JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Antioxidant and Repellent Activities of the Essential Oil from Colombian *Triphasia trifolia* (Burm. f.) P. Wilson

Beatriz E. Jaramillo Colorado,* Irina P. Martelo, and Edisson Duarte

Agrochemical Research Group, Chemical Program, Faculty Exact and Natural Sciences, University of Cartagena, Campus of Zaragocilla, Cartagena, Colombia

ABSTRACT: The chemical composition of essential oils isolated from aerial parts of *Triphasia trifolia* (Burm. f.) P. Wilson was analyzed using hydrodistillation by GC−MS. The main constituents found were β-pinene (64.36%), (+)-sabinene (8.75%), hexadecanoic acid (6.03%), α-limonene (4.24%) and *p*-cymene (2.73%). The essential oil from *T. trifolia* shows high antioxidant potential (94.53%), an effect that is comparable with ascorbic acid (96.40%), used as standard. In addition, these oils had high repellent effects on the insect *Tribolium castaneum* Herbst (99% ± 1) at 0.2 μL/cm^2 after 2 h of exposure.

KEYWORDS: *essential oils, repellent activity, Triphasia trifolia (Burm. f.) P. Wilson, antioxidant activity*

INTRODUCTION

Triphasia trifolia (Burm. f.) P. Wilson is commonly known as "limoncito", lemon red and sweet lemon. It belongs to the Rutaceae family and is a shrub about 2 m high. The leaves are trifoliate with a length setting of 2 to 4 cm; its flowers are white with oblong petals 1.2−1.6 cm long, its fruits are red at maturity, with thin skin and mucilaginous pulp.[1] Infusions of the leaves of this plant have been used to treat parasitic diseases,[2] respiratory disorders, influenza and intestinal pain.[3,4] Rasgoti et al.[5] reported the antibacterial activity of the plant.

The study of essential oils as basic raw materials for pharmaceuticals and food has become one of the most important areas of research and development for many countries; initially considered as waste material from the metabolism of plants, their importance has been recognized only recently.[6] Moreover, an important area in the field of natural products is the determination of biological activity of the plants and their extracts. Many of the chemical compounds present in vegetables are sources of antioxidants, which the pharmacology and food industries can use to neutralize the action of the radicals that have been linked to various diseases and rancidity in foods.[7,8]

In living systems, there are many redox reactions essential for life; however, oxidation can also be a source of disease, when the balance between oxidants and antioxidants is lost, which is known as oxidative stress and can cause damage to biomolecules like lipids, proteins, DNA, and others.[8−10] Due to the growing opposition to the employment of synthetic antioxidants in food, researchers have looked for natural products with antioxidant activity, which will allow replacement of synthetic antioxidants or, at least, diminishing of the necessary quantity of them to preserve the food.[11,12] The discovery of certain antioxidant substances in plant species, in which their presence was ignored, would increase the value of the plant species and might turn them into viable sources to obtain the above-mentioned.[13,14]

Several types of essential oils may be useful in keeping bugs away and preventing bites and stings.[15,16] These natural insect repellents can serve as an alternative to synthetic-chemical-based repellents, which may have toxic effects on human health and on the environment. Resistance and toxicity problems of the synthetic insecticides have resulted in the necessity of finding more effective and healthier alternatives. Thus, essential oils are the most tested products presently.[17−19] Different biological activities of plant derivatives have been demonstrated for the control of stored grain pests.[20−22]

Studies have shown that many essential oils have marked antioxidant and repellent activities;[23−25] for these reasons many branches of industry (cosmetics, agrochemicals, toiletries, food products, etc.) have been interested in them. It is very difficult to attribute the antioxidant or repellent effect of an essential oil to one or a few active principles, because an essential oil always contains a mixture of different chemical compounds. In addition to the major compounds, also, minor compounds may make a significant contribution to the oil's activity. For these reasons, it is indispensable to detect and to identify the minority compounds too; still if they are in very low concentrations, the analytical technology most used for this purpose is gas chromatography coupled to a mass spectrometer (GC−MS).[26,27]

The aim of the present work is to determine, by gas chromatography−mass spectrometry (GC−MS), the volatile chemical composition of essential oil of *Triphasia trifolia* (Burm. f.) P. Wilson grown in Bolivar, Colombia, and describe the components' repellent and antioxidant activities.

