Industrial Crops & Products 142 (2019) 111830

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Chemical composition, antioxidant capacity, toxicity and antibacterial activity of the essential oils from *Acantholippia deserticola* (Phil.) Moldenke (Rica rica) and *Artemisia copa* Phil. (Copa copa) extracted by microwave-assisted hydrodistillation

María Larrazabal-Fuentes[a,*], Jenifer Palma[a], Adrián Paredes[b], Ana Mercado[c], Iván Neira[d], Catherine Lizama[d], Bestabet Sepulveda[e], Jessica Bravo[f]

[a] Universidad de Antofagasta, Departamento de Ciencias de los Alimentos y Nutrición, FACSA, Angamos 601, Antofagasta, Chile
[b] Universidad de Antofagasta, Laboratorio Química Biológica, Instituto Antofagasta and Departamento de Química, Angamos 601, Antofagasta, Chile
[c] Universidad de Antofagasta, Departamento de Biotecnología, FACIMAR, Angamos 601, Antofagasta, Chile
[d] Universidad de Antofagasta, Departamento de Tecnología Médica, FACSA, Angamos 601, Antofagasta, Chile
[e] Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
[f] Universidad Diego Portales, Laboratorio de Productos Bioactivos, Facultad de Medicina, Santiago, Chile

ARTICLE INFO

Keywords:
Antimicrobial
Antioxidant
Essential oils
C. elegans
Toxicity
Chemical composition

ABSTRACT

Acantholippia deserticola (Rica rica; RR) and *Artemisia copa* (Copa copa; CC) are herbs native to the Chilean highlands and have historically been used for medicinal purposes by the Atacameños people. The essential oils were analized by GC/MS, the principal compounds found in RR essential oil (RR_EO) were β-thujone (45%), β-linalool (16%), and eucalyptol (9%) and were butyric acid (38%), γ-terpinene (16%), and cis-2-menthenol (12%) in CC essential oil (CC_EO). The total polyphenol content (TPC) and the antioxidant capacity (AC) were evaluated. The TPC was 25 and 20 mg/mL for CC_EO and RR_EO, respectively, and the respective AC values were 740 and 320 μg eq Trolox/mL by the ferric reducing antioxidant power (FRAP) method. The IC_{50} (1,1-diphenyl-2-picrylhydrazyl; DPPH) values were 316.9 and 485.8 μg/mL for CC_EO and RR_EO, respectively. Antimicrobial activity was evaluated against 32 bacteria using the agar disc diffusion method. CC_EO exerted a significant inhibitory effect (> 20 mm) against 9 bacteria, including *Listeria monocytogenes* and *Staphylococcus aureus,* and RR_EO significantly inhibited *Escherichia coli*. The CC_EO MIC values were 0.39 μg/mL for *Corynebacterium*. The RR_EO MIC value was 6.25 μg/mL for *S. viridans*. Neither EO showed toxicity against the nematode *C. elegans* at 2.5 mg/mL. The essential oils of RR and CC, extracted by MAHD have important bioactive potential and low toxicity, which would allow their use of active ingredients that could potentially be used in areas such as the clinic, food, cosmetics, sanitizers, and detergents, among others.

1. Introduction

Acantholippia deserticola and *Artemisia copa* are wild herbs that are collected and used by the indigenous peoples of northern Chile in infusions and/or decoctions to treat stomach pains, kidney problems and circulatory disorders (Gorzalczany et al., 2013; Miño et al., 2004). It has been proposed that developing and obtaining products with added value, such as essential oils, through efficient and environmentally friendly technologies will promote commercial interest in these native resources. In turn, these activities will encourage propagation and cultivation of these plants, ensuring a sustainable exploitation over time.

The essential oils (EOs) of some herbs have shown diverse biological effects, including vasorelaxant, anti-inflammatory (Shiva et al., 2017; Iannarelli et al., 2018), antidepressant and anxiolytic (Benites et al., 2013), anti-asthmatic (Hernández et al., 2018), anticonvulsant

Abbreviations: RR, *Acantholippia deserticola*; CC, *Artemisia copa*; MAHD, Microwave-assisted hydrodistillation; TPC, Total polyphenol content; AC, Antioxidant capacity; GC–MS, Gas chromatography–mass spectrometry; EO, Essential oil; MIC, Minimum inhibitory concentration

* Corresponding author.
E-mail addresses: maria.larrazabal@uantof.cl (M. Larrazabal-Fuentes), adrian.paredes@uantof.cl (A. Paredes), ana.mercado@uantof.cl (A. Mercado), catherine.lizama@uantof.cl (C. Lizama), bsepulveda@ciq.uchile.cl (B. Sepulveda), jessica.bravo@mail.udp.cl (J. Bravo).

https://doi.org/10.1016/j.indcrop.2019.111830
Received 16 May 2019; Received in revised form 2 September 2019; Accepted 28 September 2019

(Randrianarivo et al., 2016), antimicrobial (Kayode et al., 2018; Van Haute et al., 2017), antiparasitic (Bandeira et al., 2017), mosquitocidal, and pesticidal and repellent effects (Chellappandian et al., 2018).

The bioactivity of EOs depends on their chemical composition, which in turn depends on several factors. Some EOs are associated with the herb itself, such as the species, variety, section of the plant used, geographical and climatic conditions of cultivation and/or collection, and collection period (Gasparetto et al., 2017; García et al., 2017). Other EOs are related to the extraction method employed, such as hydrodistillation, steam distillation, solvent-free, microwave-assisted, ohmic-assisted, ultrasound or supercritical fluids (Ajayi et al., 2016; Gavahian et al., 2016; Sodeifian and Sajadian, 2017). The resulting EOs also depend on the parameters of each process, the sample size and humidity (Shahsavarpour et al., 2017), the herb: solvent mass ratio, and the time, temperature and power that are used (Thakker et al., 2016). The technique of microwave-assisted hydrodistillation (MAHD) combines microwave dielectric heating with hydrodistillation and has been used by several authors for extracting EOs from herbs (Thakker et al., 2016; Farhat et al., 2009); a previous study applied MAHD to *Acantholippia deserticola* ("Rica rica"; RR), resulting in a higher yield and bioactivity than the traditional hydrodistillation method.

In this work, the chemical composition of EOs extracted from RR and *Artemisia copa* ("Copa copa"; CC) by MAHD was determined, and the antioxidant capacity, toxicity and antibacterial activity of the EOs against 32 bacteria were evaluated.

2. Materials and methods

2.1. Collection and identification

The aerial parts of RR and CC were collected in San Pedro de Atacama, Región de Antofagasta, Chile (23°11′14.2656″ S, 68°0′16.9596″ W) in April 2017. The species was identified by Dr. Roberto Rodríguez, and a voucher specimen was deposited at the Botany Department Herbarium, School of Natural Sciences and Oceanography, University of Concepción, Chile, under the herbarium number CONC N°182473 and CONC Nº182475, respectively.

2.2. Essential oil extraction

A microwave-assisted hydrodistillation system (MADH) was used based on that described by (Golmakani and Rezaei, 2008) with modifications. A 200 g sample of ground dehydrated herb (30 °C; 72 h) was added to a flask with 1000 mL of distilled water and microwaved (LG, model S-1948 JL) for 90 min at 700 W. The extracted oils were separated from the aqueous layer using ethyl ether and dried over sodium sulfate, filtered, and then left in the oven for 1 h at 40 °C for evaporated ethyl ether. The samples were stored in the dark in amber vials at -20 °C prior to the analyses. The yield was calculated as the volume of oil obtained per mass of plant used.

