Kris Hamilton)

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Abstract: This study was designed to examine the *in vitro* antibacterial activities of the essential oils extracted from 53 aromatic plants of the Gorakhpur Division (UP, INDIA) for the control of two phytopathogenic bacteria, namely *Erwinia herbicola* and *Pseudomonas putida*, which cause several post-harvest diseases in fruits and vegetables. Out of the 53 oils screened, 8 oils, *i.e.*, *Chenopodium ambrosioides*, *Citrus aurantium*, *Clausena pentaphylla*, *Hyptis suaveolens*, *Lippia alba*, *Mentha arvensis*, *Ocimum sanctum* and *Vitex negundo*, completely inhibited the growth of the test bacteria. Furthermore, the minimum inhibitory concentration (*MIC*) and the minimum bactericidal concentration (*MBC*) values of *C. ambrosioides* oil were lower for *E. herbicola* (0.25 and 2.0 μl ml^{-1}) and *P. putida* (0.12 and 1.0 μl ml^{-1}), respectively, than those of the other 7 oils, as well as than those of agromycin and streptomycin, the drugs used in the current study. Gas chromatography (GC) and GC–mass spectroscopy (GC–MS) analysis of the *Chenopodium* oil revealed the presence of 125 major and minor compounds, of which 14 compounds were recognized. The findings led to the conclusion that *Chenopodium* oil may be regarded as a safe antibacterial agent for the management of post-harvest diseases of fruits and vegetables.

Keywords: phytopathogenic bacteria; *Chenopodium ambrosioides* oil; GC/GC–MS.

INTRODUCTION

Bacterial pathogens and their control are serious problems in agricultural practices. They cause great damage to many fruits and vegetables during transit and storage, amounting to 30–40 % losses and even much higher in some developing countries.[1] In traditional societies, appreciation of medicinal and aromatic

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plants was based largely on benefits to human beings, including those of pest control. For a long time, plants have been a valuable source of natural products for maintaining food commodities, and have proved themselves the best substitutes for synthetic drugs.[2] Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides. The use of plant essential oils and phytochemicals, both with known antibacterial properties, is of great consequence. In the past few years, investigations have been conducted worldwide to prove antimicrobial, antioxidant, anti-inflammatory, cytotoxic, antinociceptive and anti-malarial behaviour of plant essential oils.[3–5] In recent years, several reports have been published on the use of numerous plant by-products that possess antimicrobial properties towards a number of plant and human pathogenic bacteria.[6,7] Essential oils have been investigated for their antibacterial properties and were proved to be better substitutes for synthetic antibiotics due to their non-phytotoxic,[3] more systemic, renewable and easily biodegradable nature.[1] As a part of systematic research on the antibacterial properties of Indian plants, the present study deals with the evaluation of 53 essential oils for their antibacterial activities towards two test bacteria, *i.e.*, *Erwinia herbicola* and *Pseudomonas putida*. In addition, the *MIC* and *MBC* values of the more toxic oils were determined. To ascertain the chemical constituents of the most potent oil, gas chromatography (GC) and GC–mass spectroscopy (GC–MS) analysis were performed.

EXPERIMENTAL

Reference strains

The bacterial cultures used in the present work, *E. herbicola* (MTCC 3609) and *P. putida* (MTCC 1190), were procured from the Institute of Microbial Technology, Chandigarh, India. The purity of the bacterial strains was clarified using MM2Cu medium[8] for *E. herbicola* and King's B medium[9] for *P. putida*. The stock cultures were maintained on nutrient agar (NA) and Luria Bertani agar (LBA) slants at 4 °C and sub-cultured monthly. Working cultures were prepared by inoculating a loopful of each test microorganism in 3 ml of nutrient broth (NB) from NA/LBA slants. Broths were incubated at 37±2 °C for 12 h. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standards (10^8 cfu ml^{-1}).