MATERIALS AND METHODS

Plant Material. *Triphasia trifolia* (Burm. f.) P. Wilson was collected in different areas of the Department of Bolivar, Colombia. The species was identified by Dr. R. J. Francisco at the Institute of Biology, University of Antioquia, Medellin, Colombia, and a voucher specimen (HUA 167360) deposited at the Herbarium of the University of Antioquia (HUA).

Received: October 3, 2011
Revised: May 15, 2012
Accepted: May 18, 2012
Published: May 18, 2012

dx.doi.org/10.1021/jf300461k | J. Agric. Food Chem. 2012, 60, 6364−6368

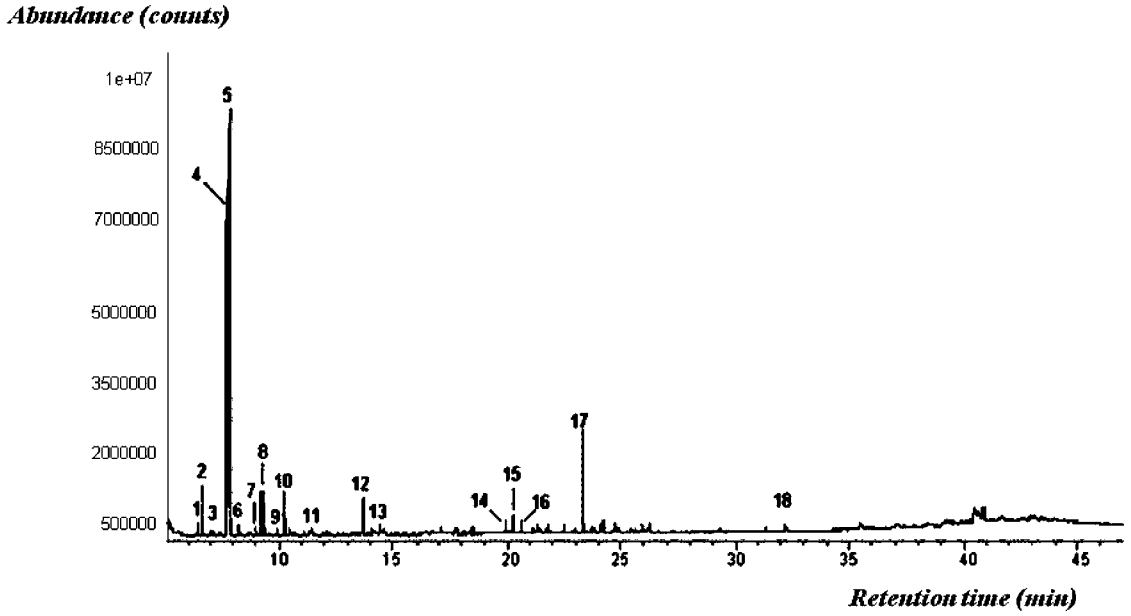

Figure 1. Typical chromatographic profile of the essential oil from *Triphasia trifolia* (Burm. f.) P. Wilson. HP-5MS column (30 m × 0.25 mm i.d. × 0.25 μm d_f), mass selective detector. (See identification of peak numbers in Table 1.)

Extraction of the Essential Oils. Essential oil was obtained by Hydrodistillation (HD) method. It was realized that employment a Clevenger distillation equipment with Dean−Stark reservoir, following the procedure described by Stashenko et al.[28] HD was performed in a 5 L round flask with 500 g of plant material and 3 L of water, using an electric heater (boiling water) for 2 h; then, the essential oil was separated from water by decantation. For GC analysis, 30 μL of essential oil was added to 1.0 mL of dichloromethane. Injection volume: 1 μL. Split 1:30.

Chromatographic Analysis. Essential oils were analyzed on a gas chromatograph, GC 7890 A (Agilent Technologies, Palo Alto, CA), coupled to a Network mass selective detector (MSD) Agilent Technologies 5975 inert GC−MS system (electron impact ionization, EI, 70 eV), equipped with an injection port split/splitless (230 °C, split ratio 30:1). For the separation of the mixtures we used a capillary column HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm d_f), stationary phase 5% phenyl-poly(methylsiloxane); and a DB-WAX (J&W Scientific) (60 m × 0.25 mm i.d. × 0.25 μm d_f), coated with poly(ethyleneglycol). The initial oven temperature was 50 °C for 2 min and then continued at the rate of 5 °C min^{-1} to 250 °C and 5 min at that temperature. The carrier gas used was helium with inlet pressure head of the column of 12.667 psi at a rate of 1.172 mL min^{-1} at 50 °C. The various compounds were identified by comparison of their Kováts retention indices, determined utilizing a linear scale on the HP-5 and DB-WAX column, and of the mass spectra of each GC component with those of standard substances, Wiley library data of the GC−MS system, and literature data.[29]

