INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 2, p. 357-365, 2015

RESEARCH PAPER OPEN ACCESS

Antibacterial effects and chemical composition of essential oils from *Cotoneaster nummularioides* pojark and *Sonchus arvensis* L. leaves extracts on typical food-borne pathogens

Somaye Kanaani, Ali Mohamadi Sani*, Fahime Yaghooti

Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, Iran

Key words: Methanolic extract, *Cotoneaster nummularioides* Pojark, *Sonchus arvensis* L., Antibacterial activity, Essential oil.

http://dx.doi.org/10.12692/ijb/6.2.357-365 Article published on January 27, 2015

Abstract

This study was designed to compare the chemical composition of essential oils and the in vitro-antibacterial activities of methanolic extracts of *Cotoneaster nummularioides* Pojark and *Sonchus arvensis* L.. The essential oils were obtained by hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis of the essential oil revealed fifteen compounds for *C. nummularioides* Pojark and thirty-five compounds for *S. arvensis* L.. The agar disk diffusion method was used to study the antibacterial activity of *C. nummularioides* methanolic extract against four bacterial strains. Between these extracts only the extract of *C. nummularioides* Pojark showed antibacterial activity against two gram-positive microorganisms tested with higher sensitivity for *Bacillus cereus* (inhibition zones of 8 and 12 mm respectively for the concentration 300 and 400 mg/ml). Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were quantified by macro-dilution method. The MIC was 3.125 and 50 mg/ml for *Bacillus cereus* and 6.25 and 50 mg/ml for *Staphylococcus aureus*, respectively. Overall, results presented here suggest that the methanolic extracts of *C. nummularioides* Pojark and *Sonchus arvensis* L. have antibacterial properties, and are therefore two potential sources of active ingredients for food and pharmaceutical industry.

* **Corresponding Author:** Ali Mohamadi Sani ✉ mohamadisani@yahoo.com

Introduction

Bacterial infectious diseases represented by important cause of morbidity and mortality worldwide. An antibiotic resistant bacterium is a threat which is increasing currently (Bokaeian *et al.*, 2013). Traditionally, plant compounds are used for treatment of hospital infections in advanced countries. Appropriate method in obviating the common problems of antibiotics side effects is using plant drugs with antimicrobial properties (Golshani & Sharifzadeh, 2013). The genus Cotoneaster (Rosaceae, Maloideae) occurs in large parts of mainly continental temperate Eurasia. Its distribution is often scattered and mainly concentrated in the mountains of the meridional and nemoral zones, while having a clear centre of diversity in China and the Himalayas (Dickore and Kasperek, 2010). The leaves of *S. wightianus* are used in earache by rural communities of India. The juice of *S. alpinus* is useful in deafness, gout and old age. The roots of *S. arvensis* L. are used in cough, bronchitis and asthma. The leaves are applied to swellings, while its latex is used for the treatment of eye diseases. The extract of *S. asper* is applied to wounds and boils. The leaves and roots of the plant are used in indigestion and as a febrifuge, while its roots act as a vermifuge (Negi *et al.*, 2004).

Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food-borne pathogens Hussain *et al.* (2010) evaluated that the chemical composition of *Sonchus eruca* and *Sonchus asper*. Also, Dickore and Kasperek (2010) Identified that species of Cotoneaster (Rosaceae, Maloideae) indigenous to, naturalising or commonly cultivated in central Europe.

The aim of the present study was to investigate the chemical composition of essential oils of *Cotoneaster nummularioides* Pojark and *Sonchus arvensis* and antibacterial effects of methanolic extracts of these plants.

Experimental

Chemicals and Plant materials

Gentamicin (Sina daroo, Iran), methanol and Dimethyl Sulfoxide (DMSO) (Merck, Germany) were purchased. The aerial parts (leaves) of plants were collected in May 2014 from the mountains of North Khorasan Province in Iran. The plants were identified by the Research Center of Natural Products Health (NPH), North Khorasan University of Medical Sciences (Iran).