Plant collection and essential oil extraction

The plant samples were collected from different forests and forest ranges of Gorakhpur Division during 2009–2010, brought to the laboratory and identified with the help of literature[10] and Departmental herbarium of Gorakhpur University, India. The plant collecting sites were located in the eastern part of Uttar Pradesh, India between latitude 27°05' and 27°25' North and longitude 83°20' and 84°10' East at an elevation of about 91 m above sea level. Voucher specimens of individual species were deposited in the Botanical Survey of India (NRC) Dehradun, India. Each fresh plant material (300 g) was separately submitted for hydrodistillation in a Clevenger's apparatus for 4 h to derive the volatile constituents in the form of essential oils. Each volatile oil was dried over anhydrous sodium sulphate and then kept separately in sealed clean glass vials at 4 °C until needed.

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Disc diffusion bioassay

The disc diffusion method of Andrews[11] was adopted for the antibacterial bioassay. The target bacterial suspensions (0.1 ml) containing 1×10^8 cfu ml^{-1} inocula were spread using a sterile spreader on Nutrient Agar (*P. putida*) and Wilbrink Agar (WA) (sucrose, 10 g l^{-1}, bacto-peptone, 5 g l^{-1}, K_2HPO_4, 0.5 g l^{-1}, $MgSO_4\cdot7H_2O$, 0.25 g l^{-1} and agar, 18 g l^{-1}) medium (*E. herbicola*). Sterile Whatman filter paper discs (6 mm diameter) soaked with 5 µl (v/v) of each essential oil were aseptically positioned separately in the centre on the bacterial suspension-seeded plates in order to define the toxicity of the volatiles in terms of the inhibition zone. The treatments also included agromycin and streptomycin. The inoculated plates were incubated at 37±2 °C for 24–72 h and the zone of inhibition, if any, around the discs were measured in mm. The experiments were replicated three times. The data were statistically analyzed.

Agar dilution bioassay

The performed agar dilution susceptibility test was based on modified methods of NCCLS and CLSI[12,13] to determine the minimum inhibitory concentration (*MIC*) of the potent oils. A series of dilutions of each potent oil and antibiotic in a final concentration ranging from 0.06–16 µl ml^{-1} for the oils and 0.06–16 µg ml^{-1} for the antibiotics were prepared in a NA or WA plate, depending on the bacterial species. Tween 80 was used as an emulsifier to ensure proper mixing of the oils with the medium. After solidification, the plates were aseptically spotted with 5 µl of overnight-grown bacterial cultures containing approximately 1×10^8 cfu ml^{-1} inocula. Plane media plates inoculated with the bacteria served as the positive control and a non-inoculated plate served as the negative control. The plates were incubated at 37±2 °C for 24–72 h. The inhibition of the bacterial growth was compared with the growth in the control plates. The *MIC* was defined as the lowest concentration of the oil inhibiting the visible growth of each bacterium on the agar plate. Furthermore, the poisoned plates showing no growth were sub-cultured onto fresh medium (without oil and antibiotic) for determination of the minimum bactericidal concentration (*MBC*).[14] The least concentration from which the bacteria did not recover growth on the fresh medium was considered as the *MBC*. Each test was replicated three times.

Gas chromatographic analysis of the Chenopodium *oil*

About 0.1 µl of pure oil sample was subjected to GC and GC–MS analysis. The GC was composed of an Agilent Technology 6890 N gas chromatograph data handling system equipped with a split–splitless injector and fitted with a flame ionization detector (FID) using N_2 as the carrier gas. The column was a HP-5 capillary column (30 m×0.32 mm, 0.25 µm film thickness) and the employed temperature program was as follows: initial temperature of 60 °C (hold: 2 min) programmed at a rate of 3 °C min^{-1} to a final temperature of 220 °C (hold: 5 min). The temperatures of the injector and FID were maintained at 210 and 250 °C, respectively.