Antioxidant Activity. The antioxidant activity was evaluated as a measure of the ability to scavenge radicals, by reacting DPPH• (1,1-diphenyl-2-picrylhydrazyl) radicals (Sigma, USA), with potential antioxidants (essential oil) and ascorbic acid (standard substance) (Merck, Germany). The procedure developed is described below:[30]

Two milliliters of a 3.6 × 10^{-5} M ethanolic solution of DPPH was added to 50 μL of an ethanolic solution of the antioxidant. The decrease in absorbance at 517 nm was recorded in a UV−vis spectrophotometer for 16 min. Antioxidant activity is expressed as percentage inhibition, which corresponds to the amount of radical DPPH• offset by essential oils, (inhibition percentage of DPPH• radical, %*I* DPPH•), according to the following equation:

$$\%I\ \mathrm{DPPH}^{\bullet} = \left[\frac{\mathrm{Abs}_0 - \mathrm{Abs}_1}{\mathrm{Abs}_0}\right] \times 100$$

The antioxidant activity was measured at 0,1, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL of *T. trifolia* extracts.

Insect Rearing. The insect colonies were reared at 27 ± 1 °C, 75 ± 5% RH, and dark conditions. Whole oat (*Avena sativa*) was employed to rear *Tribolium castaneum* Herbst.

Bioassay Method. Repellent activity was assessed by using assays on Petri dishes. The repellent effect of the essential oil against *T. castaneum* was tested using the area preference method following the procedure described by Tapondjoua et al.[19] Test areas consisted of filter paper disks (Whatman No. 1) (7 cm diameter, surface 38.5 cm^2) cut in half (19.25 cm^2). On one half were equal volumes of different concentrations of essential oil dissolved in acetone (0.00002, 0.0002, 0.002, 0.02, and 0.2 μL cm^2), and on the other was acetone only as control. These halves were attached again, and a fixed number of insects (20) were released on the center of the paper. The treatments were replicated 4 times, and the numbers of insects present on the control (N_c) and treated (N_t) areas of the disks were recorded after 2 h.

Percentage repellency (PR) values were computed as follows:

$$\mathrm{PR}\,(\%) = [(N_c - N_t/N_c + N_t)] \times 100$$

Statistical Analysis. Means and standard errors (SE) of the samples were calculated. Each treatment was carried out with three replicates. Mean differences were determined by using Fisher's protected LSD test at the 5% level of significance. All statistical analyses were performed using Minitab version 15.

RESULTS AND DISCUSSION

Volatile Chemical Composition. The yield of essential oil from Colombian *Triphasia trifolia* (Burm.) P. Wilson was 0.055% (w/w). Figure 1 presents a typical chromatographic profile of essential oil from *T. trifolia* obtained by HD method. Peak identification and relative amounts of the various compounds present in the volatile fraction appear in Table 1. It shows the main constituents found; they were β-pinene (64.36%), (+)-sabinene (8.75%), hexadecanoic acid (6.03%), α-limonene (4.24%), *p*-cymene (2.73%) and α-pinene (2,38%).

While studies realized in Brazil reported as majority compounds sabinene and pinene in the essential oils from leaves, stems and fruits of *Triphasia trifolia*,[4,31] Pino et al.[32]

Table 1. Chemical Composition of the Essential Oil from *Triphasia trifolia* (Burm. f.) P. Wilson Obtained by Hydrodistillation

peak[a]	compound	IK[b] HP-5	IK[b] DB-Wax	rel peak area[c] (%)
1	α-thujene	911	1030	0.73 ± 0.022
2	α-pinene	917	1038	2.38 ± 0.037
3	camphene	961	1075	0.19 ± 0.001
4	(+)-sabinene	964	1132	8.75 ± 0.046
5	β-pinene	981	1126	64.36 ± 0.714
6	myrcene	988	1154	0.77 ± 0.001
7	*p*-cymene	1020	1272	2.73 ± 0.004
8	α-limonene	1038	1206	4.24 ± 0.005
9	γ-terpinene	1064	1257	0.59 ± 0.032
10	terpinolene	1090	1295	1.04 ± 0.006
11	isoterpinolene	1097	1340	2.32 ± 0.090
12	(+)-*p*-menth-1-en-4-ol (-terpinen-4-ol)	1206	1638	0.79 ± 0.028
13	decanal	1207	1485	0.86 ± 0.321
14	*trans*-caryophyllene	1481	1630	1.40 ± 0.028
15	β-bisabolene	1506	1744	1.56 ± 0.042
16	caryophyllene oxide	1578	1990	0.81 ± 0.028
17	hexadecanoic acid	1964	2860	6.03 ± 0.075
18	octadecenoic acid	2140		0.45 ± 0.233