Extraction

C. nummularioides Pojark and *S. arvensis* leaves were dried at room temperature and the powdered material were then weighed (300 g), soaked in 1.5 L of methanol (MeOH) for 48 h and filtered using Whatman No.1 filter paper. The filtrate obtained was concentrated under reduced pressure (at 68°C) in a rotary evaporator to obtain the crude extracts were kept at 4°C until further uses (E Djeussi *et al.*, 2013).

Essential oil extraction

The oils of *C. nummularioides* Pojark and *S. arvensis* L. were obtained from 200 g leaves of plant, by hydro-distillation during 3hrs, using clevenger (Ben Hsouna and Hamdi, 2012). The essential oil were dried over anhydrous sodium sulfate, filtered and stored at refrigerator (Saei-dehkordi *et al.*, 2013). The essential oils were solubilized in n-Hexane for gas chromatography and mass spectrometry analysis (Ben Hsouna and Hamdi, 2012).

Gas chromatography/mass spectroscopy

The chemical composition of the essential oils was analyzed using GC–MS technique. The mass spectrometer was Agilent 6890 N GC/5973MSD-SCAN (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (EI) ionization mode (70ev) and HP-5MS (bonded and cross-linked 5% phenyl-methyl-polysiloxane, 30 mm-0.25 mm, coating thickness 0.25 mm) capillary column (Restek, Bellefonte, PA). Injector and detector temperatures were set at 220°C. The oven temperature was held at 50°C for 30 min, then programmed to 240°C at rate of 3°C/min. Helium (99.99%) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 were injected manually

(Ahmadzadeh Sani *et al*., 2014).

Organisms and Inoculation Conditions

Authentic pure cultures of bacteria were obtained from Persian Type Culture Collection (PTCC). They included gram positive bacteria; *Bacillus cereus* (PTCC 1015), *Staphylococcus aureus* (PTCC 1431) and gram-negative bacteria; *Salmonella enterica* (PTCC 1709), *Escherichia coli* (PTCC 1399). The bacteria strains were first grown on Mueller Hinton medium at 37°C for 24 hrs prior to seeding on to the nutrient agar (Zarai *et al*., 2012). Finally, suspensions were adjusted to 0.5McFarland standard turbidity. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml (Library of Congress Cataloging-in-Publication Data, 2005).

Antimicrobial assay

The Methanolic extracts of *C. nummularioides* Pojark and *S. arvensis* L. were tested for antimicrobial activity using agar disk diffusion technique to determine the diameter of growth inhibition zones while broth macro-dilution method was used to determine the MIC and MBC (Teke *et al*., 2013).

Disk-diffusion method

The antibacterial activity test was carried out on Methanolic extracts of the leaves of *C. nummularioides* Pojark and *S. arvensis* using disk diffusion method (NCCLS) against the mentioned microorganism (Zellagui *et al*., 2012). In this method, measured amount of the test samples were dissolved in definite volumes of solvent dimethyl Sulfoxide (DMSO) to give solutions of known concentrations (100, 200, 300 and 400 mg/ml). Then, sterile filter paper disks (6 mm diameters) were placed on plates (Petri dishes, 80 mm diameters) containing a suitable medium (MHA) seeded with the test organisms (1.5×10^8). The amount of 15 µL of methanolic extract was poured onto the disks. These plates were kept at low temperature (4°C) for 15 min to allow maximum diffusion. A number of events take place simultaneously, which includes absorption of water from the agar medium by dried disks and dissolving the material which is under test. The test material diffuses from the disks to the surrounding medium according to the physical law that controls the diffusion of molecules through agar gel. There is a gradual change of test material concentration on the agar surrounding each disk (Rahman and Sultana, 2011) and the solvent (DMSO) was used as a negative control (10 µl), while gentamicin was used as a positive control (10 µl) (Veljic *et al*., 2010). The plates were then inverted and incubated at 37°C for 24 hrs for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the disks and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter (Rishikesh *et al*., 2012).