Gas chromatography–mass spectrometry analysis of the Chenopodium *oil*

The GC–MS analysis was performed using a Perkin Elmer Clarus 500 GC/MS, coupled with an RTX-5 capillary column (60 m×0.32 mm, film thickness: 0.25 µm). The carrier gas was helium (1 ml min^{-1}), the injector temperature was 210 °C, and the oven temperature was programmed from 60 to 210 °C at a rate of 3 °C min^{-1} and finally held isothermally for 15 min. The ionization of the sample components was performed in the EI mode of 70 eV.

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Qualitative and quantitative analysis

Identification of the individual components was realized by matching their recorded mass spectra with those in a library (NIST/Pfleger/Wiley) provided by the instrument software, and by comparing their calculated retention indices with a GC alkane standard solution (C_8–C_{20}) as well as literature values.[15] The relative percentage area of the individual components was obtained from the GC–FID analysis.

RESULTS

Disc diffusion bioassay

The presence of a fluorescent pigment on the King's B medium and a yellowish pink colour on the MM2Cu medium showed that the procured bacteria were *P. putida* and *E. herbicola*, respectively. The present study was an attempt to investigate and evaluate 53 essential oils against plant pathogenic bacteria. The antibacterial spectra of each essential oil showing the zone of inhibition for each test bacteria are presented in Table I. All the essential oils showed more or less antagonist activity against the test pathogens while no inhibition zone was incurred by *Cleome gynandra* and *Cyperus brevifolius* oils against *E. herbicola* and *Hygrophila*, *Leonotis*, *Leonurus* and *Melia* oils against *P. putida*. The most successful results were obtained with *Chenopodium*, *Citrus aurantium*, *Clausena*, *Hyptis*, *Lippia*, *Mentha*, *Ocimum sanctum* and *Vitex* oils that completely inhibited the growth of both test bacteria at the recommended concentration. Surprisingly, *Citrus aurantifolia* completely inhibited the growth of *E. herbicola* only. Among the other oils, *Acorus calamus*, *Anisomeles indica*, *Clerodendrum inermae*, *C. viscosum* and *Curcuma zedoaria* were superior to the antibiotics against both test bacteria, having a zone of inhibition ranging from 14.0–25.8 mm.

TABLE I. Antibacterial potency of the essential oils of aromatic plants of the Gorakhpur Division (L: leaf, T: twig, W: whole plant, R: rhizome)

Plant species (essential oil)	Family	Parts used	Zone of inhibition, mm[a]	
			E. herbicola	*P. putida*
Acorus calamus Linn.	Araceae	R	14.0±2.1	15.3±1.2
Adhatoda vasica Ness	Acanthaceae	L	10.3±1.0	1.9±0.4
Aegle marmelos (L) Corr.	Rutaceae		14.3±3.8	10.9±1.9
Anethum graveolens Linn.	Apiaceae	T	8.4±1.5	10.4±0.5
Anisomeles indica (L.) Kuntz	Lamiaceae	L	24.2±2.2	23.2±0.1
Ashphodelus tenuifolius Cav.	Liliaceae	W	3.0±0.7	5.7±1.0
Callicarpa macrophylla (L.) Vahl.	Verbenaceae	L	10.4±4.3	16.1±5.7
Callistemon lanceolatus (R.Br.) DC	Myrtaceae		7.2±0.6	18.9±2.7
Cannabis sativa Linn.	Cannabinaceae	T	12.0±1.2	10.5±3.8
Chenopodium ambrosioides Linn.	Chenopodiaceae		#[b]	#
Citrus aurantifolia (Christm) Swingle	Rutaceae	L	#	12.1±0.9
C. aurantium Linn.			#	#
C. limon (L.) Burm			7.3±2.1	10.9±3.6
Clausena pentaphylla (Roxb.)DC			#	#