[a]Peak number in Figure 1. [b]Experimentally determined Kováts indices on the HP-5 and DB-Wax column. [c]Averages of five independent extractions CI = $\bar{x} \pm ts/\sqrt{n}$ (n = 5, 95% confidence).

found germacrene B (16.3%) as principal compound in the Cuban essential oil of *T. trifolia*.

It is well-known that the chemical composition of a substance may vary with harvest time, geographical location, the technique used for extraction, the plant part used and small genetic changes, including local conditions.[33]

Evaluation of the Antioxidant Activity of the Essential Oils. The antioxidant activity of *T. triflolia* essential oil has been evaluated by DPPH radical-scavenging test, commonly used for their ease, speed, and sensitivity. The presence of an antioxidant leads to the disappearance of these radical chromogens. The DPPH radical is scavenged by antioxidants through the donation of hydrogen, which forms the reduced compound, DPPH−H. The color changes from purple to yellow after reduction, which can be quantified by a decrease in the absorbance at 517 nm.[34]

Figure 2 shows that radical DPPH• was neutralized by the essential oil from *Triphasia trifolia* (Burm. f.) P. Wilson, and the maximum inhibition percent of DPPH• was 92.4% (2.0 mg/mL); a comparison was made with ascorbic acid (substance used as a reference antioxidant), where the percentage of inhibition against DPPH• radical was 96.4%.

In recent years, increasing attention has been paid by consumers to the health and nutritional benefits of plant parts. Antioxidant properties of essential oils from many plants have also been of great interest to the food processing industry, since their possible use as natural additives has emerged from a growing tendency to replace synthetic antioxidants with natural ones. The data also suggest that this essential oil may be a candidate for flavoring with functional properties in food or cosmetic products, with particular relevance for supplements in which free radicals are closely implicated. However, the efficacy and safety of this oil need to be further investigated if it is to be used as a natural agent.[35−37]

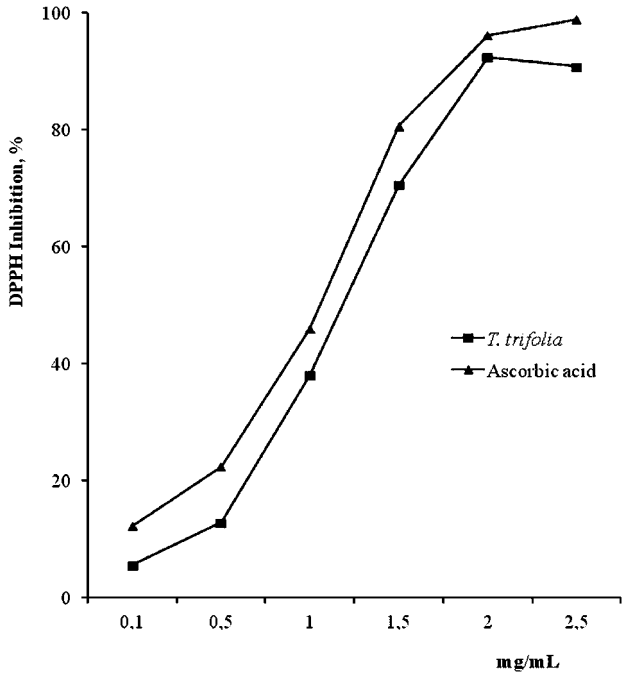

Figure 2. Antioxidant activity of *T. trifolia* essential oil measured by the DPPH• method compared with ascorbic acid.

Repellent Activity. Essential oil from *Triphasia trifolia* (Burm. f.) showed significant pest repellent activity. In Table 2, positive values represent repellency, and negative show attractant activity. The oil was repellent against *Tribolium castaneum*, highly significant at 0.2 μL/cm^2 concentration when the repellency was 99%. Thus, *T. trifolia* essential oil has potential for use with at least some stored-product insects as a repellent.

