

Antimicrobial and antioxidant activities and chemical characterization of essential oils of *Thymus vulgaris*, *Rosmarinus officinalis*, and *Origanum majorana* from northeastern México

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Abstract: There have been no reports of antifungal activity and composition of extracts from *Thymus vulgaris*, *Rosmarinus officinalis* or *Origanum majorana* from northeastern México. Antifungal activity of these oils against *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, *Microsporium gypseum*, *Microsporium canis* and *Epidermophyton floccosum* was measured by diffusion assay. Additionally, antibacterial and antioxidant activities were evaluated. Antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* was examined by microdilution. Antioxidant activity was assessed by 2,2-difenil-1-picrilhidracil reduction test. The plant oils were characterized by both GC/MS and GC/FID. Oils of *T. vulgaris* and *O. majorana* showed growth inhibition activity against dermatophytes, especially *T. vulgaris* oil, which completely inhibited growth of all tested dermatophytes. The oils also showed bioactivity against bacteria, with minimum inhibitory concentration (MIC) values between 62.5 and 500 µg/mL. The antioxidant activity of the oils was low, with effective concentration (EC₅₀) values >250µg/mL. The major components in the oils were as follows: *T. vulgaris*, *o*-cymene, γ -terpinene, thymol and carvacrol; *R. officinalis*, terpinen-4-ol and 1,8-cineole; *O. majorana*, terpinen-4-ol and thymol.

Keywords: Dermatophytes, antibacterial activity, *Thymus vulgaris*, *Rosmarinus officinalis*, *Origanum majorana*, essential oils, antioxidant activity.

INTRODUCTION

Skin infections are often caused by fungi and bacteria. Specifically, dermatophyte fungi, which primarily belong to the genera *Microsporium*, *Trichophyton* and *Epidermophyton*, cause the most common infections in skin. The infections caused by these fungi, also called ringworm, mainly affect the skin, nails and hair, but they are able to penetrate and proliferate in other tissues, causing chronic infections in deeper tissues that become difficult to eradicate. With respect to bacteria, *Staphylococcus* spp and *Streptococcus* spp., particularly *Streptococcus pyogenes*, cause impetigo and cellulitis. Other *Staphylococcus* species cause skin conditions such as erysipelas, boils, carbuncles, postoperative infections and acute inflammation. *Staphylococcus aureus* is the most common pathogen of skin infections and is resistant to many antibacterial therapies (Bensouilah and Buck, 2006).

Since ancient times, essential oils have been recognized for their therapeutic properties (Edris, 2007). Scientific research over the past two decades has demonstrated various biological activities of essential oils including

anticancer, antiviral, antioxidant, antibacterial and antifungal activities, as well as their ability to facilitate the passage of active molecules through the skin (Bakkali *et al.*, 2008).

Thymus vulgaris L. (Labiatae) carries antiseptic, anti-tussive, expectorant and deodorant properties, besides being used in aromatherapy and as a flavoring agent in foods. *Rosmarinus officinalis* subsp. *officinalis* L. (Labiatae) is used to treat lung infections, toothache and rheumatism and as a carminative, and it is widely used in perfumery and aromatherapy. *Origanum majorana* L. (Labiatae) is used as an antispasmodic, as sedative and used against digestive disorders, as well as an antidote against snake venom (Chizzola *et al.*, 2008; Dewic, 2002; Evans, 2002).

The present study is based on evaluating the antidermatophytic activity of essential oils from leaves of *T. vulgaris*, *R. officinalis* and *O. majorana* grown in the northeastern region of Mexico against *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, *Microsporium gypseum*, *Microsporium canis* and *Epidermophyton floccosum*. In addition to the antioxidant activity assessment the antibacterial activity against *Streptococcus pyogenes* and two strains of

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Staphylococcus aureus was also studied. Plus, the oils were subjected to chemical analysis.

MATERIALS AND METHODS

Microorganisms

T. tonsurans, *T. mentagrophytes*, *T. rubrum*, *E. floccosum*, *M. canis* and *M. gypseum*, *S. aureus* ATCC 12598, *S. aureus* IMSS/HE 25: 01 and *S. pyogenes*. Fungal and bacterial samples included the clinical isolates from patients. All microorganisms were maintained in the laboratory.