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TABLE I. Continued

Plant species (essential oil)	Family	Parts used	Zone of inhibition, mm[a]	
			E. herbicola	*P. putida*
Cleome gynandra (L.) Briq.	Capparidaceae	T	No inhibition	7.1±1.2
Clerodendrum inermae (L.) Gaertn.	Verbenaceae	L	16.0±2.5	17.1±1.9
Clerodendrum viscosum (L.) Vent.		L	17.6±0.7	14.9±1.4
Colebrookea oppositaefolia Sm.	Lamiaceae	L	13.0±0.5	9.8±1.6
Curcuma aromatica Salisb.	Zingiberaceae	L	7.2±0.9	9.8±1.2
C. zedoaria Rosc.		R	25.8±0.3	25.2±1.9
Cyperus brevifolius (Rottb.) Hassk	Cyperaceae	W	No inhibition	12.5±2.9
C. monocephalus Endl.			6.3±2.7	6.6±1.9
C. rotundus Linn.			3.1±0.9	6.1±1.3
C. triceps (Rottb.) Endl.			3.4±0.9	8.6±0.5
Eugenia heyneana (L.) Wall	Myrtaceae	L	15.9±1.1	11.8±0.4
Glycosmis pentaphylla (Retzz.) Corr; Hook.	Rutaceae		7.2±2.0	3.4±1.2
Hygrophila difformis Linn.	Acanthaceae	T	14.2±1.3	10.8±3.7
H. pinnatifida Linn.			3.2±2.7	No inhibition
Hyptis suaveolens (L.) Poit.	Lamiaceae		#	#
Lantana camara Linn.	Verbenaceae	L	9.1±1.3	7.9±3.1
L. indica Roxb.			11.8±0.3	9.0±2.3
Leonotis nepetaefolia R.Br.	Lamiaceae		7.8±0.6	No inhibition
Leonurus sibiricus Linn.		T	2.3±1.8	No inhibition
Leucas aspera Spreng.			5.8±0.1	3.8±0.6
L. cephalotes Spreng.			11.3±3.1	13.2±2.2
Lippia alba Rich.	Verbenaceae	L	#	#
Melia azedarach Linn.	Meliaceae		4.9±2.2	No inhibition
Mentha arvensis Linn.	Lamiaceae		#	#
Murraya koenigii Spreng.	Rutaceae		7.5±0.8	1.70±0.53
M. paniculata (L.) Jack	–		2.9±0.1	4.8±0.9
Ocimum basilicum Linn.	Lamiaceae		9.9±2.8	24.6±3.4
O. canum Sims.		T	6.7±0.8	7.2±3.2
O. gratissimum Linn.		L	2.9±0.3	6.6±0.4
O. sanctum Linn.			#	#
Piper longum Linn.	Piperaceae		10.2±1.6	13.1±1.2
P. sylvaticum Roxb.	–		7.1±0.6	11.8±1.5
Pogostemon heyneanus Benth.	Lamiaceae		3.4±1.6	3.2±1.0
P. plectranthoides Desf.			3.8±0.6	7.3±1.4
Polygonum glabrum Willd.	Polygonaceae	T	4.2±1.0	1.9±0.4
Putranjiva roxburghii Wall.	Euphorbiaceae	L	6.0±1.8	8.3±0.9
Salvia plebeia R.Br.	Lamiaceae	T	2.6±0.3	5.2±2.0
Vitex negundo Linn.	Verbenaceae	L	#	#
Zingiber officinale Rosc.	Zingiberaceae	R	5.1±3.0	14.2±3.0
Agromycin 20 µg			12.5±2.4	11.88±1.2
Streptomycin 10 µg			13.0±2.0	16.7±3.7

[a]Values given are the mean of three replicates ± standard deviation; [b]oils caused complete inhibition of the bacteria

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Agar dilution bioassay

The determination of the *MIC* and *MBC* values of the oils that completely inhibited the growth of the test bacteria showed that *Chenopodium* oil was highly effective with the lowest *MIC* and *MBC* values against *E. herbicola* (0.25 and 2.0 μl ml^{-1}) and *P. putida* (0.12 and 1.0 μl ml^{-1}) followed by *Clausena* and *Mentha* oils which had *MIC* and *MBC* values of 0.50 and 4.0 μl ml^{-1} for *E. herbicola*, and 0.25 and 2.0 μl ml^{-1} for *P. putida*, respectively (Table II). *E. herbicola* was susceptible to *C. aurantium* and *P. putida* to *Hyptis*, *Lippia* and *Vitex* oil (in terms of their *MIC* values) but *MBC* values could not be obtained within the recommended assay range. Of the antibiotics, streptomycin was more effective than agromycin against both test bacteria.

