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AROMA PROFILE AND ANTIMICROBIAL PROPERTIES OF ALCOHOLIC AND AQUEOUS EXTRACTS FROM ROOT, LEAF AND STALK OF NETTLE (*Urtica dioica* L.)

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ABSTRACT

Medicinal plant can be considered as a great source of new antimicrobial agents due to their enormous therapeutic potential and limited side effects. Nettle (*Urtica dioica* L.) is a widespread and common medicinal plant widely used in traditional medicine. The present study investigates the antimicrobial potency of alcoholic and aqueous extracts of *Urtica dioica* on some gram positive and negative bacteria and also a particular type of fungi and analyzes the extracts to find the active ingredients by gas chromatography-mass spectroscopy (GC-MS) method. Results from disc diffusion assay indicated that water extract of root, leaf and stalk had the highest antimicrobial activity respectively and caused significant inhibition zones in *P. vulgaris*, *L. monocytogenes* and *K. pneumoniae* cultures. Antimicrobial efficacy of ethanol extracts was higher in root extract which caused high growth inhibition zones in *P. vulgaris*, *K. pneumoniae* and *S. aureus* cultures. MBC and MIC experiments of the ethanol extract illustrated that the most powerful antimicrobial effect was related to the stem organ extract on *K. pnuomonae* and *S. aureus* bacteria. Highest level of antibacterial effects in root can be due to its higher concentration of contents compared to other organs. Based on these results it can be suggested that *Urtica dioica* and its water and ethanol extracts have noticeable antimicrobial effects against gram negative, positive and *Candida albicans* fungi that may be applicable as a prophylactic or therpeutic antimicrobial agent in both human and animals.

Keywords: Nettle (*Urtica dioica* L.), alcoholic extract, aqueous extract, antimicrobial properties, GC-MS

INTRODUCTION

Continuous appearance of multidrug resistant strains of bacteria and fungi adds to the urgency of continuous research to find new and effective antibiotics. Medicinal plant can be considered as a great source of new antimicrobial agents due to their enormous therapeutic potential and limited side effects **(Elzaawely et al., 2005, Neves et al., 2009)**.
Nettle (*Urtica dioica* L.) is a widespread and common medicinal plant widely used in traditional medicine. *Urtica* is annual and perennial herb with stinging hairs and can be found in wide variety of locations including Northern Iran **(Modarresi-Chahardehi et al., 2012)**. Several species of the genus *Urtica* (especially *Urtica dioica*, Urticaceae), are used medicinally to treat a variety of disease. Nettle has the great potential for providing new drug leads with new mechanisms of action **(Singh et al., 2012)**.
Different therapeutic effects of this plant have been reported in numerous studies including anti-inflammatory and antirheumatic **(Obertreis et al., 1996, Riehemann et al., 1999)**, antioxidant, antimicrobial, antiulcer, analgesic **(Gulcin et al., 2004)**, antiproliferative **(Konrad et al., 2000)** and cardiovascular **(Testai et al., 2002)** effects. Nettle extracts can act as a stimulant of proliferation in human lymphocytes **(Wagner et al., 1989)**. Therapeutic and inhibitory effect of nettle extract on prostatic hyperplasia has been shown in different studies **(Krzeski et al., 1993, Lichius and Muth, 1997)**.
On the basis of the results from the study of Gulcin *et al.*, it is clearly indicated that water extract of nettle has a powerful antioxidant activity against various oxidative systems *in vitro*; moreover, water extract of nettle can be used as accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.
Antimicrobial activity of different fractions of various *Urtica* species has been reported in several studies **(Hadizadeh et al., 2009, Modarresi-Chahardehi et al., 2012, Singh et al., 2013)** which indicate the high potential of this plant in the discovery of novel effective compounds.

The present study investigates the antimicrobial potency of alcoholic and aqueous extracts of *Urtica dioica* and analyzes the extracts to find the active ingredients by gas chromatography-mass spectroscopy (GC-MS) method.