Plant material

The fresh stems of the *T. vulgaris*, *R. officinalis* and *O. majorana* plants were purchasing from the local market in Monterrey city, Mexico in November 2010. The plants were originally collected in Matehuala, San Luis Potosí, Mexico. The specimens were deposited at the herbarium of the Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León.

Essential oil extraction

Essential oils were obtained according to method of Hussain *et al.* (2010). Ground fresh plant material was individually hydro-distilled in a Clevenger-type apparatus (100 g/L water) for 4 h. The oil obtained from *T. vulgaris* was collected as one fraction. The oils obtained from *R. officinalis* and *O. majorana* were collected as two fractions (according to their distillation order): the first fraction from each source was colorless and the second was yellow. The oils were dried with anhydrous Na₂SO₄ (Sigma-Aldrich), weighed, and kept at 4°C until use.

Antidermatophytic activities

Antidermatophytic activities were assessed by the paper disk diffusion assay according to the Clinical and Laboratory Standards Institute method (CLSI document M-44-A2, 2009). Briefly, filter paper discs of 6 mm discs containing 5 µL of oil were placed onto each of agar plates inoculated with each of the fungal strains, and the plates were incubated at 30°C for 48h. Terbinafine (Sigma-Aldrich) served as control. The growth inhibition zones were measured in mm.

Antibacterial activities

Antibacterial activities were assessed using microdilution assays according to the National Committee for Clinical Laboratory Standards protocol (NCCLS document M-100-S-12, 2002). The oil concentrations varied between 15.7 and 500 µg/mL. Oxacillin and vancomycin (Sigma-Aldrich) served as controls (4-64 µg/mL). The final concentration of each microorganism was 1 × 10⁴ colony forming units. The minimum inhibitory concentration (MIC) for each oil was defined as the minimum concentration that stopped growth.

Antioxidant activities

The percent of chemical reduction of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich) was monitored by spectroscopy at 517nm (Salazar-Aranda *et al.*, 2011). The final concentrations of the oils ranged from 0.24 to 250 µg/mL. Solutions of quercetin, rutin and pyrocatechol (Sigma-Aldrich) (0.01-250 µg/mL) served as positive controls.

Gas chromatography with a flame ionization detector (GC/FID)

For analysis of the essential oils, the method used by Hussain *et al.* (2010) was adapted with modifications. Oil samples were subjected to GC using an Autosystem XL gas chromatograph equipped with a flame ionization detector (Perkin Elmer) and an HP-5MS column (30m × 0.25mm i.d. 0.25 µm film thickness; Agilent Technologies). For GC of the *T. vulgaris* oil, the oven temperature was held at 60°C for 1min, increased by 6°C/min to 120°C and held for 1min and then increased by 25°C/min to 280°C and held for 1 min. For GC of the two oil samples from *R. officinalis*, the oven temperature was set at 60°C for 2min, increased by 11°C/min up to 80°C (and held) for 1min. For GC of the two oil samples from *O. majorana*, the oven temperature was held at 60°C for 1 min, increased by 8°C/min to 140°C and held for 1 min and then increased by 11°C/min to 250°C and held for 1min. The injector and detector temperatures were 250 and 280°C, respectively. The flow rate of the carrier gas (helium 99.999% purity, Praxair) was 2.0mL/min and the split ratio was 1: 20. The injection volume for each sample was 2 µL of an oil solution (2mg/mL in methylene chloride, Fermont). The percentage of each compound in the samples was calculated by dividing its peak area by the total peak area of the sample and multiplying by 100. It was assumed that the detector response was the same for all compounds.

GC with mass spectrometry (GC/MS)

GC/MS was performed using an Agilent Technologies 6890N gas chromatograph (column dimensions, 30m ×, 0.25 mm i.d., 0.25-µm film thickness) connected to a 5973 INERT selective mass spectrometer HP5MS. The ionization-source temperature was 230°C, the quadrupole temperature was 150°C and the injector temperature was 220°C. For each run, the GC oven temperature was held at 35°C for 9 min, increased by 3°C/min to 150°C and held for 10 min, increased by 10°C/min to 250°C and increased by 3°C/min to 270°C and held for 10 min. The flow rate of the helium carrier gas (99.999% purity) was 0.5mL/min, and the ionization energy was 70 eV. The samples were injected in the splitless mode. The injection volume for each sample was 2 µL of an oil solution (2 mg/mL in methylene chloride). MS spectra were acquired in the scan mode. The oil components were identified by comparison of their retention indices to C8-C20 *n*-alkanes (Sigma-Aldrich) and by comparison of their MS spectra