TABLE II. *MIC* and *MBC* of potent oils and the antibiotics against phytopathogenic bacteria

Oil/antibiotic	*E. herbicola*		*P. putida*	
	MIC[a]	*MBC*[a]	*MIC*	*MBC*
C. ambrosioides	0.25	2.0	0.12	1.0
C. aurantium	8.0	Static	4.0	Static
C. pentaphylla	0.50	4.0	0.25	2.0
H. suaveolens	2.0	16.0	8.0	Static
L. alba	4.0	Static	4.0	Static
M. arvensis	0.50	4.0	0.25	2.0
O. sanctum	2.0	8.0	1.0	4.0
V. negundo	4.0	16.0	8.0	Static
Agromycin	4.0	16.0	2.0	8.0
Streptomycin	8.0	Static	4.0	8.0

[a]Values in μl ml^{-1} for the oils and μg ml^{-1} for the antibiotics

Chemical constituents of Chenopodium *oil*

GC and GC–MS analysis of the *Chenopodium* oil led to the recognition of fourteen compounds comprising 97.70 % of the oil sample, with *α*-terpinene (37.74 %), *p*-cymene (16.71 %), limonene (1.90 %), ascaridole (38.03 %) and isoascaridole (2.55 %) being the major components (Table III).

TABLE III. Chemical constituents of *C. ambrosioides* oil

No.	Component	Kovats index	Composition, %
1	*α*-Thujone	924	0.01
2	*α*-Pinene	932	0.01
3	Sabinene	969	0.02
4	*β*-Myrcene	974	0.04
5	*α*-Terpinene	1014	37.74
6	*p*-Cymene	1020	16.71
7	Limonene	1024	1.90
8	*γ*-Terpinene	1054	0.34
9	Myrcenol	1103	0.02

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TABLE III. Continued

No.	Component	Kovats index	Composition, %
10	*trans*-Pinene hydrate	1139	0.14
11	Camphor	1143	0.04
12	Ascaridole	1237	38.03
13	*trans*-Chrysanthenyl acetate	1262	0.15
14	Isoascaridole	1390	2.55
Total			97.70

DISCUSSION

The test bacteria *E. herbicola* and *P. putida* used in the current study are widespread in nature as bio-control agent.[16,17] However, some strains of these bacteria have evolved into plant pathogens that cause several post-harvest evils in fruits and vegetables, resembling lesions to rot of alfalfa (*Medicago sativa* L.), internal rot of peach (*Prunus persica* L.), apricot (*Prunus armeniaca* L.), plum (*Prunus cerasifera* Ehrh.) and apple (*Malus domestica* Borkh) fruits[18] and internal discoloration to tomato (*Lycopersicon esculentum* Mill),[19] rot of potato (*Solanum tuberosum* L.).[20]

Plant based antimicrobial compounds have enormous therapeutic potential as they can serve their purpose without any of the side effects that are often associated with synthetic antimicrobials. The plants screened in the present study are used to treat various gastrointestinal disorders, diarrhoea, dysentery, cough cold and asthma.[21] A large number of volatile and non-volatile plant products have been evaluated for their toxicity against human and plant pathogenic bacteria.[22,23] However, the literature seems to be lacking in reports on the potentiality of such volatiles with respect to *E. herbicola* and *P. putida*. In the present study, some essential oils, such as *Ashphodelus tenuifolius*, *Callicarpa macrophylla*, *Clausena pentaphylla*, *Colebrookea oppositaefolia*, *Cyperus* spp., *Curcuma aromatica*, *Glycosmis pentaphylla*, *Hygrophila* spp., *Pogostemon* spp. and *Polygonum glabrum*, were subjected to preliminary screening for their antibacterial potency. It is clear from the presented results that 8 oils, namely *Chenopodium ambrosioides*, *Citrus aurantium*, *C. pentaphylla*, *Hyptis suaveolens*, *Lippia alba*, *Mentha arvensis*, *Ocimum sanctum* and *Vitex negundo*, exhibited pronounced activities against both tested bacteria through complete inhibition of their colony growth. This shows the variations in the active ingredients of the essential oils. The absence of antibacterial activities of *Cleome*, *Cyperus brevifolius*, *Leonotis*, *Leonurus*, *Hygrophila pinnatifida* and *Melia* oils indicates the poorly toxic active substances in these oils, which were less toxic than the standard antibiotics used in this study.