MATERIALS AND METHODS

Collection of plant material

The *Urtica dioica* whole plants were collected freshly from Tonekabon in Mazandaran province of Iran. Taxonomic verification was conducted in Islamic Azad University of Tonekabon's Herbarium by comparing with authentic specimen.

Preparation of extract

Root, leaf and stalk of the plant were separated, cleaned and air-dried immediately after collection in a dark room with fresh air circulation. Then all 3 parts were ground, packed separately and stored in a dark place before preparing the extracts. To prepare aqueous extract, 500 mL of distilled water pre-heated to 80°C was added to 50 g of powdered root, leaf and stem in Erlenmeyer flasks and incubated for 72 hours in 60°C water bath. After incubation the mixtures were filtered using a Whatman's No. 1 filter paper and the extracts were dried in a rotary evaporator at 45°C. The alcoholic extract prepared with percolation method and pre-heated 80% ethyl alcohol used as the solvent. 50g of each part was added to the percolator and after 72 hours of incubation remaining alcohol removed by vacuum rotary evaporator. All extracted powders dissolved in proper solvent (distilled water for aqueous extracts and dimethyl sulfoxide (DMSO) for alcoholic extracts) to reach the final concentrations of 0.5, 0.25, 0.125 and 0.0625 $g.mL^{-1}$.

Preparation of microorganisms

Test microorganisms included *Candida albicans* (ATCC 10231), *Staphylococcus aureus* (ATCC 6538), *Proteus vulgaris* (ATCC 13315), *Listeria monocytogenes* (ATCC 7644) and *Klebsiella pneumoniae* (ATCC 1053) were obtained from Iranian Research Organization for Science and Technology (IROST) and cultivated in Mueller-Hinton broth over night then sub-cultured twice for thorough recovery.

Antimicrobial activity

Antimicrobial activity of these extracts was determined by inhibition zones in disc diffusion method on Mueller-Hinton agar culture medium followed by evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in broth dilution assay. Usually, the extract with large inhibition zone diameter and low MIC can be recognized as more potent antimicrobial compound than that of small inhibition zone diameter and high MIC **(Semwal et al., 2009)**.

To perform disc diffusion assay, 30 µL of each extract was added to blank sterile antibiotic discs (Padtan-Teb Co., Iran). The suspension of microorganisms ($1.5*10^8$ CFU.mL^{-1}) was added to Mueller-Hinton agar plates then 3 extract impregnated discs were placed on each plate. Extracts were tested in triplicate and plates were incubated for 24 hours at 37°C. To demonstrate the efficacy of extracts, highest concentration of extracts were compared to commercial antibiotic discs (Ampicillin (10 mg), Ceftizoxime (30 mg), Clindamycin (2mg), Amikacin (30 mg), Gentamicin (10 mg)) as standard antimicrobial agents (Padtan-Teb Co., Iran).

To determine minimum inhibitory concentration (MIC) for tested microorganisms, two-fold serial dilutions from 0.5 g.mL^{-1} extract stock solutions were prepared and incubated for 24 hours at 37°C. Negative control contained equal amount of culture medium and 0.5 McFarland turbidity standard solution. Positive control included equal amount of culture medium and the extract. MIC was defined as the lowest concentration of extract that had no visible turbidity (optical density (OD) less than 0.05 at 660 nm).

Minimum bactericidal concentration (MBC) determination was performed after MIC test sets. As described by **Motamedi *et al.* 2009**, a loop full of broth from tubes which showed no visible growth was streaked on Mueller-Hinton agar plates then incubated at 37°C for 24 hours. The lowest concentration of extract that yielded no colonies considered as MBC.

Results were recorded and interpreted according to regulations established by National Committee on Clinical Laboratory Standards (NCCLS) **(Rex, 2008)**.