Minimum Inhibitory Concentration (MIC) Test

The MIC test was done only for the extract, which was proved for the effectiveness using the disk diffusion method (inhibition zones ≥ 10 mm). MIC was determined using micro-dilution method according to the protocol of Sahin *et al*. (2004). The 96-well plates were prepared by dispensing into each well 95 µL of MH broth and 5µL of the inoculums (Elaissi *et al*., 2012) (standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm by Shimadzu UV-120-01 spectrophotometer) (Kuete *et al*., 2011). One-hundred microliters of the extract was initially prepared at a concentration of 400 mg/ml added into the first well, followed by two-fold dilution until the 9th well. The wells of column 10 were filled with 195 µL of MHB and reserved for the bacterial growth control, whereas the 11th column wells were reserved for the control of the broth sterility. The wells of the last column were used as a negative control, and contained 195 µL of MHB and 5 µL of the inoculum. The plates were screened visually after incubation at 37°C for 24 h for broth turbidity (Elaissi *et al*., 2012). The lowest concentrations of extract with no visible microbial growth on agar plates (after incubation for 18–24 h at 37°C) were recorded as MBC (Elaissi *et al*., 2012; Islam *et al*., 2010). The minimum bactericidal concentration (MBC) is the lowest concentration of

the essential oil that can kill 99.9% of the bacterial population after incubation for 18–24 h at 37°C. It was calculated by inoculating the content of the well indicating the MIC and the wells that precede it in an agar plate (Elaissi *et al.*, 2012).

Results and discussion

The composition of the essential oils from *C. nummularioides* Pojark is shown in Table 1 which indicates fifteen different components for essential oil from *C. nummularioides* Pojark. Based on the results, Methylcyclopentane (17.05%), Eucalyptol (15.05%), Camphor (13.23%), Eucalyptol (9.65%), Camphor (7.8%) and n-Octane (5.38%) were the major constituents.

Table 1. Composition determined by GC–MS analysis of the essential oils from *C. nummularioides* Pojark that were used to formulate the combined essential oil (CEO) tested in this study.

Compound	%	KI	R.time
Methylcyclopentane	17.05	*	2.079
Cyclohexane	4.16	*	2.389
n-Octane	5.38	790	4.414
Camphene	1.65	854.505494505495	5.552
Camphene	5	950.989010989011	7.308
Eucalyptol	9.65	993.571428571429	8.083
Eucalyptol	15.05	1041.03746397695	8.912
Camphor	7.8	1140.24539877301	10.591
Camphor	13.23	1162.94478527607	10.961
Dihydrocarveol	3.95	1174.0490797546	11.142
Borneol	4.11	1180.24539877301	11.243
Terpinene-4-OL	3.12	1188.52760736196	11.378
α-terpineol	2.32	1200.45901639344	11.572
Bornyl acetate	3.25	1296.59016393443	13.038
Davanone	4.28	1599.36507936508	17.137

Also, the chemical composition of *Sonchus arvensis* L. essential oil is summarized in Table 2. According to the obtained data, thirty-five compounds of the hydro-distilled essential oil from aerial parts of *S. arvensis* L. were identified, representing 99.82 % of the total oil. High quantity of alkans including n-octane (29.82%) and n-decane (11.09%) were the main components, followed by; 3-Methylheptane (5.13%), Toluene (3.51%), 1-Hexacosanol (2.92%), p-Xylene (2.59%), Camphor (2.41%) and mono (2-ethylhexyl) phthalate (1.97%) were found in significant amounts.

Results of disk-diffusion test

These results indicated that the diameters of inhibition zones varied from 7-12 mm and 19–29 mm for the various concentrations of extract and gentamicin, respectively. Among the four bacteria, *B. cereus* was the most sensitive to the extract (the diameter of inhibition zone was 12 mm for 400 mg/ml methanolic extract). However, among the four isolates, two bacteria (*S. enterica* and *E. coli*) were resistant to the extract at all the concentrations. This method showed no growth inhibition zone for methanolic extract of *S. arvensis*.