Table 2. Percentage Repellency (PR) after Two Exposure Times for Essential Oils of *Triphasia trifolia* against *Tribolium castaneum*

concentration (μL/cm^2)	repellency, % 2 h	repellency, % 4 h
0.00002	3 ± 9	1 ± 6
0.0002	14 ± 11	11 ± 12
0.002	46 ± 5	42 ± 6
0.02	69 ± 8	64 ± 8
0.2	99 ± 1**[a]	93 ± 5**
RD_{50}	0.0028 (0.006−0.0014)	0.0043 (0.009−0.0021)

[a]**, statistically significant difference between the number of organisms in treated and untreated areas, using the paired t test (P < 0.001).

A study by Ukeh and Umoetok[20] revealed that five monoterpenoids, (*R*)-linalool, 1,8-cineole, (*S*)-2-heptyl acetate, (*S*)-2-heptanol and citral, which are natural components of the essential oils of *Aframomum melegueta* (K. Schum) and *Zingiber officinale* (Roscoe), were tested at the ratios in which they occur naturally for repellent activity against *Tribolium castaneum* (Herbst).

Some monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents of a number of essential oils

dx.doi.org/10.1021/jf300461k | J. Agric. Food Chem. 2012, 60, 6364−6368

described in the literature, as presenting mosquito repellent activity,[38−41] although repellent properties of several essential oils regularly appear to be associated with the presence of monoterpenoids and sesquiterpenes.[19,20,42,43]

In conclusion, the antioxidant and repellent activities in vitro exhibited by the essential oil from *Triphasia trifolia* (Burm. f.) P. Wilson were high, making this aromatic plant a promising source of natural antioxidants and repellents.

AUTHOR INFORMATION

Corresponding Author

*Chemistry Program, University of Cartagena, Campus of Zaragocilla, Cartagena, Colombia. Fax: 57-5-6698180. E-mail: beatrizjaramilloc@yahoo.com; bjaramilloc@unicartagena.edu.co.

Funding

This work was funded by University of Cartagena (Cartagena, Colombia).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the University of Cartagena, Colombia, especially the members of the Agrochemical Researches Group. We also thank Dr. Maritza Villalobos, Dr. Francisco Roldán and Irina Tirado.

REFERENCES

(1) Gurib-Fakim, A.; Sewraj, M. D.; Narod, F.; Menut, C. Aromatic plants of Mauritius: volatile constituents of the essential oils of *Coleus aromaticus* Benth., *Triphasia trifolia* (Burm.f.) and *Eucalyptus kirtoniana* F. Muell. *J. Essent. Oil Res.* **1995**, *7*, 215−218.

(2) Abaul, J.; Philogène, E.; Bourgeois, P.; Poupat, C.; Ahond, A.; Potier, P. Contribution à la connaissance des rutacées américaines: étude des feuilles de *Triphasia trifolia*. *J. Nat. Prod.* **1994**, *57*, 846−848.

(3) Perry, L. M. *Medicinal plants of East and Southeast Asia*; MIT Press: Cambridge, MA, 1980; 632 pp.

(4) Dos Santos, R. P.; Salles Irevisan, M. T. S.; Silveira, E. R.; Pessoa, O. D. L.; Melo, V. M. M. Chemical composition and biological activity of leaves and fruits of *Triphasia trifolia*. *Quim. Nova* **2008**, *31*, 53−58.

(5) Rastogi, N.; Abaul, J.; Goh, K. S.; Devallois, A.; Philogène, E.; Bourgeois, P. Antimycobacterial activity of chemically defined natural substances from the Caribbean flora in Guadeloupe. *Immunol. Med. Microbiol.* **1998**, *20*, 267−273.

(6) Bandoni, A. Los Recursos Vegetales Aromáticos en Latinoamérica. Ciencia y Tecnología para el Desarrollo CYTED; Ed.ial de la Universidad de La Plata: Buenos Aires, Argentina, 2000; 410 pp.

(7) Dar, M. Y.; Shah, W. A.; Rather, M. A.; Qurishi, Y.; Hamid, A.; Qurishi, M. A. Chemical composition, in vitro cytotoxic and antioxidant activities of the essential oil and major constituents of *Cymbopogon jawarancusa* (Kashmir). *Food Chem.* **2011**, *129*, 1606−1611.