Chemical analysis of extracts

The chemical composition of alcoholic extract was analyzed by gas chromatography – mass spectrometry (GC-MS). The chromatograph instrument (Agilent 6890 UK) was equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness) and the data were taken under the following conditions: initial temperature 50°C, temperature ramp 5°C.min, 240°C.min to 300°C (holding for 3 min), and injector temperature at 290°C. The carrier gas was helium and the split ratio was 0.8 mL^{-1}/min. For confirmation of analysis results, EO was also analyzed by gas chromatography–mass spectrometry (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent UK) and the same capillary column and analytical conditions as above. The MS was run in electron-ionization mode with ionization energy of 70 eV.

RESULTS

The GC/MS analysis of *S.striata* alcoholic extract disclosed 20 components which the most important ones include 5 mixtures such as Neophytadiene (25.21%), Phthalic acid (8.15%), Dibutyl phthalate (7.37%), 1,2-Benzenedithiol (7.62%) and Bis (2-ethyl) maleate (32.6%). Besides, Root had the highest level of these contents among different organs.

In general, water extracts showed more antimicrobial potency compared to ethanol extracts. Results from disc diffusion assay indicated that water extract of root, leaf and stalk had the highest antimicrobial activity respectively and caused significant inhibition zones in *P. vulgaris*, *L. monocytogenes* and *K. pneumoniae* cultures Antimicrobial efficacy of ethanol extracts was higher in root extract which caused high growth inhibition zones in *P. vulgaris*, *K. pneumoniae* and *S. aureus* cultures (Table 1). Antimicrobial susceptibility results according to standard antibiotic discs are shown in Table 2.

The lowest MIC and MBC values in water extracts were observed in leaf extract on *C. albicans* and extract of root and stem on *K. pneumoniae*. Water extract of stalk showed highest MIC and MBC values on *P. vulgaris* culture. In addition, ethanol extracts revealed the least MIC and MBC values in root extract on *P. vulgaris*, *K. pneumoniae* and *S. aureus* culture and highest values were from stalk extract on *L. monocytogenes* (Table 3).

Table 1 Means of inhibition zones of bacterial growth (mm)

Type	Section	Concentration (g.mL^{-1})	Microorganisms				
			C. albicans	*S. aureus*	*K. pneumoniae*	*P. vulgaris*	*L. monocytogenes*
Water Extract	Leaf	0.5	17.5	17	13.5	13.5	17
		0.25	16	16	11.5	11.5	15.5
		0.125	13.5	15	10	10.5	14.5
		0.0625	11	12	8.5	8.5	12
	Stem	0.5	13.5	17.5	17	11	11
		0.25	11.5	16.5	14.5	9.5	10
		0.125	9.5	15	11.5	8	9
		0.0625	8.5	14	10	7	7
	Root	0.5	13	14.5	27.5	21.5	17.5
		0.25	11	13	24	19.5	12.5
		0.125	9.5	11.5	20	15	10
		0.0625	8	9.5	17.5	12	8.5
Ethanol Extract	Leaf	0.5	10	10.5	10	11.5	11.5
		0.25	9	8.5	9.5	9.5	10.5
		0.125	7.5	7.5	8	8	8
		0.0625	7	7	7	7	7
	Stem	0.5	14	13	12.5	11	10
		0.25	12.5	12	10	9	9.5
		0.125	10.5	10	9	7.5	7.5
		0.0625	8.5	9.5	7.5	7	7
	Root	0.5	12.5	15	15	15	13.5
		0.25	10.5	14	12.5	12.5	11.5
		0.125	10	12.5	10	10.5	9.5
		0.0625	8	11	8	8.5	8

Table 2 Antimicrobial susceptibility of microorganisms toward different extracts (0.5 g.mL^{-1}) compared to standard inhibition zones of different antibiotics. S: Sensitive; I: Intermediate; R: Resistant