2.3. Composition

Gas chromatography–mass spectrometry (GC–MS) analysis was performed on a Varian gas chromatograph series 431 (Agilent Technologies, Inc., Santa Clara, CA, USA) fitted with a DB-5 ms fused silica capillary column (30 x 0.25 mm; film thickness, 0.25 µm) using split/split-less injection, and coupled to a series 220 mass detector (Agilent Technologies, Inc.). The following conditions were used: injection volume: 0.8 µL with split ratio 1:80; helium as a carrier gas at 1.5 mL/min in constant flow; injector temperature: 250 °C; oven temperature: 50–260 °C at 2 °C/min. The mass spectra electron impact (EI +) mode was set at 70 eV, with an ion source temperature of 260 °C. The mass spectra were recorded within a range of 40–300 atomic mass units. Identification of the EO constituents was accomplished based on the following: the retention index (RI) determined with respect to a homologous series of n-alkanes (C_5-C_{28}; PolyScience, Niles, IL, USA) under the same experimental conditions; co-injection with standards (Sigma-Aldrich) and standard isolates; identification using an MS library (NIST 05 and Wiley; NIST/EPA/NIH Mass Spectral Library with Search Program (data version NIST 11; software version 2.0 g), available online at: http://www.nist.gov/srd/nist1a.cfm) and comparison with previously reported MS data (Adams, 2007).

2.4. Antioxidant capacity

The total polyphenol content (TPC) and the antioxidant capacity of the oils were determined by the ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays using Trolox (53188-07-1, Sigma-Aldrich) as a standard, according to protocols described below and adapted for 96-well microplates.

2.4.1. Total polyphenol content (TPC) estimation

The TPC method was based on the 96-well microplate Folin–Ciocalteu method and adapted from (Ainsworth and Gillespie, 2007). A 20 µL sample of the diluted EO (500 µg/mL) was mixed with 100 µL of 10% (vol/vol) Folin–Ciocalteu reagent and shaken. The mixture was left for 5 min, and then 80 µL of sodium carbonate solution (700 mM) was added, and the mixture was shaken for 1 min. After 60 min at room temperature, the absorbance was measured at 765 nm using a microplate reader. Gallic acid dilutions (0–1000 µg/mL) were used as standards for calibration. The results were expressed as mg of gallic acid equivalent per ml of essential oil. All experiments were performed in triplicate.

2.4.2. FRAP assay

The FRAP assay was carried out with the following method adapted from (Akter et al., 2016). The FRAP reagent consisted of 300 mM acetate buffer pH 3.6, 10 mM 2,4,6-tris-(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ aqueous solution at a ratio of 10:1:1 (v/v). The EOs were prepared at a final concentration of 500 µg/mL. The extract solution (10 µL) was mixed with 70 µL of freshly prepared FRAP solution and incubated at 37 °C for 30 min. The absorbance of the solutions was measured at 593 nm. Trolox was used as the standard solution to construct a calibration curve over a concentration range of 0–500 µg/mL. The FRAP results were expressed as mg of Trolox equivalent per mL of EO. All experiments were performed in triplicate.

2.4.3. DPPH radical scavenging activity assay

The quantitative measurement of the EOs' radical scavenging properties was carried out by the methodology described by (Mathew and Subramanian, 2014) with some modifications. A 0.2 mM solution of DPPH in methanol was prepared, and 70 µL of this solution was added to 20 µL of EO (0–1000 µg/mL). Trolox at concentrations of 0–1000 µg/mL was used as a reference antioxidant. Discoloration of reaction mixture was measured at 517 nm after incubation for 30 min. The results were expressed as IC_{50} values (concentration of EO in µg/mL required to inhibit 50% of DPPH radical present in solution). The analyses were carried out in triplicate.

2.5. Antibacterial activity

2.5.1. Microbial strains

The antimicrobial activity of 2 oil samples of RR and CC were tested against 32 bacterial strains: (*Acinetobacter baumanii* ATCC 747, *Aeromonas caviae, Aeromonas veronii, Alcaligenes* sp., *Bacillus cereus, Corynebacterium diphtheriae, Enterobacter cloacae, Escherichia coli, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella oxytoca, Klebsiella pneumoniae* (bleomycin resistant), *Listeria monocytogenes, Morganella morganii, Providencia alcaligenes, Proteus mirabilis, Proteus vulgaris, Pseudomonas putida, Pseudomonas aeruginosa, Serratia rubidaea, Shigella*

boydii, Shigella flexneri, Shigella putre, Shigella sonnei, Staphylococcus aureus CECT 976, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis, Staphylococcus xylosus, Salmonella typhi, Streptococcus viridans, Streptococcus pyogenes* (Group A), and *Yersinia enterocolitica*. All strains were obtained from the collection of Laboratorio de Microbiología, Departamento de Tecnología Médica, Universidad de Antofagasta. Some bacterial strains were from the American Type Culture Collection (ATCC). The strains were grown in Mueller Hinton Agar and incubated at 37 °C.

2.5.2. Agar disk diffusion assay

The agar disk diffusion technique has been widely used to assay plant extracts for antimicrobial activity. In this method, 6 mm sterilized filter paper disks (Whatman N° 1) were saturated with 10 µL of filter-sterilized oil plant extract. The impregnated discs were then placed onto the surface of a suitable solid agar medium, such as Mueller Hinton BD®. The media was pre-inoculated with test organisms. A standard inoculum size of 1×10^8 CFU/mL of bacteria was used to inoculate the diffusion plates, which is equal to the McFarland 0.5 turbidity standard. The plates were incubated overnight at 37 °C, and the diameter of the inhibition zone around each disk (diameter of inhibition zone plus diameter of the disk) was measured in millimeters. A Sensydisc was used as a positive control for Gram + and Gram- strains (CLSI, 2012).

2.5.3. Microplate assay

The microdilution method was performed in ELISA plates according to instructions approved by the Clinical and Laboratory Standards Institute. For this purpose, the oil was added in 10-fold decreasing serial concentrations into Mueller Hinton broth BD®. Fresh bacterial suspensions (equivalent to 1×10^8 bacteria/mL) were used to inoculate the microplates and were incubated as described above (Morcillo et al., 2004).

To enhance EO solubility, they were dissolved in 2% (v/v) dimethyl sulfoxide (DMSO) (this amount does not affect bacterial growth) and then added to the microplates. The inoculated microplates were incubated as described above for agar plates, but for only 1 day. ELISA readings at 600 nm were recorded to calculate the MIC values as described by the Clinical and Laboratory Standards Institute (CLSI, 2012) protocol. Control microplates of bacteria without the EO and without bacteria were also included in these assays. To identify significant differences between treated and untreated groups, data comparison by the ANOVA test was performed using the GraphPad Prism 5 program.

2.6. Toxicity

2.6.1. Maintenance of Caenorhabditis elegans culture

The N2 wild-type strain of *C. elegans* was used to investigate toxicity. The nematodes were maintained on nematode growth medium (NGM) agar plates, with an established layer of the *E. coli* OP50 strain. The plates were maintained at 20 °C for 3 days. The gravid nematodes were collected and treated for 5 min in a chlorine solution (0.45 N NaOH, 2% HOCl) to isolate eggs. The eggs were placed in plates with OP50; once hatched they were left for 3 days to obtain synchronized adult nematodes. These were collected in M9 saline solution (1.5 g KH_2PO_4, 3 g Na_2HPO_4, 2.5 g NaCl, 0.5 mL of 1 M $MgSO_4$ and distilled water for a final volume of 500 mL) (Brenner, 1974).