Table 1: Antimicrobial activity of the essential oils from *Thymus vulgaris*, *Rosmarinus officinalis* and *Origanum majorana*

Microorganism	<i>Thymus vulgaris</i>	<i>Rosmarinus officinalis</i>		<i>Origanum majorana</i>	
	Leaves	Leaves		Leaves	
		Colorless	Yellow	Colorless	Yellow
<i>Trichophyton rubrum</i>	FI	NI	NI	FI	36 ^a
<i>Trichophyton tonsurans</i>	FI	NI	NI	40 ^a	FI
<i>Trichophyton. mentagrophyte</i>	FI	NI	NI	FI	FI
<i>Microscopurum gypseum</i>	FI	NI	NI	27 ^a	42 ^a
<i>Microsporium canis</i>	FI	NI	NI	NI	47 ^a
<i>Epidermophyton floccosum</i>	FI	NI	NI	FI	FI
<i>Staphylococcus. aureus</i> ^b	250	NA	NA	250	500
<i>Staphylococcus. aureus</i> ^c	NA	NA	NA	NA	NA
<i>Streptococcus pyogenes</i>	62.5	NA	NA	125	125

^amm of growth inhibition; ^bATCC 12598; ^cIMSS/HE 25:01; NA, Not active at 500mg/ml; NI, No inhibition, FI, Full inhibition. Oxacillin, antibacterial control; Terbinafina, antifungal control

with those of the US National Institute of Standards and Technology (NIST) library and reference data (Adams, 2012).

RESULTS

Table 1 shows the results of the antifungal activity assays. Oil from *T. vulgaris* completely inhibited the growth of all six fungi. The colorless oil of *O. majorana* completely inhibited the growth of three of the fungi tested, i.e. *T. rubrum*, *T. mentagrophytes* and *E. floccosum* and partially inhibited *T. tonsurans* and *M. gypseum* (inhibition zones of 40 and 27 mm, respectively). This oil did not inhibit the growth of *M. canis*. The yellow oil of *O. majorana* also showed activity against dermatophytes, completely inhibiting the growth of *T. tonsurans*, *T. mentagrophytes* and *E. floccosum* and partially inhibiting the dermatophytes *T. rubrum*, *M. gypseum* and *M. canis* (inhibition zones of 36, 42 and 47mm, respectively). Oils of *R. officinalis* did not inhibit any of the tested fungi.

Table 1 also shows the results of the antibacterial activity assays. The oil of *T. vulgaris* had an MIC of 250µg/mL against *S. aureus* (ATCC 12598) and 62.5µg/mL against *S. pyogenes*. Both oils of *O. majorana* showed activity against *S. aureus* (ATCC 12598) and *S. pyogenes*, with the colorless oil having MICs of 125 and 250µg/mL, respectively, and the yellow oil having MICs of 125 and 500 µg/mL. The two oils from *R. officinalis* did not show antibacterial activity even at 500µg/mL.

In the test for antioxidant activity, the essential oil of *T. vulgaris*, colorless and yellow oils of *R. officinalis*, and colorless and yellow oils of *O. majorana* reduced the free radical 1,1-diphenyl-2-picrylhydrazyl by 20, 0, 6, 12 and 10%, respectively, which indicates low activity, with EC₅₀ values >250µg/mL.

Table 2 shows the chemical composition of each of the essential oils. For *T. vulgaris* oil, 17 GC signals were

observed and identified, the major components being *o*-cymene (37.1%), γ -terpinene (20.0%), thymol (17.0%) and carvacrol (4.9%). For the colorless *R. officinalis* oil, 15 GC signals were observed, of which 14 were identified, with the major components being terpinen-4-ol (45.9%) and 1,8-cineole (23.8%). For the yellow *R. officinalis* oil, 16 GC signals were detected, of which only 13 were identified, with terpinen -4-ol being the main compound (87.6%). For *O. majorana*, terpinen-4-ol and thymol were the major components in both oils, making up 23.1 and 16.3% of the colorless fraction and 27.7 and 24.6% of the yellow fraction, respectively.

DISCUSSION

The antimicrobial activity of several plant oils was tested against six fungal and two bacterial strains that cause infections of the skin (table 1). Was found that the dermatophytic fungi were more sensitive to the oils than the bacteria examined.