Type	Section	Microorganisms	Antibiotics				
			Ampicillin (10mg)	Ceftizoxime (30mg)	Amikacin (30mg)	Clindamycin (2mg)	Gentamicin (10mg)
Water Extract	Leaf	*C. albicans*	S	I	S	S	S
		S. aureus	S	I	S	S	S
		K. pneumoniae	S	R	R	R	I
		P. vulgaris	S	R	R	R	I
		L. monocytogenes	S	I	S	S	S
	Stem	*C. albicans*	S	R	R	R	I
		S. aureus	S	I	S	S	S
		K. pneumoniae	S	I	S	S	S
		P. vulgaris	R	R	R	R	R
		L. monocytogenes	R	R	R	R	R
	Root	*C. albicans*	I	R	R	R	I
		S. aureus	S	R	R	R	I
		K. pneumoniae	S	S	S	S	S
		P. vulgaris	S	S	S	S	S
		L. monocytogenes	S	I	S	S	S
Ethanol Extract	Leaf	*C. albicans*	R	R	R	R	R
		S. aureus	R	R	R	R	R
		K. pneumoniae	R	R	R	R	R
		P. vulgaris	R	R	R	R	R
		L. monocytogenes	R	R	R	R	R
	Stem	*C. albicans*	S	R	R	R	I
		S. aureus	I	R	R	R	I
		K. pneumoniae	I	R	R	R	R
		P. vulgaris	R	R	R	R	R
		L. monocytogenes	R	R	R	R	R
	Root	*C. albicans*	I	R	R	R	R
		S. aureus	S	I	I	I	S
		K. pneumoniae	S	I	I	I	S
		P. vulgaris	S	I	I	I	S
		L. monocytogenes	I	R	R	R	I

Table 3 Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values (g.mL^{-1})

Type	Section	Microorganisms									
		C. albicans		*S. aureus*		*K. pneumoniae*		*P. vulgaris*		*L. monocytogenes*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Water Extract	Leaf	0.004	0.0625	0.313	0.0625	0.0625	0.25	0.313	0.125	0.313	0.25
	Stem	0.0156	0.125	0.313	0.125	0.0156	0.0625	0.125	0.25	0.125	0.25
	Root	0.008	0.0625	0.313	0.125	0.004	0.313	0.008	0.0625	0.0625	0.25
Ethanol Extract	Leaf	0.125	0.25	0.125	0.25	0.125	0.25	0.0625	0.25	0.125	0.25
	Stem	0.0156	0.125	0.0625	0.25	0.0625	0.25	0.25	0.25	0.25	0.25
	Root	0.0625	0.25	0.008	0.0625	0.0156	0.125	0.008	0.125	0.0625	0.25

DISCUSSION

In this research the antimicrobial impact of the nettle plant extract on some gram positive and negative bacteria and also a particular type of fungi has been studied which signified potent antimicrobial effects. Water extracts exhibited a powerful antimicrobial effect against gram positive and negative bacteria. High sensitivity of gram positive bacteria (*S. aureus* and *L. monocytogenes*) was obvious in all dilutions of water extract of leaf but gram negative bacteria (*P. vullgaris* and *K. pneumoniae*) were more susceptible to water extract of root in all dilutions. Less antibacterial effect was visible in ethanol extract compared to water extract in different organs of the nettle plant. The alcoholic extract of stem had the highest effect on the gram positive bacteria. In addition, this extract caused more antibacterial potency on gram negative bacteria and *Candida albicans* compared to the extract of other organs. Higher antimicrobial efficacy of aqueous extracts may be due to the boiling step of extracion which causes a more efficient isolation of active ingredients. According to the **Gulcin *et al.* (2004)**, **Motamedi *et al.* (2009)** and **Dar *et al.* (2012)** water extracts of nettle have antimicrobial activity against some microorganisms such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

Based on the MIC and MBC value, the water extract of root had the good antimicrobial effects on *P. vulagaris*, *K. pneumoniae* and *C. albicans* bacteria. Also the ethanol extract of root revealed the lowest MBC and MIC value on *P. vulagaris* and *S. aureus* bacteria. Which can be a result of higher active ingredients content in root organ.