2.6.2. Test preparation

The RR and CC essential oils were prepared at concentrations of 2.5, 5.0, 10.0 and 50.0 mg/mL and placed in a final volume of 100 µL in 96-well plates. The control assays were performed with M9 and 1% DMSO. Ten individuals of *C. elegans*/well were used in each trial. The plates were incubated at 20 °C for 24 and 48 h. The experiments were performed in triplicate and repeated twice. After incubation at 20 °C, all the nematodes were counted at 24 and 48 h to determine their survival. They were considered alive if they showed some type of motility and were considered dead when they showed no movement, either from their tail, head or pharynx, for 5 s of observation. The count was performed to establish the degree of mortality (Skantar et al., 2005).

3. Results and discussion

The EO yields obtained using the MADH process were 2% for RR and 1.5% for CC, and these differ from those reported by other authors who extracted the EOs by hydrodistillation from the same herbs. For RR, the reports range between 2.7% and 5.5% (Zarria et al., 2010; Sampietro et al., 2016; Rojo et al., 2006), while for CC Lopez et al. (2004) reported values of 0.375%. These differences can be due to both the extraction method and to factors associated with the collection, location, and season (Gasparetto et al., 2017; Lopez et al., 2004).

Regarding EO yields according to the extraction processes, using the MADH method Torrenegra et al. (2015) reported values of 0.95% for oregano, Leon et al. (2015) reported 0.51% for *Citrus sinensis*, Gavahian et al. (2016) reported 2.27% for peppermint, and Thakker et al. (2016) reported 2.44% for oil extracted from *Cymbopogon martinii* (Palmarosa).

3.1. Composition

The sample compositions obtained by MAHD (Table 1) indicated that the *Artemisia copa* essential oil (CC_EO) contained 19 compounds, while the *Acantholippia deserticola* essential oil (RR_EO) contained 13 compounds. The highest yielding compounds in RR were β-thujone (45%), β-linalool (16%), and eucalyptol (9%), and the highest yielding compounds in CC were butyric acid (38%), γ-terpinene (16%), and cis-2-menthenol (12%). Rojo et al. (2006), reported that the RR_EO extracted by hydrodistillation (HD) contained 22 compounds, with α-thujone (10.5%) and β-thujone (77.9%) being the highest, while Sampietro et al. (2016) reported 24 compounds, most of which were oxygenated monoterpenes (93%), where β-thujone (66.5 ± 0.2%) and trans-sabinyl acetate (12.1 ± 0.2%) constituted the majority.

According to Lopez et al. (2004), for CC_EO extracted by HD, β-thujone (42%) and chamazulene (6.5%) constituted the majority. Ajayi et al. (2016) found more compounds in EOs extracted by MAHD (16) than by HD (7) but found higher yields by HD (0.73%) than by MAHD (0.64%) in *Cymbopogon citratus*. The reported differences in yield and composition are due to different extraction methods.

In the current study, the differences in the yield and composition of the RR and CC EOs compared to those in the literature could be due to both the extraction method and the collection time (García et al., 2017; Tohidi et al., 2017). The chemical composition of the oils affects their bioactivity (Jordán et al., 2013; Jiao et al., 2012), even though their bioactivity should not be attributed to a particular compound (Lopez et al., 2004).

Biological effects are the result of a synergism of all molecules contained in an essential oil, even if it is possible that the activity of the main components is modulated by other minor molecules; however, the activity of the isolated constituents is also notable (Bilia et al., 2014).

Some of the compounds found in this work, such as eucalyptol, a major compound in RR oil and a minor compound in CC oil, has been reported in other EOs, such as that obtained from the leaves of *Cryptocarya alba* (peumo), demonstrating antifungal activity against *Nosema ceranae* (Bravo et al., 2017). Linalool, present in RR_EO, has been reported to show antitumor activity, and it has also been shown that using it in nanoencapsulation inhibits cancer cells in a time-dosage-dependent manner (Rodenak-Kladniew et al., 2017).

β-Thujone, present in RR oil, has been reported to decrease the cell viability and exhibit potent anti-proliferative, pro-apoptotic, and anti-angiogenic effects *in vitro*. *In vivo* assays have shown that α/β-thujone promotes the regression of neoplasia and inhibits the angiogenic markers VEGF, Ang-4 and CD31 in the tumor (Quezada et al., 2016). Bayramoglu et al. (2008) and Li et al. (2012) reported no significant changes in the components of the obtained oils, but they showed

Table 1
Summary of compounds from CG- MS analyses of essential oil of A) RR (*Acantholippia deserticola*) and (B) CC (*Artemisia copa*) EOs and the respective calculated Kovats index (KI cal) and from the literature (KI lit).

(A) RR essential oil

Fraction Number	Retention time (min)	CAS	KI cal	KI lit	[M+]	Fragment	%	Name
1	11.5	3387-41-5	–	976	136	93 (100) 91 (40) 77 (40)	7.40	sabinene
2	13.6	99-86-5	1088	1018	136	121 (100) 93 (85) 77 (359	0.58	α-terpinene
3	13.9	99-87-6	1043	1026	134	119 (100) 91 (15) 117 (10)	8.33	ρ-cymene
4	14.3	470-82-6	1024	1031	154	43 (100) 81 (65) 108 (55)	9.07	eucalyptol
5	15.6	99-85-4	1037	1062	136	93 (100), 91 (35), 77 (30)	3.40	γ-terpinene
6	17.8	78-70-6	1079	1098	136	71 (100) 93 (75) 55 (65)	16.70	β-linalool
7	18.4	1125–12-8	1026	1124	152	81 (100) 41 (90) 68 (70)	45.65	β -thujone
8	20.1	513-23-5	1057	–	154	43 (100) 55 (90) 93 (85)	0.08	isothujol
9	21.4	562-74-3	1065	1177	154	71 (100), 111 (50), 93 (45)	6.02	4-terpineol
10	22.1	98-55-5	1013	1189	154	93 (100), 59 (90), 121 (86)	0.45	terpenol
11	27.0	3228-02-2	1071	–	150	135 (100) 150 (35) 91 (15)	0.17	ρ-thymol
12	29.0	554-61-0	1026	1001	136	93 (100) 121 (90) 136 (55)	0.66	2-carene
13	34.6	5989-08-2	1481	1334	204	119 (100) 105 (55) 133 (45)	1.52	α-longipinene

(B) CC essential oil

Fraction Number	Retention time (min)	CAS	KI cal	KI lit	[M+]	Fragment	%	Name
1	14.4	470-82-6	–	1007	154	43 (100) 81 (65) 108 (55)	0.11	eucalyptol
2	15.2	13877-91-3	1067	1037	136	93 (100) 91 (50) 79 (45)	1.27	o-cymene
3	15.6	99-85-4	1020	1056	136	93 (100), 91 (35), 77 (30)	16.63	γ-terpinene
4	17.2	41519-23-7	1070	1385	170	43 (100) 82 (87) 67 (83)	38.00	butyric acid
5	17.9	1224-46-0	1747	–	–	–	1.46	2-(4-methylcyclohex-3-en-1-yl)propan-2-yl N-phenylcarbamate
6	18.7	29803-81-4	1080	1040	154	43 (100) 93 (45) 139 (35)	5.87	trans-2-menthenol
7	18.9	29803-82-5	1014	1039	154	43(100) 93 (45) 139 (38)	12.83	cis-2-menthenol
8	20.1	1786-08-9	1048	1153	152	68 (100) 41 (55) 83 (54)	4.85	nerol oxide
9	21.4	562-74-3	1059	1177	154	71 (100), 111 (50), 93 (45)	1.58	4-terpineol
10	22.3	80-26-2	1269	1328	196	43 (100) 121 (91) 93 (83)	2.98	α-terpineol acetate
11	22.7	18309-32-5	1067	1116	150	107 (100) 81 (88) 39 (75)	0.59	D-verbene
12	22.9	16721-38-3	1017	1177	154	84 (100) 93 (24) 43 (28)	5.57	cis-piperitol
13	23.9	89-81-6	1048	1189	152	82 (100) 110 (72) 39 (35)	3.10	carvomenthenone
14	25.0	33522-69-9	1565	–	–	–	1.69	trans-verbenyl acetate
15	25.6	16750-82-6	1586	–	–	–	1.97	ρ-mentha-1,8-dien-3-one
16	25.7	16750-88-2	1503	–	–	–	0.44	methyl (3z)-3,7-dimethyl-3,6-octadienoate
17	29.6	123-35-4	1534	–	–	–	0.54	β-myrcene
18	37.2	77171-55-2	1564	1619	220	43 (100) 41 (68) 91 (43)	0.24	spathulenol
19	41.4	473-04-1	1489	1693	222	43 (100) 41 (65) 81 (59)	0.29	juniper camphor

