Middle-East Journal of Scientific Research 9 (4): 467-471, 2011
ISSN 1990-9233

Comparative Study of the Antimicrobial Activity of *Rosmarinus officinalis* L. Essential Oil and Methanolic Extract

S. Tavassoli, S.M. Mousavi, Z. Emam-Djomeh and S.H. Razavi

Department of Food Science, Technology and Engineering,
Faculty of biosystems engineering Agricultural,
Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Abstract: The goal of this work was to test the antimicrobial activity of the essential oil and methanolic extract of *R. officinalis* against *Leuconostoc mesenteroides*, *Lactobacillus delbruekii*, *Saccharomyces cerevisia and Issatchenikia orientalis* (*Candida krusei*). Extract of rosemary leaves was prepared with microwave assisted extraction at concentration of methanol 60%. Extraction Yield was 21.5% and the total phenolics were found to be 5.138±0.072 gm as gallic acid/100gm dry leaves. The antimicrobial activity of the essential oil and methanolic extract were determined by the minimum inhibitory concentration (MIC). The results indicated that the tested microbes were highly sensitive to the essential oil and moderately sensitive to the methanolic extract. Minimum inhibitory concentration values of essential oil for both bacteria *Leuconostoc mesenteroides* and *Lactobacillus delbruekii* ranged between 0. 5 and 1.0 mg/ml. The oil was analyzed by GC and GC/MS. The major components of *R. officinalis* oil were 1,8-Cineole (23.14%), camphor (12.35%), α-pinene (9.87%), β-pinene(6.10%), borneol (5.61%), camphene (5.58%) and α-terpineol (4.30%), respectively. These results indicate the latent potency of essential oil of *R. officinalis* as a natural preservative in food products against *L. mesenteroides*, *L. delbruekii*, *S. cerevisia* and *C.krusei*.

Key words: *Rosmarinus Officinalis* L. · Essential Oil · Extract · Chemical Composition · Antimicrobial Activity

INTRODUCTION

Medicinal plants have been used by human and their components are widely used in since ages in traditional medicine due to their constituents of different medical products, in the food therapeutic potential [1]. Despite synthetic additives have been widely used in processed food products to extend their shelf lives, the leaning is to diminish their use because of the growing apprehension among consumers about this type of products [2]. Currently, there is a growing interest to use natural additives such as herbs and spices extracts for the preservation of foods due their special flavor and eventual antioxidant as well as antimicrobial activities [3].

The antimicrobial activities of essential oils have been known since the dawn of early medicinal practices to the present time of investigation. [4] Several researches have established the antimicrobial activity of essential oils (EOs) in both model and real food systems [2, 5]. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food [6]. Since EOs are generally regarded as safe (GRAS), the possibility of strengthening their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be a way to achieve an equilibrium between their sensorial attributes and antimicrobial efficiency [7].

Rosemary (*Rosmarinus officinalis* L.), belonging to the Lamiaceae family, is a pleasant-smelling perennial shrub growing in several regions all over the world [8]. It is a well-known valuable medicinal herb that is widely used in pharmaceutical products and traditional medicine as a digestive, tonic, astringent, diuretic, diaphoretic and is useful for urinary ailments [9]. The wild area in which *R. officinalis* grows is mainly in the areas around the Mediterranean Sea and in many islands, particularly Sicily, Sardinia, Corsica, Baleari and Elba [10]. It is used either in form of dried herb or oil as spicy and flavoring agents in several food formula for its desirable flavor, high antioxidant activity and recently as antimicrobial agent

Corresponding Author: Sedigheh tavasoli, Department of Food Science, Technology & Engineering, Faculty of biosystems engineering Agricultural, Campus of Agriculture & Natural Resources, University of Tehran, Karaj, Iran.

[11]. *R. officinalis* contains a great amount of essential oil (up to 1%). Moreno *et al.* [12] reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria [12]. Its strong antimicrobial activity may be attributed to the presence of carnosic acid and carnosol. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply described [12].

The aim of our work was to investigate antimicrobial activity of essential oils and methanolic extracts of *R. officinalis* in concentrations that can be used as natural additives in foods.

MATERIALS AND METHODS

Materials: Methanol used in the experiments was HPLC grade and purchased from Merck. The oil of rosemary was prepared by Zardband Company of Iran. The oils were stored in dark glass bottles in the refrigerator at 4°C until they were used.

Rosemary leaves were collected from Tehran University campus area and dried at 25-30°C for 4 days without applying any heat treatment to reduce the loss of active components. Dried leaves were separated from the branches, then blended in a blender and kept in refrigerator at 4°C until use.