Shale *et al.* (1999) investigated antimicrobial properties in ethanol and methanol extracts of the stem and leaf organs of *Urtica dioica* against *E. coli* ,*S. aureus* and *Pseudomonas aeruginosa* by disc diffusion assay and they declared that the *E. coli* and *P. aeruginosa* were completely resistant to the nettle extract. In our research also, all tested microorganisms were resistant to the alcoholic extract of the nettle stem. A study by **Kukrić *et al.* (2012)** revealed that higher content of chlorophyll, carotenoids and proteins in young nettle leaves and also the extract of nettle had inhibitory effect on various gram positive and gram-negative bacteria including: *Bacillus subtilis, Lactobacillus plantarum, Pseudomonas aeruginosa*, and *Escherichia coli*.

Palic *et al.* (2002), declared that plants containing Neophytadies have antibacterial activity. **Lalitharani *et al.* (2010)**, reported that the neophytadiene compounds which has antibacterial activity is suitable for treatment of some skin diseases. **Roy *et al.* (2010)** reported Aromatic compounds including carboxylic acid and ester in the nettle plant have the same effects as neophytadiene compounds.

Many fatty acids have antibacterial and antifungal properties **(Russell, 1991)**. **Li *et al.* (2004)** reported fatty acids including dibutyl Ester Bis (2-ethyl hexyl) Maleat, Phatalic acid and 1, 2-benzendi carboxylic acid, which are present in ur extracts, have antiseptic and antimicrobial activities.

It has been suggested that ethyl acetate, hexane and chloroform extracts showed higher antimicrobial activity than the other crude extracts. For example, ethyl acetate extract showed the highest growth inhibition against *B. cereus*, methicillin resistant *Staphyloccocus aureus* and *Vibrio parahaemolyticus*

(Modarresi-Chahardehi et al., 2012). Terpens and phenols of nettle are one of the major groups associated with the inhibition of microbial infections and cancers and also, terpens improves the treatment of headache, rheumatism and some skin diseases **(Dar et al., 2012, Lalitharani et al., 2010, Taylor et al., 1996)**. Phenols also have been reported to be associated with the inhibition of atherosclerosis and cancer, as well as age-related degenerative brain disorders **(Cheung et al., 2003, Wang et al., 2009)**. Phenols probably play a synergistic role toward other components **(Singh et al., 2012)**. The study of **Ghaima *et al.* (2013)** revealed that ethyl acetate extract of nettle had obvious antibacterial and antioxidant activities and it was more effective on all bacterial isolates such as *Aeromonas hydrophila*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* with highest inhibition zone towards *B. cereus* but *A hydrophila* was more resistant than other bacteria. This bacterial inhibition may be due to high phenolic content. Moreover, phytochemical qualitative screening exhibited flavonoid, glycosides and phenols were present in nettle and presence of active compounds such as alkaloids, tannins and terpenoids in nettle was verified **(Ahmed et al., 2012)**. On the contrary, some studies revealed that the ethanol extract of nettle leaves did not inhibit the growth of *E. coli* which was assumed to be a result of outer membrane of cell wall of gram negative bacteria acting as barrier to many substances including antibiotics and caused high resistant to plant extracts **(Marino et al., 2001, Sánchez et al., 2009)**.

Extraction method of active ingredients, the plant type, the geographical and ecological status, climate, seasonal and experimental conditions, the age of plant, environmental stresses on the plant, and the inter species differences are effective on the amount and the type of the effective plant chemicals and it is reason of diversities in different studies **(Otles and Yalcin, 2012, Özkan et al., 2011, Pourmorad et al., 2006)**. Different antimicrobial properties of alcoholic and aqueous extract might be the result of isolation of different compounds in different solvents and also different extraction efficiencies and possible chemical degradations by polar and non-polar solvents.