NIST 2018.

differences in the percentages of some compounds depending on the extraction method used. Okoh et al. (2010) reported a greater antimicrobial effect of rosemary oils when extracted by MADH than by HD.

3.2. Antioxidant capacity

The CC_EO had an antioxidant capacity that was significantly ($p < 0.05$) greater than that of RR_EO in both measurement methods (Table 2), even though β-linalool, which is highly antioxidant (Jiao et al., 2012), was the major constituent in RR_EO. Baschieri et al. (2017) noted that some nonphenolic components of the essential oil, such as linalool, act as antioxidants below a critical concentration (4%) and while above this threshold they act as pro-oxidants, which would explain the results obtained.

On the other hand, CC_EO contained γ-terpinene and terpinen-4-ol, which reportedly have high antioxidant activity (Olmedo et al., 2013; Guala et al., 2009). The IC_{50} (DPPH) of both EOs were significantly higher than of the Trolox standard. For RR, Morales et al. (2008) reported an IC_{50} of 18 ± 0.5 μg/mL (DPPH) in ethanol extracts (50%), and Rojo et al. (2006) reported antioxidant capacities of 5600 mg eq Trolox/L (FRAP) and 222 μmol/L (Trolox equivalent antioxidant capacity (TEAC)-DPPH) for aqueous extracts.

Regarding CC, Altunkaya et al. (2014) reported values between 3.4 and 4.9 mM Trolox (2,2′-azino-bis-(ABTS)) for essential oils of other *Artemisia* species extracted by HD. Youssef et al. (2015) reported differences in the antioxidant capacity of *Artemisia abyssinica* depending on the type of extract, with the best activity in aqueous extracts. No reports of the antioxidant activity of RR or CC EOs obtained by MAHD were found.

The TPC of RR_EO was higher than that reported by Rojo et al.

Table 2
Total polyphenol content and antioxidant capacity, by FRAP and DPPH, of RR (*Acantholippia deserticola*) and CC (*Artemisia copa*) EOs obtained by MAHD.

	TPC (mg eq gallic acid/mL EO)	FRAP (μg eq Trolox/mL EO)	DPPH IC_{50} (μg/mL)
CC (*Artemisia copa*)	25 ± 3*	730 ± 30**	3162 ± 22**
RR (*Acantholippia deserticola*)	20 ± 3*	310 ± 10*	6309 ± 45***
Trolox			56,2 ± 4*

Values are the mean ± SEM (n = 3). Statistically significant differences: **$p < 0.01$, $p < 0.01$ and ***$p < 0.001$.

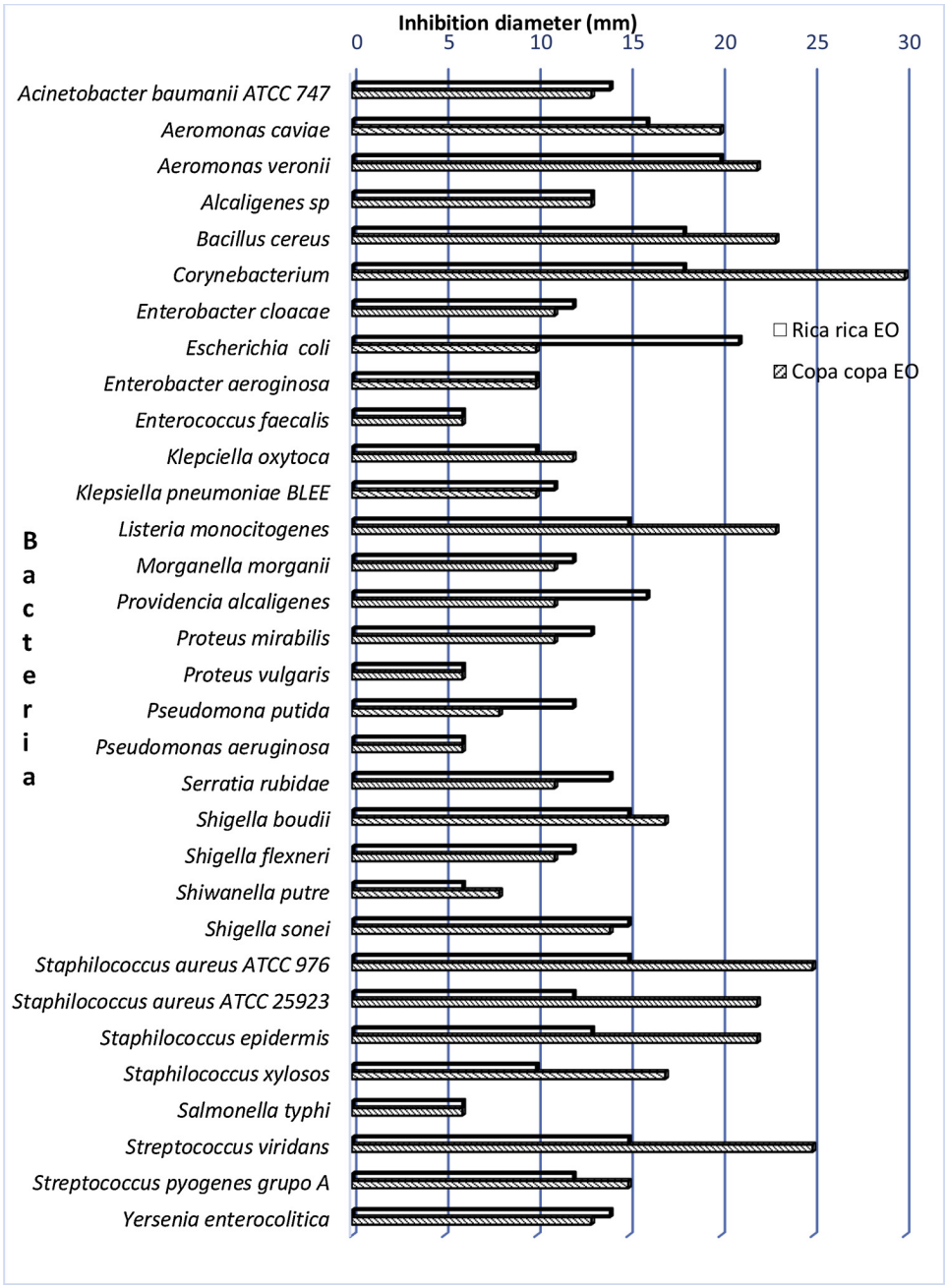

Fig. 1. Zone of Inhibition (mm) resulting from RR (*Acantholippia deserticola*) and CC (*Artemisia copa*) essential oils obtained by MAHD.