Has been described that the activity against the dermatophytes is related to the damage of the thymol and carvacrol on the membrane of these fungi; Pinto *et al.* (2006) have suggested that this effect occurs through the inhibition of ergosterol synthesis, this could explain why fungi are more sensitive than bacteria. Although the antibacterial activity is associated with the presence of thymol and carvacrol, such activity is not related to its concentration, but is suggested its interaction with other compounds present in the oils (Rota *et al.*, 2008).

Regarding the activity against dermatophytes, oil from *T. vulgaris* was the most effective, as it completely inhibited the growth of the six fungi tested. This result is similar to those reported by Sajjad *et al.* (2011) and Shin and Lim, (2004), who found that essential oil from *T. vulgaris* has strong antidermatophytic activity against several species of *Trichophyton* in a disc diffusion assay.

Table 2: Chemical composition (relative% of peak area) of essential oils from *Thymus vulgaris*, *Rosmarinus officinalis* and *Origanum majorana*.

Constituents	RI ^a	RI ^b	<i>Thymus vulgaris</i>	<i>Rosmarinus officinalis</i>		<i>Origanum majorana</i>	
				Colorless	Yellow	Colorless	Yellow
Monoterpene hydrocarbons							
α -Thujene	923	924	0.7	--	--	--	--
α -Pinene	929	932	0.9	3.8	1.5	--	--
Camphene	943	946	0.7	2.2	0.9	--	--
Sabinene	971	969	--	--	--	1.0	--
β -Pinene	972	974	--	--	0.8	--	--
Myrcene	989	988	2.1	1.8	0.9	0.9	--
α -Phellandrene	1000	1002	--	2.8	1.2	--	--
d-Carene	1011	1008	--	--	--	--	0.7
α -Terpinene	1012	1014	1.1	--	--	3.4	--
r-Cymene	1020	1020	--	2.4	0.3	5.5	--
o-Cymene	1021	1022	37.1	--	--	--	0.8
b-Phellandrene	1025	1025	--	8.4	1.3	--	--
E- β -Ocimene	1047	1044	--	--	--	2.1	1.5
γ -Terpinene	1056	1054	20.0	1.1	0.5	11.5	4.7
Terpinolene	1085	1086	--	--	--	2.2	2.1
allo-Ocimene	1127	1128	--	--	--	1.9	--
Total			62.6	22.5	7.4	28.5	9.8
Oxygenated monoterpenes							
1,8-Cineole	1027	1026	--	23.8	2.3	--	--
cis-hydrate Sabinene	1064	1065	--	--	--	3.0	--
Linalool	1098	1095	6.2	0.4	--	--	--
trans-hydrate Sabineno	1095	1098	--	--	--	15.3	10.5
cis-r-Menth-2-en-1-ol	1118	1118	--	--	--	0.5	1.6
β -trans-dihydro Terpeneol	1137	1138	--	--	--	--	3.6
Camphor	1141	1141	0.2	0.9	0.3	--	--
Borneol	1163	1165	1.9	--	0.2	0.7	--
Terpinen-4-ol	1174	1174	4.1	45.9	87.6	23.1	27.7
α -Terpineol	1188	1186	--	--	1.2	4.5	1.4
Verbenene	1206	1204	--	1.4	--	--	--
Thymol methyl ether	1232	1232	--	--	--	3.4	3.9
Carvacrol methyl ether	1242	1241	1.3	--	--	2.6	4.6
Bornyl acetate	1287	1284	--	0.6	--	--	--
Thymol	1299	1289	17.0	--	--	16.3	24.6
Carvacrol	1299	1298	4.9	--	--	--	--
Total			35.6	73.0	91.4	69.4	77.9
Sesquiterpene hydrocarbons							
E-Cariophyllene	1418	1417	2.0	--	--	--	--
4,8 β - epoxy-Cariophyllane	1419	1423	--	--	--	--	3.5
Germacrene D	1480	1480	--	--	--	--	1.0
Bicyclgermacrene	1496	1500	--	--	--	--	1.9
β - Bisabolene	1505	1505	--	--	--	--	1.3
Total			2.0	0	0	0	7.7
Oxygenated sesquiterpenes							

Continued...