Higher Antimicrobial activity of aqueous extract from stem compared to standard Antibiotics (Gentamycin, Ampicillin, Ceftizoxim, Amikacin, and Clindamycin), shows that these extracts have the potential to be used as efficient and strong antibiotic.

In addition, urtica dioca water extract is very rich with various salts, especially with magnesium. Also, some apolar extracts (chloroform, benzene, ethyl acetate) are very rich with sterols (which are very good for hair; most of shampoos contains extract of urtica dioca; reason is vasodilatator activity of sterols in combination with various salts).

With regards to the fact that nettle plant has growing ability in diverse and rough environmental conditions, industrial scale propagation of this plant is practical and economical. Also by choosing the species with more active ingredients, it is possible to obtain suitable proficiency from this plant.

CONCLUSION

Based on these results it can be suggested that *Urtica dioica* and its water and ethanol extracts have noticeable antimicrobial effects against the gram negative, positive and *Candida albicans* fungi and it is an interesting source of biologically active compounds and have the potential for being used in controlling and treating infections caused by some bacterial species that may be applied for prophylaxis and therapy, in both humans and animals.

REFERENCES

AHMED, A., ZAIN, U., ABJULUZIZ, M., RIUS, U., IUBUL, H. & MUHAMMAD, T. 2012. Evaluation of the chemical composition and element analysis of Urtica dioica. *African Journal of Pharmacy*, 6**,** 1555-1558. http://dx.doi.org/10.1046/j.1469-8137.1999.00428.x

CHEUNG, L., CHEUNG, P. C. & OOI, V. E. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, 81**,** 249-255. http://dx.doi.org/10.1016/S0308-8146(02)00419-3

DAR, S. A., GANAI, F. A., YOUSUF, A. R., BHAT, T. M. & BHAT, F. A. 2012. Bioactive potential of leaf extracts from Urtica dioica L. against fish and human pathogenic bacteria. *African Journal of Microbiology Research*, 6**,** 6893-6899.

ELZAAWELY, A. A., XUAN, T. D. & TAWATA, S. 2005. Antioxidant and antibacterial activities of Rumex japonicus HOUTT. Aerial parts. *Biological and Pharmaceutical Bulletin*, 28**,** 2225-2230. http://dx.doi.org/10.1248/bpb.28.2225

GHAIMA, K. K., HASHIM, N. M. & ALI, S. A. 2013. Antibacterial and antioxidant activities of ethyl acetate extract of nettle (Urtica dioica) and dandelion (Taraxacum officinale).

GULCIN, I., KUFREVIOGLU, O. I., OKTAY, M. & BUYUKOKUROGLU, M. E. 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). *J Ethnopharmacol*, 90**,** 205-15. http://dx.doi.org/10.1016/j.jep.2003.09.028

HADIZADEH, I., PEIVASTEGAN, B. & KOLAHI, M. 2009. Antifungal activity of nettle (Urtica dioica L.), colocynth (Citrullus colocynthis L. Schrad), oleander (Nerium oleander L.) and konar (Ziziphus spina-christi L.) extracts on plants pathogenic fungi. *Pak J Biol Sci*, 12**,** 58-63.

KONRAD, L., MULLER, H. H., LENZ, C., LAUBINGER, H., AUMULLER, G. & LICHIUS, J. J. 2000. Antiproliferative effect on human prostate cancer cells by a stinging nettle root (Urtica dioica) extract. *Planta Med*, 66**,** 44-7. http://dx.doi.org/10.1055/s-2000-11117

KRZESKI, T., KAZON, M., BORKOWSKI, A., WITESKA, A. & KUCZERA, J. 1993. Combined extracts of Urtica dioica and Pygeum africanum in the treatment of benign prostatic hyperplasia: double-blind comparison of two doses. *Clin Ther*, 15**,** 1011-20.