(2006) (0.072 mg/mL for aqueous extracts) and lower than that reported by Morales et al. (2008) (725 ± 12 mg/g of dried extract in water-ethanol extracts (50%).

3.3. Antimicrobial activity

Rota et al. (2008) indicated that the antimicrobial effectiveness of essential oils depends on the type of microorganism evaluated and classified this effect into 3 categories: growth inhibition zone (mm, disk diameter included): ⩾20 mm inhibition zone is strongly inhibitory; < 20–12 mm inhibition zone is moderately/mildly inhibitory; and < 12 mm is not inhibitory.

The preliminary study results of antimicrobial activity (Fig. 1) showed that CC_EO (extracted by MAHD) exerted a strongly inhibitory effect (> 20 mm) on 8 bacteria, particularly against *L. monocytogenes*, a pathogen responsible for serious health problems, and *S. aureus*, known for its virulence and resistance to antibiotics. Lopez-Arce et al., (2004) reported a negligible antimicrobial effect (< 12 mm of inhibition) of the CC essential oil when extracted by HD, although in some cases it was comparable to the gentamicin control.

Youssef et al. (2015) reported that n-hexane and aqueous extracts of *A. abyssinica* showed strong antibacterial activity, with MICs of 32 µg/mL for both *E. coli* and *S. aureus*, coinciding with Donato et al. (2015) who reported the antimicrobial effect of oil extracted by HD from *Artemisia annua*. On the other hand, Altunkaya et al. (2014) found lower inhibition zone values (< 15 mm) for oils from 2 *Artemisia* species. Morales et al. (2003) found antibacterial and antifungal activity for alcohol and chloroform CC extracts.

Regarding RR, an important antimicrobial effect (> 20 mm) of the essential oil extracted by MADH was observed for *E. coli, a* pathogen associated with gastrointestinal diseases, with a significantly greater effect than that observed with CC_EO. Rojo et al. (2006) found no

Table 3
MIC_{50} for CC_EO and RR_EO using M-MTT.

EO	MIC_{50} (μg/mL) CC_EO	MIC_{50} (μg/mL) RR_EO
S. viridans	1.56	6.25
L. monocytogenes	6.25	25
S. epidermidis	12.5	25
S. aureus	6.25	25
B. cereus	1.56	25
Corynebacterium	0.39	25
E. coli	6.25	25

ANOVA analysis including the Tukey test did not reveal significant differences between CC and RR. The values were an average of 7 species, and the whole experiment was performed in triplicate.

antimicrobial activity for HD-extracted RR oil against *S. aureus, E. coli*, or *Candida albicans*.

This was similar to the results of Sampietro et al. (2016) who reported only a moderate antibacterial activity of this oil against *Septoria glycines* and found an inverse correlation between the increase of the β-thujone, trans-sabinyl acetate and trans-sabinol contents with the MIC100 values.

The differences found in this work can be caused by the effect of the location and time of collection (Palá-Paúl et al., 2004; Sá et al., 2016), as well as by extraction methods, where MADH would promote the extraction of certain compounds (Jordán et al., 2013; Ajayi et al., 2016).

The antibacterial activity of monoterpenes (Jiao et al., 2012; Jordán et al., 2013) is associated with the ability to break and penetrate the bacterial cell membrane, with a greater effect on gram-positive than on gram-negative bacteria, probably due to differences in their cellular membranes (Okoh et al., 2010).

The presence of oxygenated monoterpenes in essential oils of other *Artemisia* species extracted by HD, such as camphor, 1,8-cineole, and 1-terpinen-4-ol in *A. copa* (Lopez et al., 2004) and 1,8-cineole, camphor, 1-terpinen-4-ol, linalool, α-terpinol, and borneol in RR_EO (Altunkaya et al., 2014), are linked to this activity.

Watanabe et al. (2008) studied the effect of several monoterpenes, demonstrating the antibacterial activity of eucalyptol against 28 strains of *S. aureus*. Linalool and eucalyptol are compounds especially present in RR oil, and the authors reported that these monoterpenes, present as the majority in *Myrtus communis* L., showed bactericidal and bacteriostatic activity against the resistant clinical pathogen *A. baumanii* strains (Aleksic et al., 2014). Sonboli et al. (2014) reported the activity of these compounds against gram-negative bacteria, such as *E. coli* 25922.

Bouyahya et al. (2017) demonstrated the antimicrobial activity of another compound present in RR oil, γ-terpinene, against pathogens such as *Bacillus subtilis*, *Listeria* sp., and *S. aureus*. Other authors (Marchese et al., 2017; Kisko and Roller, 2005; Ultee et al., 2002; Rattanachaikunsopon and Phumkhachorn, 2010) have shown that p-cymene, present in RR, could be responsible for the antimicrobial activity of essential oils, especially against *E. coli* O157:H7, *Bacillus cereus* and *Salmonella enterica* serovar Thyphi.

The different compounds present in the EOs can affect the bacteria in different ways. In studies with rosemary EO, Jordán et al. (2013) reported that eucalyptol and α-pinene strongly inhibited *Salmonella typhimurium* and that α-pinene increases the effectiveness of the oil against *S. aureus*. However, eucalyptol considerably decreased the efficiency of rosemary EO. The above findings justify the need to evaluate compounds and/or groups of compounds, specifically on different bacteria.

The development of natural antimicrobials will help to decrease the negative effects (residues, resistance, and environmental pollution) of synthetic drugs. In this respect, natural antimicrobials may also be effective, selective, biodegradable, and less toxic to environment.

However, the safety and toxicity of these compounds will need to be addressed (Rattanachaikunsopon and Phumkhachorn, 2010). Donato et al. (2015) found up to 10 times more antibacterial activity of *A. annua* EO against *Y. enterocolitica* and *Salmonella enteritidis* compared with the pure compounds. They reported lower MIC values for this essential oil (17.6, 0.18 and 17.6 mg/mL) against *E. coli*, *Salmonella* and *Listeria,* respectively, than the isolated compounds.

According to the classification of Duarte et al. (2007), the antibacterial potential for both essential oils indicates that they would both be strong inhibitors according to the MICs shown in Table 3. There was a greater antibacterial effect of CC_EO against *Corynebacterium* strains, with MIC values of 0.39 μg/mL. On the other hand, RR_EO showed greater activity against *S. viridans*, with a MIC value of 6.25 μg/mL.

3.4. Toxicity

Both EOs showed low or no toxicity against *C. elegans* (Fig. 2). RR_EO only showed toxicity at the highest concentration (50 mg/mL), with approximately 80% mortality, both at 24 and 48 h. CC_EO was toxic at 24 h at 50 and 25 mg/mL, with approximately 100% mortality. However, at 5 mg/mL it showed toxicity at 48 h, with 55% mortality.

Although some level of toxicity was observed, it was only at the highest concentrations, higher than those recommended for studies in *C. elegans* (1 mg/mL) (Dal Forno et al., 2016), which demonstrates that both oils have low or no toxicity.