Constituents	RI ^a	RI ^b	<i>Thymus vulgaris</i>		<i>Rosmarinus officinalis</i>		<i>Origanum majorana</i>	
					Colorless	Yellow	Colorless	Yellow
Globulol	1573	1590	--	--	--	--	--	1.7
Total			0	0	0	0	0	1.7
Otros								
Octen-3-ol	ND	974	--	1.3	--	--	--	--

a IR= Retention indices relative to *n*-alkanes on HP-5MS column. b IR= Retention indices from literature.

The oils of *O. majorana* also showed good antifungal activity, with the colorless fraction completely inhibiting *T. rubrum*, *T. mentagrophytes* and *E. floccosum* and partially inhibiting *T. tonsurans* and *M. gypseum*, and the yellow oil showing a high degree of bioactivity against *T. tonsurans*, *T. mentagrophytes* and *E. floccosum* and less activity against *T. rubrum*, *M. gypseum* and *M. canis*.

Oils of *R. officinalis* did not inhibit any of the tested fungi. However, alcoholic extracts of *R. officinalis* have been reported to have activity against other fungi such as the filamentous fungi *Aspergillus ochraceus* and *Aspergillus flavus* (Centeno et al., 2010). It may be that the alcoholic extracts contain compounds that are not in the essential oil that confer antifungal activity.

The antibacterial activity was tested at oil concentrations between 15.7 and 500 µg/mL. This concentration range was chosen based on a review of antimicrobial activity studies by Salazar et al. (2013), which indicated that the maximum concentration that should be used for testing antimicrobial activity is 500 µg/mL.

In this study, the oil of *T. vulgaris* was active against *S. aureus* and *S. pyogenes*, which is consistent with previous reports of antibacterial activity of oils and extracts of this plant from Columbia and Serbia against Gram-positive and Gram-negative bacteria (Roldán et al., 2010; Soković et al., 2007).

The oils of *O. majorana* were also active against *S. aureus* and *S. pyogenes*, which is consistent with previous reports that some Gram-positive and Gram-negative bacteria are susceptible to the essential oil of *O. majorana* (from Egypt), especially against *Bacillus subtilis* (Busatta et al., 2008). As in the present study, terpinen-4-ol was identified as a major component of the essential oil in that study. Plants of several other species of the genus *Origanum* show broad-spectrum antibacterial activity. Oil from *Origanum vulgare*, for example, shows activity against *Listeria monocytogenes*, *S. aureus*, *Enterococcus faecium*, *Salmonella choleraesuis*, *B. subtilis*, *Rhodococcus equi* and *Micrococcus luteus* (Faleiro et al., 2005; Sartoratto et al., 2004).

The oil extracts from *R. officinalis* showed no antibacterial activity, even at the highest concentration

tested. These results are contrary to a previous report that the essential oil of *R. officinalis* from Pakistan is active against Gram-positive and Gram-negative strains (Hussain et al., 2010).

None of the essential oils tested showed appreciable antioxidant activity, which was determined by reduction of the free radical 1,1-diphenyl-2-picrylhydrazyl assay. However, varying degrees of antioxidant activity have been reported for essential oils from these plants (Faleiro et al., 2005; Genena et al., 2008; Grigore et al., 2010; Woo et al., 2001).

Chemical composition analysis indicated that the main components of *T. vulgaris* oil were monoterpene hydrocarbons (62.6%), primarily *o*-cymene (37.1%), γ -terpinene (20.0%), thymol (17.0%) and carvacrol (4.9%). These results appear to be, partially consistent with those of Rota et al. (2008), who found that thymol (57%), carvacrol (2.8%) and γ -terpinene (1.9%) are the major components of the essential oil from *T. vulgaris* from Spain. However, the results reported in the present study differ from those reported by Sartoratto et al. (2004), who found geranial to be the major component (21.8%) in essential oil from *T. vulgaris* from Brazil, although they also found γ -terpinene and thymol (2.6 and 17.5%, respectively) to be present. It may be that the geographic location and/or environment in which *T. vulgaris* grows may affect the proportions of compounds in the plant.