Results of MIC and MBC

The MIC values for *C. nummularioides* Pojark are summarized in Table 4, which show that the methanolic extract was able to prevent the growth of all the tested bacteria. Generally, all the microorganisms were sensitive to the extract. The

MIC values of the extract ranged from 3.125-75 mg ml^{-1}, with *B. cereus* being the most sensitive while *S. enterica* was the least sensitive. MIC for *B. cereus* was 3.125 and for *S. aureus* obtained 4.16 mg ml^{-1}, so the gram-positive bacteria were more sensitive than the gram-negative bacteria.

Table 2. Composition determined by GC–MS analysis of the essential oils from *S. arvensis* that were used to formulate the combined essential oil (CEO) tested in this study.

Compound	%	KI	R.time
3-Methylhexane	4.897	*	2.878
cis-1,3-Dimethylcyclopentane	2.714	*	2.941
trans-1,3-Dimethylcyclopentane	1.6815	*	2.969
trans-1,2-Dimethylcyclopentane	2.0355	*	2.998
n-Heptane	1.652	700	3.085
Toluene hexahydride	0.7965	718.6301	3.372
Toluene	3.5105	764.863	4.047
3-Methylheptane	5.133	772.1233	4.153
1-Ethyl-3-methylcyclopentane	1.5635	788.2192	4.388
1-Ethyl-3-methylcyclopentane	1.7995	791.6438	4.438
n-Octane	29.8245	812.5275	4.788
α-Methyltoluene	0.6195	863.3516	5.713
p-Xylene	2.596	873.0769	5.89
o-Xylene	1.6815	896.7033	6.32
α-pinene	1.062	937.6923	7.066
5-Methylnonane	0.6195	960.9341	7.489
3-Methylnonane	0.7375	972.3077	7.696
n-Decane	11.092	1007.781	8.335
EUCALYPTOL	0.7375	1037.867	8.857
Camphor	2.419	1157.055	10.865
BORNEOL	0.826	1177.239	11.194
n-Dodecane	3.8645	1203.475	11.618
n-Tetradecane	1.475	1401.324	14.543
3-(2-Methoxyethyl)-1-nonanol	0.767	1496.544	15.838
n-Hexadecane	0.708	1600	17.145
2-PENTADECANON, 6,10,14-TRIMETHYL	0.9735	1850.323	20.046
Isobutyl phthalate	0.649	1882.396	20.394
Palmitic acid, methyl ester	0.6195	1928.213	20.877
Palmitic acid	1.888	1977.488	21.387
Heneicosane	1.239	2102.434	22.633
Linolenic acid, methyl ester	1.003	2112.063	22.724
PHYTOL	2.36	2125.714	22.853
mono(2-ethylhexyl) phthalate	1.9765	2570.875	26.727
1-Hexacosanol	2.9205	2910.141	29.267
Nonacosane	1.3865	*	34.881

Table 5 also reports the Minimum Inhibitory Concentration (MIC) and the Minimum Bacterial Concentration (MBC) values of the essential oils against four Gram positive and Gram negative bacterial strains. All extracts showed dose dependent activity which increases with increase in concentration. Antibiotic gentamicin was positive control and DMSO was the negative control.

As the table shows, methanolic extract of *S. arvensis* L. has prevented from the growth of all bacteria. The results determined that in tested bacteria, there was a difference in terms of sensitivity to methanolic extracts. In other words, the most sensitivity was observed in *B. cereus* and *S. aureus* and the least was seen in *S. enterica* and *E. coli*. So, as it shown in this table, MIC is between 12.5 to 100 mg/ml and MBC is between 100 to even more than 400 mg/ml. The MIC and MBC against the bacteria *B. cereus* and *S. aureus* was satisfactory.