The determination of bactericidal and bacteriostatic concentrations are more sensitive techniques than the disc diffusion technique, which was used purely as a screening tool, to eliminate those oils with no or very slight inhibitory activities

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against the test pathogens.[24] The obtained results illustrate that of the 53 screened oils, 8 oils have a greater inhibitory effect, hence, they were considered for *MIC* and *MBC* determination. Additionally, during the *MIC* determination, the addition of the emulsifying agent Tween 80 introduced an extra component with respect to the activity and possible interaction, its use was unavoidable.[25] Earlier the oils of *Chenopodium*, *Citrus*, *Clausena*, *Hyptis*, *Lippia*, *Mentha*, *Ocimum* and *Vitex* were reported to be strong fungicidal agents[26–30] and are now being reported as strong antibacterial agents against both the studied phytopathogenic bacteria. The present findings are also in accordance with Vasinauskiene *et al.*[31] who found streptomycin to be a poorer antibacterial agent than essential oils against several phytopathogenic bacteria. These might be due to the presence of monoterpenes, sesquiterpenes, phenolic compounds, ketones and coumarins.[32] *Chenopodium* oil was the most toxic and was found to be bacteriostatic and bactericidal in nature at lower concentration than the other potent oils.

Essential oil from the fresh plant part of *C. ambrosioides* was previously found to contain ascaridole (40–70 %) as the major component, followed by *α*--terpinene, *p*-cymene, glycol and isoascaridole.[33] Similarly in the present research, *α*-terpinene and ascaridole were found in higher percents, followed by *p*--cymene and isoascaridole. In another investigation, *cis*-ascaridole was found as a major peroxy-monoterpenoid (up to 46.9 %) in the oil. Three minor isomers *cis*-isoascaridole (1.1–6.4 %), *trans*–ascaridole (1–2 %) and *trans*-isoascaridole (1–2 %) were also detected.[34] However, no such components were detected in the current study. This may be due to the period of isolation and the places of origin.

The herb *C. ambrosioides* (Chenopodiaceae) is a plant widely known in popular medicine as anthelminthic, vermifuge, emmenagogue and abortifacient.[21] It is used for the treatment of digestive, respiratory, uro–genital, vascular and nervous disorders, for metabolic disturbances such as diabetes and hypercholesterolemia and as a sedative, antipyretic and antirheumatic.[34] The oil of the plant contains ascaridole as a major component. Ascaridole (also known as ascarisin; 1,4-epidioxy-*p*-menth-2-ene) is a bicyclic monoterpene that has an unusual bridging peroxide functional group. Ascaridole has been documented with sedative and pain-relieving properties as well as antifungal effects, and was found to be a potent inhibitor of the *in vitro* development of *Plasmodium falciparum*, *Trypanosoma cruzi* and *Leishmania amazonensis*.[35,36] The findings are thus a hint that ascaridole may be an interesting novel candidate drug against several pathogens.