The major components of the *R. officinalis* colorless oil, were terpinen-4-ol (45.9%) and 1,8-cineole (23.8%) and for the *R. officinalis* yellow oil, terpinen-4-ol was the main (87.6%) compound. For both *R. officinalis* oils, majority of the compounds were oxygenated monoterpenes (73% and 91.4%, respectively). The colorless oil also contained a substantial amount of monoterpene hydrocarbons (22.5%; table 2). The two oils had very similar composition, varying only in the proportion of each compound. One of the major differences was the low proportion of 1-8-cineole in yellow oil (2.3%) in comparison to the colorless oil (23.8%). Genena et al. (2008) reported isocarnosol as a major component of essential oil of *R. officinalis* from Brazil, followed by borneol and camphor; whereas Hussain et al. (2010) reported 1,8-cineol as the major component followed (in proportion) by limonene,

camphene, linalool and borneol. Thus, some of the major components of *R. officinalis* identified in the present analysis of the essential oil, namely borneol and 1,8-cineol, overlap with these (other) studies.

Oils of *O. majorana* had relatively high proportions of oxygenated monoterpenes, 69.4% for the colorless oil and 77.9% for the yellow oil (table 1). The colorless oil also had a high percentage of hydrocarbon monoterpenes (28.5%). Terpinen-4-ol and thymol were the major components in both oils and these are consistent with the findings of Bussata *et al.* (2008) that terpinen-4-ol is the major component and of Sellami *et al.* (2009) that terpinen-4-ol is the main compound, followed by *cis*-hydrate sabinene, *trans*-hydrate sabinene, γ -terpinene, bornyl acetate and linalool, most of which are oxygenated monoterpenes. Consequently, present findings for both *O. marjoram* oil fractions from plants cultivated in northeastern Mexico are appreciably similar to the previous reports, except for the detection of sesquiterpene hydrocarbons in the yellow *O. marjoram* fraction (as per the present findings).

T. vulgaris and *O. majorana* oils showed antidermatophytic activity and both contained thymol, whereas oils of *R. officinalis* showed no antifungal activity and did not contain this compound (thymol). These data are in agreement with those of Pinto *et al.* (2006), who found that thymol was the major constituent in the essential oil of *Thymus pulegioides* and those of López *et al.* (2007), who found that vapor-phase thymol from *T. vulgaris* completely inhibits the growth of filamentous fungi i.e. *Aspergillus flavus* and *Penicillium islandicus*. In contrast, the high proportion of γ -terpinene in the active oils suggests that this compound may also be active against the dermatophytes; However, Pinto *et al.* (2006) reported low activity of this compound against some dermatophytes.

CONCLUSION

Taken together, the chemical characterization and antimicrobial activity assays revealed that oils with a higher percentage of aliphatic and oxygenated monoterpenes, mainly phenolic monoterpenes such as thymol, showed antimicrobial activity. It's concludes that oils from *T. vulgaris* and (to a lesser degree) *O. marjoram*, grown in northeastern Mexico are highly bioactive against the dermatophytes tested in this study and slightly active against *S. aureus* (ATCC 12598) and *S. pyogenes*. However, none of the plant oils (studied) displayed antioxidant activity.

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REFERENCES

- Adams RP (2012). Identification of essential oil components by gas Chromatography/Mass Spectrometry, 4th ed., Allured Business Media. Illinois, pp.100-503.
- Bakkali F, Averbeck S, Averbeck D and Idaomar M (2008). Biological effects of essential oils A review. *Food Chem. Toxicol.*, **46**: 446-475.
- Bensouilah J and Buck P (2006). Aromadermatology: Aromatherapy in the treatment and care of common skin conditions, 1st ed., Radcliffe Publishing Ltd., Abidong, pp.116-145.
- Busatta C, Vidal RS, Popiolski AS, Mossi AJ, Dariva C, Rodrigues MR, Corazza FC, Oliveira V and Cansian RL (2008). Application of *Origanum majorana* L. essential oil as an antimicrobial agent in sausage. *Food Microbiol.*, **25**: 207-211.
- Centeno S, Calvo MA, Adelantado C and Figueroa S (2010). Antifungal activity of extracts of *Rosmarinus officinalis* and *Thymus vulgaris* against *Aspergillus flavus* and *Aspergillus ochraceus*. *Pak. J. Biol. Sci.*, **13**: 425-455.
- Chizzola R, Michitsch H and Franz C (2008). Antioxidative properties of *Thymus vulgaris* leaves: Comparison of different extracts and essential oil chemotypes. *J. Agric. Food Chem.*, **56**: 6897-6904.
- CLSI (2009). Method for antifungal disk diffusion susceptibility testing of yeast: Approved guideline-Second edition. CLSI document M-44-A2. Wayne, PA: Clinical and Laboratory Standards Institute, pp. 3-6.
- Dewic PM (2002). Medicinal Natural Products a Biosynthetic Approach, 2nd ed., John Wiley & Sons Ltd., Chinchester, pp.184-1855.
- Edris AE (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. *Phytother. Res.*, **21**: 308-323.
- Evans WC (2002). Treasse and evans Pharmacognosy, 15th ed., W. B. Saunders. London, pp.456-474.
- Faleiro L, Miguel G, Gómes S, Costa L, Venâncio F, Figueiredo C, Barroso JG and Pedro LG (2005). Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L and *Origanum vulgare* L. *J. Agric. Food Chem.*, **53**: 8162-8168.
- Genena A, Hense H, Smania A and Machado S (2008). Rosemary (*Rosmarinus officinalis*) a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. *Ciênc. Tecnol. Aliment.*, **28**: 463-469.
- Grigore A, Paraschiv I, Colceru-Mihul S, Bubueanu C, Draghici E and Ichim M (2010). Chemical composition and antioxidant activity of *Thymus vulgaris* volatile oil obtained by two different methods. *Rom. Biotechnol. Lett.*, **15**: 5436-5443.
- Hussain AI, Anwar F, Chatha SAS, Jabbar A, Mahboob S and Nigam OS (2010). *Rosmarinus Officinalis* Essential Oil: Antiproliferative, Antioxidant and