Table 3. Results of disk-diffusion test and inhibition zones (mm) for methanolic extract of *C. nummularioides*.

Microorganism	Concentrations of methanolic extract (mg/ml)				Positive control	Negative control
	100	200	300	400	Gentamicin	DMSO
B. cereus	6	6	8	12	29	6
S. aureus	6	6	7	11	19	6
S. enterica	6	6	6	6	20	6
E. coli	6	6	6	6	21	6

Food contamination is enormous public health problem, but it could be controlled by the use of natural preservatives such as essential oils obtained from plants (M. Skrinjar and Nemet, 2009). The antimicrobial activities of *C. nummularioides* methanolic extract against microorganism examined in the present study and their potency were qualitatively and quantitatively assessed by the presence of inhibition zones and MIC values. The maximal inhibition zones and related MIC values for the bacterial strains, which were sensitive to the methanolic extract of *C. nummularioides* Pojark, were respectively in the range of 7-12 mm, and 3.125-75 mg ml^{-1}.

Table 4. MIC and MBC for methanolic extract of *C. nummularioides* Pojark and *S. arvensis* (mg ml^{-1}).

Microorganism	*B. cereus*	*S. aureus*	*S. enterica*	*E. coli*
MIC	3.125	4.16	75	66.67
MBC	108.33	102.08	400	233.33

Table 5. MIC and MBC for methanolic extract of *S. arvensis* and *S. arvensis* (mg ml^{-1}).

Microorganism	*B. cereus*	*S. aureus*	*S. enterica*	*E. coli*
MIC	50	50	100	100
MBC	400	400	>400	>400

A review of the literature on the antimicrobial activity of different plant extracts shows that methanolic extract has a high level of activity (Askun *et al.*, 2009). Moreover, several authors have studied antibacterial activities of different herbal extracts using methanol as an extraction solvent and observed comparatively better activity of methanolic extract than the other solvents (Islam *et al.*, 2010). Negi *et al.* (2004) showed that the methanolic extract was the most effective, followed by chloroform and acetone extracts (Negi *et al.*, 2004).

S. enterica was the most resistant species to the methanolic extracts of *C. nummularioides* Pojark and *S. arvensis* with the highest MIC value (75 and 100 mg ml^{-1}). In the present study, bacterial species including gram (+) and gram (-) bacteria exhibited different degrees of sensitivity to the extracts which may be due to the differences in the chemical composition and structure of cell wall of both types of microorganisms (Goyal *et al.*, 2009). The higher resistance of gram-negative bacteria to external agents has been earlier reported, and it is attributed to the presence of lipopolysaccharides in their outer membranes, which make them inherently resistant to antibiotics, detergent and hydrophilic dyes. The reason for higher sensitivity of the gram-positive bacteria than negative bacteria could be ascribed to the presence of an outer peptidoglycan layer which is an ineffective permeability barrier (Negi *et al.*, 2004). The hydrophilic cell wall structure of gram-negative bacteria, constituted essentially by a lipo-polysaccharide, blocks the penetration of hydrophobic components of oils and for this reason, gram-positive bacteria are found to be more sensitive to the essential oils effects (M. Ojeda-Sana *et al.*,

2012).

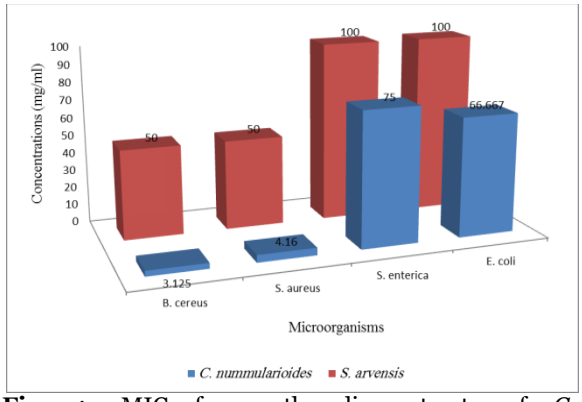

Fig. 1. MIC for methanolic extracts of *C. nummularioides* Pojark and *S. arvensis* (mg ml^{-1}) against different bacteria.