Lei et al. (2010) reported nematicidal activity against *C. elegans* from thymol and p-cymene, both present in RR_EO. Khanavi et al. (2017) reported a greater toxic effect of p-cymene (present in RR) than of γ-terpinene (present in CC). Furthermore, Morales et al. (2003) reported high biotoxicity of alcohol and chloroform extracts of CC against

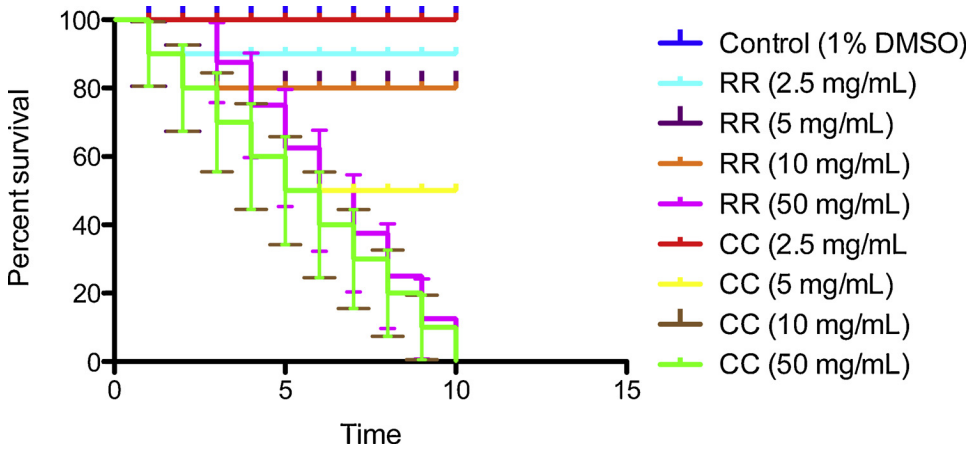

Fig. 2. Percent effect of RR (*Acantholippia deserticola*) and CC (*Artemisia copa*) essential oils against *C. elegans*.

Artemia salina.

4. Conclusions

The essential oils of RR and CC extracted by MADH have a high antioxidant activity and show strong inhibition against food-associated pathogenic bacteria. In addition to their low toxicity, these findings enable us to propose the evaluation of their use in the food sector, either as a functional ingredient in food, as an edible carrier, or as a natural sanitizer.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgement

This work was possible thanks to the Proyecto Semillero (5310) and the support of workshops for writing scientific articles, Direction of Research Management, Universidad de Antofagasta, Chile.

References

Adams, R.P., 2007. 4th ed. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry Vol.8. Allured Publishing Corporation, Carol Stream, pp. 804.

Ajayi, E.O., Sadimenko, A.P., Afolayan, A.J., 2016. GC–MS evaluation of *Cymbopogon citratus* (DC) Stapf oil obtained using modified hydrodistillation and microwave extraction methods. Food Chem. 209, 262–266. https://doi.org/10.1016/j.foodchem.2016.04.071.

Aleksic, V., Mimica-Dukic, N., Simin, N., Nedeljkovic, N.S., Knezevic, 2014. Synergistic effect of *Myrtus communis* L. essential oils and conventional antibiotics against multi-drug resistant *Acinetobacter baumannii* wound isolates. Phytomedicine. 21 (12), 1666–1674. https://doi.org/10.1016/j.phymed.2014.08.013.

Altunkaya, A., Yıldırım, B., Ekici, K., Terzioğlu, Ö., 2014. Determining essential oil composition, antibacterial and antioxidant activity of water wormwood extracts. GIDA 39 (1), 17–24. https://doi.org/10.5505/gida.00710. (2014) GD13061.

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2 (4), 875–877. https://doi.org/10.1038/nprot.2007.102.

Akter, K., Barnes, E.C., Loa-Kum-Cheung, W.L., Yin, P., Kichu, M., Brophy, J.J., Barrow, R.A., Imchen, I., Vemulpad, S.R., Jamie, J.F., 2016. Antimicrobial and antioxidant activity and chemical characterisation of *Erythrina stricta* Roxb. Fabaceae). Journal of Ethnopharmacology 185, 171–181. https://doi.org/10.1016/j.jep.2016.03.011.

Bandeira, G., Pês, T.S., Saccol, E.M.H., Sutili, F.J., Rossi, W., Murari, A.L., Baldisserotto, B., 2017. Potential uses of *Ocimum gratissimum* and *Hesperozygis ringens* essential oils in aquaculture. Ind. Crops Prod. 97, 484–491. https://doi.org/10.1016/J.INDCROP.2016.12.040.

Baschieri, A., Daci Ajvazi, M., Folifack, J., Valgimigli, L., Amorati, R., 2017. Explaining the antioxidant activity of some common non-phenolic components of essential oils. Food Chem. 232, 656–663. https://doi.org/10.1016/j.foodchem.2017.04.036.

Bayramoglu, Beste, Sahin, Serpil, Gulum, Sumnu, 2008. Solvent-free microwave extraction of essential oil from oregano. J. Food Eng. 88 (4), 535–540. https://doi.org/10.1016/j.jfoodeng.2008.03.015.

Benites, Julio, Bustos, Luis, Rios, David, Bravo, Felipe, López, José, Gajardo, Sandra, Rojo, Leonel, Buc-Calderon, Pedro, 2013. Antidepressant and anxiolytic-like effects of essential oil from *Acantholippia deserticola* Phil in female rats. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 12 (4), 413–419. http://www.redalyc.org/articulo.oa?id=85628141009.

Bilia, A., Santomauro, F., Sacco, C., Bergonzi, M., Donato, R., 2014. Essential oil of *Artemisia annua* L.: an extraordinary component with numerous antimicrobial properties. Evid. Complement. Alternat. Med. 7. https://doi.org/10.1155/2014/159819. Article ID 159819.

Bouyahya, A., Dakka, N., Talbaoui, A., Et-Touys, A., El-Boury, H., Abrini, J., Bakri, Y., 2017. Correlation between phenological changes, chemical composition and biological activities of the essential oil from Moroccan endemic Oregano (*Origanum compactum* Benth). Ind. Crops Prod. 108 (1), 729–737. https://doi.org/10.1016/j.indcrop.2017.07.033.

Bravo, J., Carbonell, V., Sepúlveda, B., Delporte, C., Valdovinos, C.E., Martín-Hernández, R., Higes, M., 2017. Antifungal activity of the essential oil obtained from *Cryptocarya alba* against infection in honeybees by *Nosema ceranae*. J Invert Pathol 2017. https://doi.org/10.1016/j.jip.2017.08.012.

Brenner, S., 1974. The genetics of Caenorhabditis elegans. Genetics 77, 71–94.

Chellappandian, M., Vasantha-Srinivasan, P., Senthil-Nathan, S., Karthi, S., Thanigaivel, A., Ponsankar, A., Hunter, W.B., 2018. Botanical essential oils and uses as mosquitocides and repellents against dengue. Environ. Int. 113, 214–230. https://doi.org/10.1016/J.ENVINT.2017.12.038.

CLSI, 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA CLSI document M02-A11. 2012.

Dal Forno, A., Câmara, D., Parise, B., Rodrigues, C., Soares, J., Wagner, R., Ribeiro, S., Folmer, V., Puntel, R., Haas, S.E., 2016. Antioxidant and lipid lowering effects of dried fruits oil extract of *Pterodon emarginatusin Caenorhabditis elegans*. Arab. J. Chem. https://doi.org/10.1016/j.arabjc.2016.04.001. In Press.

Donato, R., Santomauro, F., Bilia, A.R., 2015. Antibacterial activity of Tuscan *Artemisia annua* essential oil and its major components against some foodborne pathogens. Lwt - Food Sci. Technol. 64, 1251–1254.

Duarte, M.C.T., Leme, E.E., Delarmelina, C., Soares, A.A., Figueira, G.M., Sartoratto, A., 2007. Activity of essential oilsfrom Brazilian medicinal plants on Escherichia coli. J. Ethnopharmacol. 111 (2), 197–201.