KUKRIĆ, Z. Z., TOPALIĆ-TRIVUNOVIĆ, L. N., KUKAVICA, B. M., MATOŠ, S. B., PAVIČIĆ, S. S., BOROJA, M. M. & SAVIĆ, A. V. 2012. Characterization of antioxidant and antimicrobial activities of nettle leaves (Urtica dioica L.). *Acta periodica technologica***,** 257-272. http://dx.doi.org/10.2298/APT1243257K

LALITHARANI, S., MOHAN, V. R. & REGINI, G. S. 2010. GC-MS analysis of ethanolic extract of Zanthoxylum rhetsa (roxb.) dc spines. *Journal of Herbal Medicine and Toxicology*, 4**,** 191-192.

LI, R. W., LEACH, D. N., MYERS, S. P., LEACH, G. J., LIN, G. D., BRUSHETT, D. J. & WATERMAN, P. G. 2004. Anti-inflammatory activity, cytotoxicity and active compounds of Tinospora smilacina Benth. *Phytotherapy Research*, 18**,** 78-83. http://dx.doi.org/10.1002/ptr.1373

LICHIUS, J. J. & MUTH, C. 1997. The Inhibition Effects of Urtica dioica Root Extracts on Experimentally Induced Prostatic Hyperplasia in the Mouse. *Planta Med*, 63**,** 307-310. http://dx.doi.org/10.1055/s-2006-957688

MARINO, M., BERSANI, C. & COMI, G. 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *International Journal of Food Microbiology*, 67**,** 187-195. http://dx.doi.org/10.1016/S0168-1605(01)00447-0

MODARRESI-CHAHARDEHI, A., IBRAHIM, D., FARIZA-SULAIMAN, S. & MOUSAVI, L. 2012. Screening antimicrobial activity of various extracts of Urtica dioica. *Rev Biol Trop*, 60**,** 1567-76.

MOTAMEDI, H., SAFARY, A., MALEKI, S. & SEYYEDNEJAD, S. 2009. Ziziphus spina-christiy a Native Plant from Khuzestan, Iran, as a Potential Source for Discovery New Antimicrobial Agents. *Asian Journal of Plant Sciences*, 8. http://dx.doi.org/10.3923/ajps.2009.187.190

NEVES, J. M., MATOS, C., MOUTINHO, C., QUEIROZ, G. & GOMES, L. R. 2009. Ethnopharmacological notes about ancient uses of medicinal plants in Tras-os-Montes (northern of Portugal). *J Ethnopharmacol*, 124**,** 270-83. http://dx.doi.org/10.1016/j.jep.2009.04.041

OBERTREIS, B., GILLER, K., TEUCHER, T., BEHNKE, B. & SCHMITZ, H. 1996. Anti-inflammatory effect of Urtica dioica folia extract in comparison to caffeic malic acid. *Arzneimittelforschung*, 46**,** 52-6.

OTLES, S. & YALCIN, B. 2012. Phenolic compounds analysis of root, stalk, and leaves of nettle. *The Scientific World Journal*, 2012.

ÖZKAN, A., YUMRUTAŞ, Ö., SAYGIDEĞER, S. D. & KULAK, M. 2011. Evaluation of Antioxidant Activities and Phenolic Contents of Some Edible and Medicinal Plants from Turkey's Flora. *Advances in Environmental Biology*, 5**,** 231-236.

PALIC, R., STOJANOVIC, G., ALAGIC, S., NIKOLIC, M. & LEPOJEVIC, Z. 2002. Chemical composition and antimicrobial activity of the essential oil and CO2 extracts of the oriental tobacco, Prilep. *Flavour and Fragrance Journal*, 17**,** 323-326. http://dx.doi.org/10.1002/ffj.1084

POURMORAD, F., HOSSEINIMEHR, S. & SHAHABIMAJD, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*, 5.