Conclusion

This is the first study to provide data on the methanolic extracts of *C. nummularioides* Pojark and *S. arvensis* L. plant evaluated against four pathogenic bacteria. Our findings may indicate that the methanolic extracts of *C. nummularioides* Pojark and *S. arvensis* L. to be used as a natural preservative in food systems against the well-known causal agents of food-borne diseases and food spoilage such as *B. cereus* and *S. aureus*.

References

Ahmadzadeh Sani T, Golmakani E, Mohammadi A, Feyzi P, Kamali H. 2014. Optimization of pressurized hot water extraction on the extract yield and antioxidant activity from *Biebersteinia multifida* DC using a modified supercritical fluid extractor. The Journal of Supercritical Fluids **94,** 130-137. http://dx.doi.org/10.1016/j.supflu.2014.07.006.

Askun T, Tumen G, Satil F, Ates M. 2009. In vitro activity of methanol extracts of plants used as spices against Mycobacterium tuberculosis and other bacteria. Food Chemistry **116,** 289-294. http://dx.doi.org/10.1016/j.foodchem.2009.02.048

Ben Hsouna A, Hamdi N. 2012. Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from *Pelargonium graveolens* growing in Tunisia: BioMed Central Ltd. Lipids in Health and Disease **11(167),** 1-7. http://dx.doi.org/10.1186/1476-511X-11-167.

Bokaeian M, Saboori E, Saeidi S, Niazi A, Amini N, Khaje H, Bazi S. 2013. Phytochemical analysis, antibacterial activity of *Marrubium vulgare* L. against *Staphylococcus aureus* in vitro. Zahedan Journal Research in Medical Science **16(10),** 60.

Dickore WB, Kasperek G. 2010. Species of Cotoneaster (Rosaceae, Maloideae) indigenous to naturalizing or commonly cultivated in Central Europe. Willdenowia **40,** 13-45. http://dx.doi.org/10.3372/wi.40.40102.

Djeussi ED, Noumedem J, Seukep AJ, Fankam AG, Voukeng KI, Tankeo BS, Nkuete HLA, Kuete V. 2013. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complementary and Alternative Medicine **13(164),** 1-8. http://dx.doi.org/10.1186/1472-6882-13-164.

Elaissi A, Rouis Z, Ben Salem N A, Mabrouk S, Ben Salem Y, Bel Haj Salah K, Aouni M, Farhat F, Chemli R, Harzallah-Skhiri F, Larbi Khouja M. 2012. Chemical composition of 8 eucalyptus species essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC Complementary and Alternative Medicine **12,** 81. http://dx.doi.org/10.1186/1472-6882-12-81.

Golshani Z, Sharifzadeh A. 2013. Evaluation of antibacterial activity of alcoholic extract of rosemary leaves against pathogenic strains. Zahedan Journal Research in Medical Science **16(3),** 12-15.

Goyal P, Chauhan A, Kaushik P. 2009. Laboratory evaluation of crude extracts of *Cinnamomum tamala* for potential antibacterial activity. Electronic Journal of Biology **5(4),** 75-79.

Heravi MM, Rodi S, Ardalan P. 2013. Study of Antioxidant and Free Radical Scavenging Activities of

Cotoneaster medicus and *Glycyrrhiza glabra* Plants. J Chem Health Risks **3(2),** 27-34.

Hussain J, Muhammad Z, Ullah R, Ullah Khan F, Ullah Khan I, Khan N, Ali J, Jan S. 2010. Evaluation of the Chemical Composition of *Sonchus eruca* and *Sonchus asper*. Journal of American Science **6(9), 231.**

Islam S, Rahman A, Sheikh MI, Rahman M, Jamal AHM, Alam F. 2010. *In vitro* Antibacterial Activity of Methanol Seed Extract of *Elettaria cardamomum* (L.) Maton. Agriculturae Conspectus Scientificus **25(3),** 113-117.