Preparation of Rosemary Extract: Dry rosemary leaves were soaked in 60% methanol for 90 min. as recommended by Pan *et al.* [13] in ratio of liquid to solid 20:1. The suspensions were heated with microwaves (1000 W, Butan, Iran) as the following conditions: 2 min. 10% full power on (heating to the desired temperature, lower than 40°C) and 3 min. power off and then 1 min. 10% full power on (for heating) and 3 min. power off (for cooling) and so on to the pre-set extraction time (4 min.). Super boiling of the solution did not occur. The extract was then filtered through SandS No. 604 paper, evaporated to dryness under reduced pressure (40°C). The remaining extract was finally dried in a vacuum oven at 30°C for two hours to ensure the removal of any residual solvent. The extract was weighted to calculate the extraction yield (%). Final extract kept in dark bottle in the refrigerator at 4°C until use.

Determination of Total Phenols Contents: Total phenols contents in the obtained extracts were estimated by a colorimetric analysis based on procedures described by Singleton and Rossi [14] with some modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (CEILE CE 2502, 2000 spectrophotometer, England). Gallic acid was used for constructing the standard curve (0.01-0.4 mM). The results were uttered as gm of gallic acid equivalents/100 gm of extract.

Oil Analysis: GC analyses were performed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector. The analysis was carried out using a HP-5MS column (30 m×0.25 mm, film thickness 0.25 ìm). The operating conditions were as follows: injector and detector temperature, 290°C and 220°C, respectively; and carrier gas, Helium. Oven temperature program was 50°C at the rate of 3°C/min, held for 5 min, then increased to 240°C at 15°C/min, increased to 300 °C, held for 3 min in this temperature. Mass spectrometer conditions were: ionization potential 70 eV; and electron multiplier energy 2000 V. The identities of the oil components were established from their GC retention indices, relative to C7-C88 n-alkanes, by comparison of their MS spectra with those reported in the literature and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Antimicrobial Activity

Microorganisms and Culture Conditions: The organisms used were *Saccharomyces cerevisia* PTCC 5269, *Candida krusei (I. orientalis*) PTCC 5295, *Leuconostoc mesenteroides* PTCC 1591, *Lactobacillus delbruekii* PTCC 1333.Yeasts were maintained on Sabouraud Dextrose Agar (Merck, Germany) at 4°C and bacteria were maintained on MRS (de Man, Rogosa and Sharpe) Agar (Merck, Germany) at 4°C.

Antibacterial Activity of* Essential Oil and Extract *of Rosemary: The concentrations of essential oil and extract were estimated using the broth dilution susceptibility test [14]. The purpose of this method is testing decreeing accentuations of the antimicrobial agent(s), which usually are prepared in serial tow fold dilutions and placed in tubes of a broth medium that will support the growth of the test microorganism. MRS agar and MRS broth obtained from MST- England were used in this method. To perform the broth dilution susceptibility test, a standard inoculums of the microorganism (organisms 1×10^6 ml, a 1:500 dilution of a suspension of turbidity

equal to a McFarland standard 1), was added to an equal volume of each concentration of antimicrobial agent and to a tube of the growth medium without antimicrobial agent, which has been served as a growth control adding a bacterial suspension dilutes both the suspension and the concentration of antimicrobial agent in the tube. An un-inoculated tube of medium has been incubated to serve as a negative growth control. After overnight incubation, the tubes were cultured on the plats containing MRS agar and were incubated at 37°C (*Lactobacillus delbruekii*) and 26°C (*Leuconostoc mesenteroides*) for overnight [15].

Anti Yeast Activity **Essential Oil and Extract** ***of Rosemary:*** The described method with some modification was also used for evaluating anti yeast activity. The concentrations of essential oil and extract were estimated using the broth dilution susceptibility test [15]. The purpose of this method is testing decreeing accentuations of the antimicrobial agent (s), which usually are prepared in serial tow fold dilutions and placed in tubes of a broth medium that will support the growth of the test microorganism. Sabouraud Dextrose Agar and Sabouraud Dextrose broth obtain from MST- England were used in this method. To perform the broth dilution susceptibility test, a standard inoculums of the microorganism (organisms 1×10^6 ml, a 1:500 dilution of a suspension of turbidity equal to a McFarland standard 1), was added to an equal volume of each concentration of antimicrobial agent and to a tube of the growth medium without antimicrobial agent, which has been served as a growth control adding a bacterial suspension dilutes both the suspension and the concentration of antimicrobial agent in the tube. An un-inoculated tube of medium has been incubated to serve as a negative growth control. After overnight incubation, the tubes were cultured on the plats containing Sabouraud Dextrose Agar and were incubated at 35°C (*Saccharomyces cerevisia*) and 25°C (*Candida krusei*) for overnight [15].