- Antibacterial activities. *Braz. J. Microbiol.*, **41**: 1070-1078.
- López P, Sánchez C, Batlle R and Nerin C (2007). Vapor-phase activities of cinnamon, thyme and oregano essential oils and key constituents against foodborne microorganisms. *J. Agric. Food Chem.*, **55**: 4348-4356.
- National Committee for Clinical Laboratory Standards (2002). Performance standards for antimicrobial susceptibility testing. In: 12th Informational Supplement. NCCLS document M-100-S-12, pp.96 and 110.
- Pinto E, Pina-Vaz C, Salgueiro L, Goncalves MJ, Costa-de-Oliveira S and Cavaleiro C *et al.*, (2006). Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. *J. Med. Microbiol.*, **55**: 1367-1373.
- Roldán LP, Díaz GJ and Durringer JM (2010). Composition and antibacterial activity of essential oils obtained from plants of the *Lamiaceae* family against pathogenic and beneficial bacteria. *Rev. Colomb. Cienc. Pecu.*, **23**: 451-461.
- Rota MC, Herrera A, Martínez RM, Sotomayor JA and Jordán MJ (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control*, **19**: 681-687.
- Sajjad M, Khan A and Ahmad I (2011). Antifungal activity of essential oils and their synergy with fluconazole against drug-resistant strains of *Aspergillus fumigatus* and *Trichophyton rubrum*. *Appl. Microbiol. Biotechnol.*, **90**: 1083-1094.
- Salazar-Aranda R, Pérez-López L, Rivas-Galindo V and Waksman N (2013). Antimicrobial activity of Plants Used in México for Gastrointestinal and Respiratory Disorders. In: Shahid M, Shahzad A, Malik A, Sahai A editors. *Recent Trends in Biotechnology and Therapeutic Applications of Medicinal Plants*, 1st ed., Springer. Dordrecht, pp.131-188.
- Salazar-Aranda R, Pérez-López LA, López-Arroyo J, Alanís-Garza B and Waksman N (2011). Antimicrobial and antioxidant activities of plants from Northeast Mexico. *Evid. Based Complement. Alternat. Med.*, 536139.
- Sartoratto A, Machado AL, Delarmelina C, Figueira GM, Duarte MC and Rehder VL (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz. J. Microbiol.*, **35**: 275-280.
- Sellami H, Maamouri E, Chahed T, Wannas WA, Kchouk ME and Marzouk B (2009). Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (*Origanum majorana* L.). *Ind. Crop. Prod.*, **30**: 395-402.
- Shin S. and Lim S (2004). Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. *J. Appl. Microbiol.*, **97**: 1289-1296.
- Soković M, Marin P, Brkic D and van Griensven L (2007). Chemical composition and antimicrobial activity of essential oil of ten aromatic plants against human pathogenic bacteria. *Food*, **1**: 1-7.
- Woo JJ, Bok KH, Kwang WY, Moo SK, Ih SC and Hee YK *et al.* (2001). Antioxidant effects of *Origanum majorana* L. on superoxide anion radicals. *Food Chem.*, **7**: 439-444.