Kuete V, Kamga J, Sandjo PL, Ngameni B, Poumale MPH, Ambassa P, Ngadjui TB. 2011. Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita Vahl.* (Moraceae). BMC Complementary and Alternative Medicine **11(6),** 1-6. http://dx.doi.org/10.1186/1472-6882-11-6.

Library of Congress Cataloging-in-Publication Data. 2005. Manual of antimicrobial susceptibility testing, 39-41.

Skrinjar MM, Nemet NT. 2009. Antimicrobial effects of spices essential oils. Acta periodica technologica **40,** 195-209. http://dx.doi.org/10.2298/APT0940195S.

Ojeda-Sana MA, Van Baren CM, Elechosa MA, Juarez MA, Moreno S. 2012. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control **31,** 189-195. http://dx.doi.org/10.1016/j.foodcont.2012.09.022

Negi PS, Chauhan AS, Sadia GA, Rohinishree YS, Ramteke RS. 2004. Antioxidant and antibacterial activities of various sea buckthorns (*Hippophae rhamnoides* L.) seed extracts. Food Chemistry **1(92),** 119-124. http://dx.doi.org/10.1016/j.foodchem.2004.07.009

Rahman SM, Sultana S. 2011. Antimicrobial, Antioxidant and Cytotoxic Effects of the Bark of TERMINALIA ARJUNA. Inter J Pharma Sci Res **3(1),** 130-137.

Rishikesh M, Rahman M, Siddiqul Islam SM, Rahman MM. 2012. Phytochemical Screening and In Vitro Antimicrobial Investigation of the Methanolic Extract of *Centella Asiatica* Leaves. Inter J Pharma Sci Res **3(9),** 3323-3330.

Saei-dehkordi S, Khalighi-sigaroodi F, Pirali-kheirabadi K, Saei-dehkordi S, Alimardani-naghani F, Fallah A. 2014. Chemical composition, antioxidative capacity and antimicrobial activity of *Zeravschaina membranacea* (boiss.) pimenov essential oil. Journal of Food Safety **34,** 87–94. http://dx.doi.org/10.1111/jfs.12100.

Sahin F, Gulluce M, Daferera D, Sokmen A, Sokmen M, Polissiou M, Agar G, Ozar H. 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. Vulgare in the Eastern Anatolia region of Turkey. Food Control **15,** 549-557. http://dx.doi.org/10.1016/j.foodcont.2003.08.009

Teke GN, Elisee KN, Roger KJ. 2013. Chemical composition, antimicrobial properties and toxicity evaluation of the essentioal oil of *Cupressus Lusitanica* Mill. Leaves from Cameroon. BMC Complementary and Alternative Medicine **13(130),** 1-9. http://dx.doi.org/10.1186/1472-6882-13-130.

Veljic M, Ciric A, Sokovic M, Janackovic P, Marin PD. 2010. Antibacterial and Antifungal Activity of the Liverwort (*PTILIDIUM PULCHERRIMUM)* Methanol Extract. Arch Biol Sci Belgrade **62(2),** 381-395. http://dx.doi.org/10.2298/ABS1002381V.

Zarai Z, Ben Chobba I, Ben Mansour R, Bekir A, Gharsallah N, Kadri A. 2012. Essential oil of the leaves of *Ricinus communis* L.: in vitro

cytotoxicity and antimicrobial properties. Lipids in Health and Disease **11(102),** 1-7. http://dx.doi.org/10.1186/1476-511X-11-102.

Zellagui A, Gherraf N, Rhouati S. 2012. Chemical composition and antibacterial activity of the essential oils of *Ferula vesceritensis* Cosset Dur. Leaves, endemic in Algeria. Organic and Medicinal Chemistry Letters **2(2),** 1-4. http://dx.doi.org/10.1186/2191-2858-2-2.