(8) Chung, M. J.; Kang, A. Y.; Park, S. O.; Park, K. W.; Jun, H. J.; Lee, S. J. The effect of essential oils of dietary wormwood (*Artemisia princeps*), with and without added vitamin E, on oxidative stress and some genes involved in cholesterol metabolism. *Food Chem. Toxicol.* **2007**, *4*, 1400−1409.

(9) Madhavi, D. L., Seshpande, S. S., Salunkhe, D. K., Eds. *Food Antioxidants: technological, and health perspectives*; Marcel Dekker, Inc.: New York, 1996; pp 6−9, 41, 86−106.

(10) Horváthová, E.; Slameňová, D.; Navarová, J. Administration of rosemary essential oil enhances resistance of rat hepatocytes against DNA-damaging oxidative agents. *Food Chem.* **2010**, *123*, 151−156.

(11) Prakash, D.; Upadhyay, G.; S.ingh, B. N.; Singh, H. B. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (*Glycine max*). *Food Chem.* **2007**, *104*, 783−790.

(12) Finley, J. W.; Kong, A. N.; Hintze, K. J.; Jeffery, E. H.; Ji, L. L.; Lei, X. G. Antioxidants in Foods: State of the Science Important to the Food Industry (review). *J. Agric. Food Chem.* **2011**, *59*, 6837−6846.

(13) Pellegrini, N.; Colombi, B.; Salvatore, S.; Brenna, O. V.; Galaverna, G.; Del Rio, D. Evaluation of antioxidant capacity of some fruit and vegetable foods: efficiency of extraction of a sequence of solvents. *J. Sci. Food Agric.* **2007**, *87*, 103−111.

(14) Beretta, G.; Artali, R.; Maffei Facino, R.; Gelmini, F. An analytical and theoretical approach for the profiling of the antioxidant activity of essential oils: the case of *Rosmarinus officinalis* L. *J. Pharm. Biomed. Anal.* **2011**, *5*, 1255−1264.

(15) González-Coloma, A.; Delgado, F.; Rodilla, J. M.; Silva, L.; Sanz, J.; Burillo, J. Chemical and biological profiles of *Lavandula luisieri* essential oils from western Iberia Peninsula populations. *Biochem. Syst. Ecol.* **2011**, *39*, 1−8.

(16) Ukeh, D. A.; Birkett, M. A.; Pickett, J. A.; Bowman, A. S.; Mordue, A. J. Repellent activity of alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale*, against the maize weevil, *Sitophilus zeamais*. *Phytochemistry* **2009**, *70*, 751−758.

(17) Papachristos, D. P.; Stamopoulos, D. C. Toxicity of vapours of three essential oils to the immature stages of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *J. Stored Prod. Res.* **2002**, *38*, 365−373.

(18) Umoetok, S. B. A.; Gerard, M. B. Comparative efficacy of *Acorus calamus* powder and two synthetic insecticides for control of three major insect pests of stored cereal grains. *Global J. Agric. Sci.* **2003**, *2*, 94−97.

(19) Tapondjou, A. L.; Adler, C.; Fontem, D. A.; Bouda, H.; Reichmuth, C.. Bioactivities of cymol and essencial oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du val. *J. Stored Prod. Res.* **2005**, *41*, 91−102.

(20) Ukeh, D. A.; Umoetok, S. B. A. Repellent effects of five monoterpenoid odours against *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) in Calabar, Nigeria. *Crop Prot.* **2011**, *30*, 1351−1355.

(21) Suthisut, D.; Fields, P. G.; Chandrapatya, A. Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. *J. Stored Prod. Res.* **2011**, *47*, 222−230.

(22) Zoubiri, S.; Baaliouamer, A. Chemical composition and insecticidal properties of some aromatic herbs essential oils from Algeria. *Food Chem.* **2011**, *129*, 179−182.

(23) Schultz, T .P; Nicholas, D. D; Henry, W. P.; Pittman, C. U., Jr; Wipf, D:O; Goodell, B. Review of laboratory and outdoor exposure efficacy results of organic biocide: antioxidant combinations, an initial economic analysis and discussion of a proposed mechanism. *Wood Fiber Sci.* **2005**, *37*, 175−184.

(24) Singh, G.; Marimuthu, P.; de Heluani, C. S.; Catalan, C.A. N. Antioxidant and biocidal activities of *Carum nigrum* (seed) essential oil, oleoresin, and their selected components. *J. Agric. Food Chem.* **2006**, *54*, 174−181.