REX, J. H. 2008. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard*, Clinical and Laboratory Standards Institute.

RIEHEMANN, K., BEHNKE, B. & SCHULZE-OSTHOFF, K. 1999. Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-κB. *FEBS letters*, 442**,** 89-94. http://dx.doi.org/10.1016/S0014-5793(98)01622-6

ROY, S., RAO, K., BHUVANESWARI, C., GIRI, A. & MANGAMOORI, L. N. 2010. Phytochemical analysis of Andrographis paniculata extract and its antimicrobial activity. *World Journal of Microbiology and Biotechnology*, 26**,** 85-91. http://dx.doi.org/10.1007/s11274-009-0146-8

RUSSELL, A. D. 1991. Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. *Journal of Applied Bacteriology*, 71**,** 191-201. http://dx.doi.org/10.1111/j.1365-2672.1991.tb04447.x

SÁNCHEZ, D. O. S., NAJERA, G. L. A., RIVERA, I. L., RAMÍREZ, O. D., CISNEROS, M. G. V. & GARCÍA, V. M. N. 2009. Antimicrobial activity of medicinal plants from the Huautla Sierra Biosphere Reserve in Morelos (México). *Polibotánica*, 28**,** 213-225. http://dx.doi.org/10.1186/1746-4269-8-45

SEMWAL, D., RAWAT, U., BAMOLA, A. & SEMWAL, R. 2009. Antimicrobial activity of Phoebe lanceolata and Stephania glabra; preliminary screening studies. *Journal of Scientific Research*, 1**,** 662-666. http://dx.doi.org/10.3329/jsr.v1i3.2423

SHALE, T., STIRK, W. & VAN STADEN, J. 1999. Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. *Journal of Ethnopharmacology*, 67**,** 347-354. http://dx.doi.org/ 10.1016/S0378-8741(99)00035-5

SINGH, R., DAR, S. & SHARMA, P. 2012. Antibacterial Activity and Toxicological Evaluation of Semi Purified Hexane Extract of Urtica dioica Leaves. *Research Journal of Medicinal Plant*, 6. http://dx.doi.org/10.3923/rjmp.2012.123.135

SINGH, R., HUSSAIN, S., VERMA, R. & SHARMA, P. 2013. Anti-mycobacterial screening of five Indian medicinal plants and partial purification of active extracts of Cassia sophera and Urtica dioica. *Asian Pac J Trop Med*, 6**,** 366-71. http://dx.doi.org/10.1016/S1995-7645(13)60040-1

TAYLOR, R. S. L., EDEL, F., MANANDHAR, N. P. & TOWERS, G. H. N. 1996. Antimicrobial activities of southern Nepalese medicinal plants. *Journal of Ethnopharmacology*, 50**,** 97-102. http://dx.doi.org/10.1016/0378-8741(95)01335-0

TESTAI, L., CHERICONI, S., CALDERONE, V., NENCIONI, G., NIERI, P., MORELLI, I. & MARTINOTTI, E. 2002. Cardiovascular effects of Urtica dioica L. (Urticaceae) roots extracts: in vitro and in vivo pharmacological studies. *J Ethnopharmacol*, 81**,** 105-9. http://dx.doi.org/10.1016/S0378-8741(02)00055-7

WAGNER, H., WILLER, F. & KREHER, B. 1989. Biologically active compounds from the aqueous extract of Urtica dioica. *Planta medica*, 55**,** 452-454. http://dx.doi.org/10.1055/s-2006-962062

WANG, B.-G., ZHANG, W.-W., DUAN, X.-J. & LI, X.-M. 2009. *In vitro* antioxidative activities of extract and semi-purified fractions of the marine red alga, *Rhodomela confervoides* (Rhodomelaceae). *Food Chemistry*, 113**,** 1101-1105. http://dx.doi.org/10.1016/j.foodchem.2008.08.078