Farhat, A., Ginies, C., Romdhane, M., Chemat, F., 2009. Eco-friendly and cleaner process for isolation of essential oil using microwave energy. Experimental and theoretical study. J. Chromatography. 1216, 5077–5085. https://doi.org/10.1016/j.chroma.2009.04.084.

García, L.T., Leal, A.F., Moreno, É.M., Stashenko, E.E., Arteaga, H.J., 2017. Differential anti-proliferative effect on K562 leukemia cells of *Lippia alba* (Verbenaceae) essential oils produced under diverse growing, collection and extraction conditions. Ind. Crops Prod. 96, 140–148.

Gasparetto, A., Bella Cruz, A., Wagner, T.M., Bonomini, T.J., Correa, R., Malheiros, A., 2017. Seasonal variation in the chemical composition, antimicrobial and mutagenic potential of essential oils from *Piper cernuum*. Ind. Crops Prod. 95, 256–263. https://doi.org/10.1016/J.INDCROP.2016.10.030.

Gavahian, M., Farahnaky, A., Farhoosh, R., Javidnia, K., Shahidi, F., 2016. Extraction of essential oils from *Mentha piperita* using advanced techniques: microwave versus ohmic assisted hydrodistillation. Food Bioprod. Process. 94, 50–58. https://doi.org/10.1016/j.fbp.2015.01.003.

Golmakani, M., Rezaei, K., 2008. Comparison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extraction of essential oils from *Thymus vulgaris* L. Food Chem. 109 (4), 925–930. https://doi.org/10.1016/j.jfoodeng.2008.03.015.

Gorzalczany, Susana, Moscatelli, Valeria, Ferraro, Graciela, 2013. *Artemisia copa* aqueous extract as vasorelaxant and hypotensive agent. J. Ethnopharmacol. 148, 56–61. https://doi.org/10.1016/j.jep.2013.03.061.

Guala, M., Elder, H., Perez, G., Chiesa, A., 2009. Evaluación del poder antioxidante de fracciones de aceite esencial crudo de *Schinus molle* L. Obtenidas por destilación al vacío. Inf. Tecnolã³gica 20 (2), 83–88.

Hernández, J.J., Ragone, M.I., Bonazzola, P., Bandoni, A.L., Consolini, A.E., 2018. Antitussive, antispasmodic, bronchodilating and cardiac inotropic effects of the essential oil from *Blepharocalyx salicifolius* leaves. J. Ethnopharmacol. 210, 107–117. https://doi.org/10.1016/J.JEP.2017.08.013.

Iannarelli, R., Marinelli, O., Morelli, M.B., Santoni, G., Amantini, C., Nabissi, M., Maggi, F., 2018. Aniseed (*Pimpinella anisum* L.) essential oil reduces pro-inflammatory cytokines and stimulates mucus secretion in primary airway bronchial and tracheal epithelial cell lines. Ind. Crops Prod. 114, 81–86. https://doi.org/10.1016/J.INDCROP.2018.01.076.

Jiao, Jiao, Fu, Yu-Jie, Zu, Yuan-Gang, Luo, Meng, Wang, Wei, Zhang, Lin, Ji, Li., 2012. Enzyme-assisted microwave hydro-distillation essential oil from *Fructus forsythia*, chemical constituents, and its antimicrobial and antioxidant activities. Food Chemistry, Volume 134, Issue 1 (1 September), 235–243. https://doi.org/10.1016/j.foodchem.2012.02.114. 2012.

Jordán, M., Lax, V., Rota, M., Lorán, S., Sotomayor, J., 2013. Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. Food Control 30 (2), 463–468. https://doi.org/10.1016/j.foodcont.2012.07.029. April 2013.

Kayode, R.M., Azubuike, C.U., Laba, S.A., Dauda, A.O., Balogun, M.A., Ajala, S.A., 2018. Chemical composition and anti-microbial activities of the essential oil of *Adansonia digitata* stem-bark and leaf on post-harvest control of tomato spoilage. LWT. 93, 58–63. https://doi.org/10.1016/J.LWT.2018.03.014.

Khanavi, M., Laghaei, P., Murray, B., 2017. Essential oil composition of three native Persian plants and their inhibitory effects in the cabbage looper, *Trichoplusia ni*. J. Asia Pac. Entomol. 20 (4), 1234–1240.

Kisko, G., Roller, S., 2005. Carvacrol and p-cymene inactivate Escherichia coli O157:H7 in apple juice. BMC Microbiol. 5, 36. https://doi.org/10.1186/1471-2180-5-36.

Lei, J., Leser, Michael, Enan, Essam, 2010. Nematicidal activity of two monoterpenoids and SER-2 tyramine receptor of *Caenorhabditis elegans*. Biochem. Pharmacol. 79 (7), 1062–1071. https://doi.org/10.1016/j.bcp.2009.11.002.

Leon, G., Osorio, R., Ruby, S., Useche, M., 2015. Comparación de dos métodos de extracción del aceite esencial de *Citrus sinensis* L, vol.49. Facultad de ciencias farmacéuticas, revista cubana de Farmacia, Colombia, Cartagena, pp. 742–750 (4).

Li, Xiao-Juan, Wang, Wei, Luo, Meng, Li, Chun-Ying, Zu, Yuan-Gang, Mu, Pan-Song, Yu-Jie, Fu, 2012. Solvent-free microwave extraction of essential oil from *Dryopteris fragrans* and evaluation of antioxidant activity. Food Chem. 133 (2012), 437–444. https://doi.org/10.1016/j.foodchem.2012.01.056.

Lopez, J., Collin, G., Garneau, F., Jean, F., Gagnon, H., 2004. Essential oils from Bolivia. III. Asteraceae: *artemisia copa* philippi. Journal of Essential Oil. 16 (6) p554.

Marchese, A., Arciola, C.R., Barbieri, R., Silva, A.S., Nabavi, S.F., Tsetegho Sokeng, A.J., Izadi, M., Jafari, N.J., Suntar, I., Daglia, M., 2017. Update on monoterpenes as antimicrobial agents: a particular focus on p-Cymene. Materials. 10 (8), 947. https://doi.org/10.3390/ma10080947.

Mathew, M., Subramanian, S., 2014. *In vitro* screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. PLoS One 9 (1), e86804. https://doi.org/10.1371/journal.pone.