(25) Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils: a review. *Food Chem. Toxicol.* **2008**, *46*, 446−475.

(26) Stashenko, E. E.; Jaramillo, B. E.; Martínez, J. R. Analysis of volatile secondary metabolites from Colombian *Xylopia aromatica* (Lamarck) by different extraction and headspace methods and gas chromatography. *J. Chromatogr., A* **2004**, *1025*, 105−113.

(27) Zheljazkov, V. D.; Callahan, A.; Cantrell, C. L. Yield and oil composition of 38 Basil (*Ocimum basilicum* L.) accessions grown in Mississippi. *J. Agric. Food Chem.* **2008**, *56*, 241−245.

(28) Stashenko, E. E.; Jaramillo, B. E.; Martínez, J. R. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Colombia, and evaluation of its in vitro antioxidant activity. *J. Chromatogr., A* **2004**, *1025*, 105−113.

(29) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th ed.; Allured Publishers: Carol Stream, IL, 1995; 804 pp.
(30) Guerrero, J.; Guirado, C.; Fuentes, M.; Pérez, A. Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annuun*) varieties. *Eur. Food Res. Technol.* **2006**, *224*, 1−9.
(31) Zoghbi, M. D. B.; Andrade, E. H. A. Chemical composition of the leaf, stem and fruit essential oils from *Triphasia trifolia* (Burm. f.) P. Wilson cultivated in north of Brazil. *J. Essent. Oil-Bear. Plants* **2009**, *12*, 81−86.
(32) Pino, J. A.; Marbot, R.; Fuentes, V. Aromatic plants from western Cuba. VI. Composition of the leaf oils of *Murraya exotica* L., *Amyris balsamifera* L., *Severinia buxifolia* (Poir.) Ten. and *Triphasia trifolia* (Burm. f.) P. Wilson. *J. Essent. Oil Res.* **2006**, *18*, 24−28.
(33) Paolini, J.; Barboni, T.; Desjobert, J. M.; Djabou, N.; Muselli, A.; Costa, J. Chemical composition, intraspecies variation and seasonal variation in essential oils of *Calendula arvensis* L. *Biochem. Syst. Ecol.* **2010**, *38*, 865−874.
(34) Dung, N. T.; Kim, J. M.; Kang, S. C. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food Chem. Toxicol.* **2008**, *46*, 3632−3639.
(35) Lin, K. H.; Yeh., S. Y.; Lin, M. Y.; Shih, M. C.; Yang, K. T.; Hwang, S. Y. Major chemotypes and antioxidative activity of the leaf essential oils of *Cinnamomum osmophloeum* Kaneh. from a clonal orchard. *Food Chem.* **2007**, *105*, 133−139.
(36) Cortes, A.; Prieto, J. M. Application of artificial neural networks to the prediction of the antioxidant activity of essential oils in two experimental in vitro models. *Food Chem.* **2010**, *118*, 141−146.
(37) Suhaj, M. Spice antioxidants isolation and their antiradical activity: a review. *J. Food Compos. Anal.* **2006**, *19*, 531−537.
(38) Jaenson, T. G.; Palsson, K.; Borg-Karlson, A. K. Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and Guinea-Bissau. *J. Med. Entomol.* **2006**, *43*, 113−119.
(39) Gillij, Y. G.; Gleiser, R. M.; Zygadlo, J. A. Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. *Bioresour. Technol.* **2008**, *99*, 2507−2515.
(40) Park, B. S.; Choi, W. S.; Kim, J. H.; Lee, S. E. Monoterpenes from thyme (*Thymus vulgaris*) as potential mosquito repellents. *J. Am. Mosq. Control Assoc.* **2005**, *21*, 80−83.
(41) Kiran, S.; Devi, P. Evaluation of mosquitocidal activity of essential oil and sesquiterpenes from leaves of *Chloroxylon swietenia* DC. *Parasitol. Res.* **2007**, *101*, 413−418.
(42) Zapata, N.; Smagghe, G. Repellency and toxicity of essential oils from the leaves and bark of *Laurelia sempervirens* and *Drimys winteri* against *Tribolium castaneum*. *Ind. Crops Prod.* **2010**, *32*, 405−441.
(43) Mikhaiel, A. A. Potential of some volatile oils in protecting packages of irradiated wheat flour against *Ephestia kuehniella* and *Tribolium castaneum*. *J. Stored Prod Res.* **2011**, *47*, 357−364.