RESULTS AND DISCUSSIONS

Total Phenolic Compounds: There are diverse reports on the concentration of total compounds in rosemary. For methanol extracts of rosemary leaves and stems [16] it was reported that a phenolic concentration of 5.07 gm gallic acid equivalents/100 gm of herb (dry weight). For dry rosemary leaves, Dorman *et al.* [17] found the concentration in water extracts to be 185 mg gallic acid equivalents/gm of extract. Similar values were reported by Kosar *et al* [16] for methanol extracts.

Table 1: Composition percentage of *Rosmarinus officinalis* L. essential oil

NO.	RT(retention time)	Compounds	Concentration (%)
1	7.82	Tricyclene	0.24
2	8.64	α- pinene	9.87
3	9.18	Camphene	5.58
4	9.33	Verbenene	0.11
5	10.51	β-pinene	6.10
6	10.97	3-Octanone	0.44
7	11.23	β-myrcene	1.10
8	11.74	1-Phellandrene	0.54
9	13.45	1,8-Cineole	23.14
10	14.51	ã-Terpinene	0.05
11	15.76	L-Fenchone	0.05
12	15.93	Benzene	0.19
13	16.25	3- Oxatricyclo	0.07
14	16.99	Linalool	1.38
15	17.62	Unkhnown	0.58
16	18.88	Camphor	12.35
17	19.37	Isoborneol	1.04
18	20.00	Borneol	5.61
19	20.40	3- cyclohexen	1.15
20	20.66	Unkhnown	0.21
21	21.29	α-terpineol	4.30
22	21.52	Myrtenol	0.48
23	21.95	Unkhnown	3.84
24	22.40	Trans-carveol	0.17
25	22.60	Endobornyl acetate	0.27
26	22.94	β-citronellol	0.09
27	23.41	2-cyclohexen-1-one	0.75
27	23.81	α-terpinene	0.13
29	24.11	Geraniol	0.26
30	24.71	Z-citral	0.11
31	25.36	Unkhnown	2.87
32	25.61	Adamantane	0.12
33	26.04	Delta.3-carene	0.50
34	26.35	2-octanene	0.08
35	26.82	1,3-cyclopentadiene	0.15
36	27.52	α-terpinene	0.04
37	29.22	Copanene	0.09
38	29.72	Geranyl acetate	0.11
39	30.58	Benzene	0.10
40	31.15	Trans-caryoohyllene	3.47
41	31.85	Aromadendrene	0.06
42	32.45	α-humulene	0.27
43	35.33	Delta-candinene	0.07
44	37.66	Caryophllene oxide	2.44
45	38.59	Naphthalene	0.25
46	71.87	Dodecane	0.35
47	72.41	Quinazoline	1.83
48	72.70	Unkhnown	6.40
49	74.59	Cis-jasmone	0.39
50	74.80	Unkhnown	0.16

Table 2: MIC and MBC values (mg ml^{-1}) of *Rosmarinus officinalis* L. essential oil against tested microorganisms.

Species of bacteria or yeast	MIC	MBC
Leuconostoc mesenteroides	0.5	1.0
Lactobacillus delbruekii	1.0	1.5
Saccharomyces cerevisia	1.5	1.75
Candida krusei (Ssatchenikia orientalis)	1.5	2.0

Table 3: MIC and MBC values (mg ml^{-1}) of Rosemary extract against tested microorganisms.

Species of bacteria or yeast	MIC	MBC
Leuconostoc mesenteroides	1.25	1. 5
Lactobacillus delbruekii	1.5	2.25
Saccharomyces cerevisia	2.25	2.7 5
Candida krusei (Ssatchenikia orientalis)	2.5	3.0

In this work we found, for methanol concentration 60% (v/v) extract of rosemary leaves, a phenolic concentration of 5.13 gm gallic acid equivalents/ 100 gm of herb (dry weight). This result is similar to values reported by Shan *et al.* [16].

Chemical Composition and Antimicrobial Activities of Essential Oil: Chemical analysis of the components of oil led to the identification of 20 components in *R. officinalis* (Table 1). The major components were 1,8-cineole (23.14%), camphor (12.35%), α-pinene (9.87%), β-pinene (6.10%), borneol (5.61%), camphene (5.58%) and α-terpineol (4.30%). The antimicrobial efficacy of *R. officinalis* essential oils may be attributed to its composition. The high antimicrobial capacity of Rosemary may be explained by the high content of phenolic compounds found in its essential oil analyzed in the present study. Activity of rosemary is mainly due to borneol and other phenolics in the terpene fraction. The volatile terpenes carvacrol and p-Cymene are reported to be probably responsible for the antimicrobial activity of some essential oils [18]. A group of terpenes (borneol, camphore, 1,8 cineole, α-pinene, camphene, verbenone and bornyl acetate) in rosemary which are responsible of its antimicrobial activity. However, the role of other minor compounds should not be neglected. Gill *et al.* [19], have concluded that whole essential oils have a greater antibacterial activity than a mixture of major components of the same essential oils which suggests that the minor components may have a synergistic effect or potentiating influence. The volatile oils of *R. officinalis* were screened against two (*L. mesenteroides and L. delbruekii*) bacterial strains and two (*S. cerevisia and C. krusei*) yeast strains. The results of the effect of the essential oil from *R. officinalis* on tested microbial strains are shown in Table 2. The essential oil inhibited the growth of both bacteria and yeast at MIC values ranging between 0.5 and 1.5 mg ml^{-1}. These results indicate potential of essential oil of *R. officinalis* as natural preservatives in food against *L. mesenteroides*, *L. delbruekii*, *S. cerevisia* and *C. krusei*.