0086804.
Miño, J., Moscatelli, V., Hnatyszyn, O., Gorzalczany, S., Acevedo, C., Ferraro, G., 2004. Antinociceptive and antiinflammatory activities of *Artemisia copa* extracts. Pharmacol. Res. 50, 59–63. https://doi.org/10.1016/j.phrs.2003.12.016.
Morales, G., Sierra, P., Mancilla, A., Paredes, A., Loyola, L.A., Gallardo, O., Borquez, J., 2003. Secondary metabolites from four medicinal plants from northern chile: antimicrobial activity and biotoxicity against *Artemia salina*. J. Chil. Chem. Soc. 48 (2), 13–18. https://doi.org/10.4067/S0717-97072003000200002. [online]. 2003.
Morales, G., Paredes, A., Sierra, P., Loyola, L., 2008. Antioxidant activity of 50% aqueous - ethanol extract from *Acantholippia deserticola*. Biol. Res. 41 (2), 151–155. https://doi.org/10.4067/S0716-97602008000200004.
Morcillo, N., Di Giulio, B., Testani, B., 2004. A microplate indicator-based method for determining drug-susceptibility of multidrug resistant *Mycobacterium tuberculosis* to antimicrobial agents. Int. J. Tuberc. Lung Dis. 8, 253–259.
Okoh, O.O., Sadimenko, A.P., Afolayan, A.J., 2010. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. Obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem. 120 (1), 308–312. https://doi.org/10.1016/j.foodchem.2009.09.084.
Palá-Paúl, J., García-Jiménez, R., Pérez-Alonso, M.J., Velasco-Negueruela, A., Sanz, J., 2004. Essential oil composition of the leaves and stems of Meum athamanticum Jacq., from Spain. J. Chromatogr. A 1036 (2), 245–247. https://doi.org/10.1016/j.chroma.2004.02.064.
Quezada, C., Torres, A., Vargas, Y., Uribe, D., Carrasco, C., Torres, C., Rocha, R., Oyarzún, C., San Martín, R., 2016. Pro-apoptotic and anti-angiogenic properties of the α/β-thujone fraction from *Thuja occidentalis* on glioblastoma cells. J. Neurooncol. (February 22). https://doi.org/10.1007/s11060-016-2076-2.
Olmedo, R., Nepote, V., Grosso, N.R., 2013. Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils. Food Science and Technology – LWT 53, 409–417.
Randrianarivo, E., Maggi, F., Nicoletti, M., Rasoanaivo, P., 2016. Evaluation of the anticonvulsant activity of the essential oil of *Myrothamnus moschatus* in convulsion induced by pentylenetetrazole and picrotoxin. Asian Pac. J. Trop. Biomed. 6 (6), 501–505. https://doi.org/10.1016/J.APJTB.2016.01.017.
Rattanachaikunsopon, P., Phumkhachorn, P., 2010. Assessment of factors influencing antimicrobial activity of carvacrol and cymene against *Vibrio cholerae* in food. J. Biosci. Bioeng. 110, 614–619. https://doi.org/10.1016/j.jbiosc.2010.06.010.
Rodenak-Kladniew, B., Islan, G., Bravo, M., Durán, N., Castro, G., 2017. Design, characterization and in vitro evaluation of linalool-loaded solid lipid nanoparticles as potent tool in cancer therapy. Colloids Surf. B Biointerfaces 154, 123–132. https://doi.org/10.1016/j.colsurfb.2017.03.021.
Rojo, L., Benites, J., Rodrigues, A., Venâncio, F., Ramalho, L., Teixeira, A., Feio, S., Costa, M., 2006. Composition and antimicrobial screening of the essential oil of *Acantholippia deserticola* (Phil.eX F. Phil.) Moldenke. J. Essent. Oil Res. 18, 1–3.
Rota, M.C., Herrera, A., Martınez, R.M., Sotomayor, J.A., Jordan, M.J., 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control 19, 681–687. https://doi.org/10.1016/j.foodcont.2007.07.007.
Sá, S., Fiuza, T., Borges, L., Ferreira, H., Tresvenzol, L., Ferri, P., Rezende, M., 2016. Chemical composition and seasonal variability of the essential oils of leaves and morphological analysis of *Hyptis carpinifolia*. Rev. Bras. Farmacogn. 26, 688–693. https://doi.org/10.1016/j.bjp.2016.05.011.
Sampietro, D., Lizarraga, E., Ibatayev, Z., Omarova, A., Suleimen, Y., 2016. Chemical composition and antimicrobial activity of essential oils from *Acantholippia deserticola*, *Artemisia proceriformis*, *Achillea micrantha* and *Libanotis buchtormensis* against phytopathogenic bacteria and fungi. Nat. Prod. Res. 30 (17), 1950–171955. https://doi.org/10.1080/14786419.2015.1091453.
Shahsavarpour, M., Lashkarbolooki, M., Eftekhari, Mohammad Javad, Esmaeilzadeh, F., 2017. Extraction of essential oils from *Mentha spicata* L. (Labiatae) via optimized supercritical carbon dioxide process. The Journal of Supercritical Fluids, In Press, Accepted Manuscript. https://doi.org/10.1016/j.supflu.2017.02.004.
Shiva, A., Jeyaprakash, K., Chellappan, D.R., Murugan, R., 2017. Vasorelaxant and cardiovascular properties of the essential oil of *Pogostemon elsholtzioides*. J. Ethnopharmacol. 199, 86–90. https://doi.org/10.1016/J.JEP.2017.01.036.
Skantar, A.M., Agama, K., Meyer, S.L.F., 2005. Effects of Geldanamycin on hatching and juvenile motility in *Caenorhabditis elegans* and *Heterodera glycines*. J. Chem. Ecol. 31, 2481–2491.
Sodeifian, G., Sajadian, S.A., 2017. Investigation of essential oil extraction and antioxidant activity of *Echinophora platyloba* DC. using supercritical carbon dioxide. J. Supercrit. Fluids 121, 52–62. https://doi.org/10.1016/J.SUPFLU.2016.11.014.
Sonboli, A., Sefidkon, F., Yousefzadi, M., 2014. Antimicrobial activity and composition of the essential oil of *gontscharovia popovii* from Iran. Zeitschrift für Naturforschung C 61 (9–10), 681–684. https://doi.org/10.1515/znc-2006-9-1012. Retrieved 10 May. 2019, from.
Thakker, R., Parikh, K., Desai, A., 2016. Microwave assisted extraction of essential oil from the leaves of Palmarosa: multi-response optimization and predictive modelling. Ind. Crops Prod. 86, 311–319. https://doi.org/10.1016/j.indcrop.2016.03.055.
Tohidi, B., Rahimmalek, M., Arzani, A., 2017. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. Food Chemistry. Volume 220 (April 1), 153–161. https://doi.org/10.1016/j.foodchem.2016.09.203. 2017.
Torrenegra, M., Granados, C., Osorio, M., León, G., 2015. Comparación de la Hidrodestilación Asistida por Radiación de Microondas (MWHD) con Hidro-destilación Convencional (HD) en la Extracción de Aceite Esencial de *Minthostachys mollis*. Inf. Tecnol. 26 (1), 117–122. https://doi.org/10.4067/S0718-07642015000100013.
Ultee, A., Bennik, M.H., Moezelaar, R., 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68, 1561–1568. https://doi.org/10.1128/AEM.68.4.1561-1568.2002.
Van Haute, S., Raes, K., Devlieghere, F., Sampers, I., 2017. Combined use of cinnamon essential oil and MAP/vacuum packaging to increase the microbial and sensorial shelf life of lean pork and salmon. Food Packag. Shelf Life 12, 51–58. https://doi.org/10.1016/J.FPSL.2017.02.004.
Watanabe, E., Tanomaru, J.M.G., Nascimento, A.P., Matoba, F., Tanomaru, M., Ito, I.Y., 2008. Determination of the maximum inhibitory dilution of *cetylpyridinium* chloride-based mouthwashes against *Staphylococcus aureus*: an in vitro study. J. Appl. Oral Sci. 16 (4), 275–279. https://doi.org/10.1590/S1678-77572008000400009.
Youssef, M.M., Mahmoud, A.A., Al-Faiyz, Y.S., 2015. Antimicrobial and antioxidant activities of *Artemisia abyssinica* extracts and DNA degradation effects. Asian J. Biochem. Pharm. Res. 10 (1), 31–41.
Zarria, K., Gajardo, S., Benites, J., Rojas, M., 2010. Formulación y evaluación de la estabilidad de una serie de vehículos, para el desarrollo de un biopesticida a base de aceite esencial de *Acantholippia Desertícola*, vol. 18. Universidad Arturo Prat, Revista Biofarbo, Iquique, Chile, pp. 20–30. (2). http://www.revistasbolivianas.org.bo/pdf/rbfb/v18n2/a03_v18n2.pdf.