It has been shown that essential oils from different plants possessed a wide range of antibacterial properties because they inhibited the growth and the diameter of inhibition varied depending upon the susceptibility of the test organisms. The increased awareness of the environmental problems associated with conventional non-biodegradable agrochemicals has led to the search for non-con-

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ventional chemicals of biological origin for the management of post-harvest disease in fruits and vegetables. The observed antibacterial properties of *C. ambrosioides* essential oil show its potential for the practical use of the essential oil towards plant pathogenic bacteria as a natural bactericide. The obtained results suggest that the use of *Chenopodium* oil as antibacterial agent may be judiciously applied to prevent the decay of fruits and vegetables due to bacteria.

CONCLUSIONS

To best of our knowledge, this is the first study to provide data on the evaluation of essential oils against *E. herbicola* and *P. putida*. According to the obtained results, it is possible to conclude that *C. ambrosioides* has a strong antibacterial activity. The antibacterial activities of *Chenopodium* oil reported herein could be associated with the presence of *α*-terpinene (37.74%), *p*-cymene (16.71 %) and ascaridole (38.03 %). Based on the observed results, *C. ambrosioides* oil could be used as a preservative in food products, to protect them from microbial spoilage.

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ИЗВОД

АНТИБАКТЕРИЈСКА АКТИВНОСТ ЕТАРСКИХ УЉА АРОМАТИЧНИХ БИЉАКА СПРАМ БАКТЕРИЈА *Erwinia herbicola* (LOHNIS) И *Pseudomonas putida* (KRIS HAMILTON)

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У овој студији испитана је *in vitro* антибактеријска активност етарских уља из 53 ароматичне биљке из колекције Универзитета у Горакпуру (Индија) према фитопатогеним бактеријама *Erwinia herbicola* и *Pseudomonas putida*, изазивачима болести воћа и поврћа. Осам уља је инхибирало раст бактерија; из биљака *Chenopodium ambrosioides*, *Citrus aurantium*, *Clausena pentaphylla*, *Hyptis suaveolens*, *Lippia alba*, *Mentha arvensis*, *Ocimum sanctum* и *Vitex negundo*. *MIC* и *MBC* вредности за уље из *C. ambrosioides* су биле најниже; за *E. herbicola* 0,25 и 2,0 μl ml^{-1} и за *P. putida* 0.12 и 1.0 μl ml^{-1}. GC и GC–MS анализом уља биљке *Chenopodium* нађено је 125 састојака, од којих је 14 идентификовано. Резултати су показали да се уље биљке *Chenopodium* може сматрати погодним антибактеријским агенсом за третирање болести воћа и поврћа.

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REFERENCES

1. L. Claflin, *Control of Pseudomonas syringae pathovars*, in *Pseudomonas syringae and related pathogens*, N. S. Iacobellis, A. Collmer, S. W. Hutcheson, J. W. Mansfield, C. E.

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Morris, J. Murillo, N. W. Schaad, D. E. Stead, G. Surico, M. S. Ullrich, Eds., Kluwer, Dordrecht, The Netherlands, 2001, p. 423
2. P. Steiner, in *Fire Blight: The disease and its causative agent, Erwinia amylovora*, J. L. Vanneste, Ed., CABI Publishing, New York, 2000, p. 339
3. N. N. Tripathi, N. Kumar, *J. Stored Prod. Res.* **43** (2007) 435
4. S. Dordevic, S. Petrovic, S. Dobric, M. Milenkovic, D. Vucicevic, S. Zizic, J. Kukic, *J. Ethnopharmacol*. **109** (2007) 458
5. T. Patharakorn, T. Arpornsuwan, N. Nuanchawee Wetprasit, A. Promboon, S. Ratanapo, *J. Med. Plants Res*. **4** (2010) 837
6. P. L. Cantore, N. S. Iacobellis, A. D. Marco, F. Capasso, F. Senatore, *J. Agric. Food Chem*. **52** (2004) 7862
7. R. Karami-Osboo, M. Khodaverdi, F. Ali-Akbari, *J. Agric. Sci. Tech*. **12** (2010) 35
8. S. Bereswill, S. Jock, P. Bellemann, K. Geider, *Plant Dis*. **82** (1998) 158
9. E. O. King, M. K. Ward, D. E. Raney, *J. Lab. Clin. Med*.**44** (1954) 301
10. T. N. Srivastava, *Flora Gorakhpurensis*, Today & Tomorrow Printers and Publishers, New Delhi, 1976
11. J. M. Andrews, *J. Antimicrob. Chemother*. **48** (Suppl. S1) (2001) 43
12. National Committee for Clinical Laboratory Standards (NCCLS), *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, Approved Standard - NCCLS document 6th ed., M7-A6, Wayne, PA, USA, 2003
13. Clinical and Laboratory Standards Institute (CLSI), *Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, Approved standard - 8th ed., CLSI document M07-A8, Wayne, PA, USA, 2009
14. A. K. Mishra, A. Mishra, S. Tripathi, N. N. Tripathi, *J. Microbial World* **10** (2008)108
15. R. P. Adams, *Identification of essential oils components by gas chromatography/mass spectroscopy*, Allured Pub Corp., Carol Stream, IL, 2007
16. J. Handelsman, W. J. Brill, *Appl. Environ. Microbiol*. **49** (1985) 818
17. P. D. Colyer, M. S. Mount, *Plant Dis*. **68** (1984) 703
18. G. T. I. Abdel, S. A. M. El-Sadek, A. A. Galal, J. D. Janse, *Egypt. J. Microbiol*. **36** (2001) 477
19. A. A. Galal, in *Proceedings of 10th Congress of Phytopathology*, Giza, Egypt, 2003, p. 325
20. D. K. Pandey, A. Asthana, N. N. Tripathi, S. N. Dixit, *Indian Perfumer* **25** (1982) 10
21. S. P. Ambasta, *The Useful Plants of India*, National Institute of Science Communication Dr K. S. Krishnan Marg, New Delhi, India, 2006
22. T. Mangena, N. Y. O. Muyima, *Lett. Appl. Microbiol*. **28** (1999) 291
23. S. Kizil, F. Uyar, *Asian J. Chem.* **18** (2006) 1455
24. A. Smith-Palmer, J. Stewart. L. Fyfe, *Lett. Appl. Microbiol*. **26** (1998) 118
25. A. M. Janssen, J. J. C. Scheffer, A. B. Svendsen, *Planta Med*. **53** (1987) 395
26. D. K. Pandey, N. N. Tripathi, R. D. Tripathi, S. N. Dixit, *J. Plant Dis. Prot*. **89** (1981) 344
27. N. K. Dubey, K. S. Bhargava, S. N. Dixit, *Int. J. Trop. Plant Dis*. **1** (1983) 177
28. R. Kumar, A. K. Mishra, N. K. Dubey, Y. B. Tripathi, *Int. J. Food Microbiol*. **115** (2007) 159
29. R. Kumar, N. K. Dubey, O. P. Tiwari, Y. B. Tripathi, K. K. Sinha, *J. Sci. Food Agric*. **87** (2007) 1737

2012 Copyright (CC) SCS

30. A. K. Pandey, P. Singh, U. T. Palni, N. N. Tripathi, in *Proceedings of* 33rd *Conference of Indian Botanical Society and International Symposium on the New Horizons of Botany*, (2010), Shivaji University, Kolhapur (MS), India, 2010, p. 19
31. M. Vasinauskiene, J. Radusiene, I. Zitikaite, E. Surviliene, *Agronomy Res*. **4** (2006) 437
32. M. M. Cowan, *Clin. Microbiol. Rev*. **12** (1999) 564
33. J. F. Cavalli, F. Tomi, A. F. Bernardini, J. Casanova, *Phytochem. Anal*. **15** (2004) 275
34. V. Dembitsky, I. Shkrob, O. L. Hanus, *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub*. **152** (2008) 209
35. Y. Pollack, R. Segal, J. Golenser, *Parasitol. Res*. **76** (1990) 570
36. L. Monzote, A. M. Montalvo, S. Almanonni, R. Scull, M. Miranda, J. Abreu, *Chemotherapy* **52** (2006) 130.

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