Antimicrobial Activity of Rosemary Extract: The study of antimicrobial power of plant phenolics is well known [20]. Some reports found that the mainly apolar phenolic compounds from rosemary extracts may be responsible of their antibacterial activity [3]. In this study, the total phenol content was significant. In general, methanol extract inhibited the growth of tested microbes but exhibited less antimicrobial activities compared to the essential oil (Table 3).

Essential oil of rosemary can inhibit the growth of bacteria and yeasts. Therefore it can be used as a potential antimicrobial agent of natural origin in foods. It is recommended that further investigation can be carried out to study the antimicrobial properties of essential oil and extract of *R. officinalis* against yeasts and its applications in foods.

REFERENCES

1. Derwich, E., A. Manar, Z. Benziane and A. Boukir, 2010. GC/MS Analysis and *In vitro* Antibacterial Activity of the Essential Oil Isolated from Leaf of *Pistacia lentiscus* Growing in Morocoo. World Applied Sci. J., 8: 1267-1276. Make references like this style.
2. Tsigarida, E., P. Skandamis and G.J.E. Nychas, 2000. Behaviour of L. monocytogenes and autochthonous fora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5°C. J. Applied Microb., 89: 901-909.

3. Fernandez-Lopez, J., N. Zhi, L. Aleson-Carbonell, J.A. Perez-Alvarez and V. Kuri, 2005. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. J. Meat Sci., 69: 371-380.
4. Rahman, M.U., S. Gul and E.A. Odhano, 2008. Antimicrobial Activities of *Ferula assafoetida* Oil Against Gram Positive and Gram Negative Bacteria. American-Eurasian J. Agric. and Environ. Sci., 4(2): 203-206.
5. Koutsoumanis, K., C.C. Tassou, P.S. Taoukis and G.J.E. Nychas, 1998. Modelling the effectiveness of a natural antimicrobial on Salmonella enteritidis as a function of concentration, temperature and pH, using conductance measurements. J. Applied Microbiol., 84: 981-987.
6. Del Campo, J., M.J. Amiot and C. Nguyen-The, 2000. Antimicrobial effect of rosemary extracts. J. Food Protect. 10: 1359-1368.
7. Rasooli, I., M.H. Fakor, D. Yadegarinia, L. Gachkar, A. Allameh and M.B. Resaei, 2008. Antimycotoxigenic characteristics of Rosmarinus officinalis and Trachyspermum copticum L. essential oils. J. Food Microbiol., 122: 135-139.
8. Özacan, M.M. and J.C. Chalchat, 2008. Chemical composition and antifungal activity of rosemary (Rosmarinus officinalis L.) oil from Turkey. J. Food Sci. Nutrition, 59: 691-698.
9. Mahomoud, A.A., S.S. Al-Shihry and B.W. Son, 2005. Diterpenoid quinines from Rosemary (Rosmarinus officinalis L.). J. Phytochemistry, 66: 1685-1690.
10. Pintore, G., M. Usai, P. Bradesi, C. Juliano, G. Boatto, F. Tomi, M. Chessa, R. Cerri and J. Casanova, 2002. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and corsical. J. Flavor and Fragrence. 17: 15-19.
11. Ouattara, B ., R.E. Simard, R.A. Holley, G.J.P. Piette and A. Begin, 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. J. Food Microbiol., 37: 155-162.
12. Moreno, S., T. Scheyer, C.S. Romano and A.A. Vojnov, 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Res., 40: 223-231.
13. Pan, X., G. Niu and H. Liu, 2003. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chem. Eng. and Process. 42: 129-133.
14. Singleton, V.L. and J.A. Rossi, 1965. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol and Viticul., 16: 144-158.
15. Barnon, E.J. and S.M. Fineg Old, 1990. Method for testing antimicrobial effectiveness. In: Diagnostic Microbiology. 8thed. the Moby Company, pp: 172-184.
16. Shan, B., Y.Z. Cai, M. Sun and H. Corke, 2005. Antioxidant capacity 26 spice extracts and characterization of their phenolic constituent. J. Agri and Food Chem., 53: 7749-7759.
17. Dorman, H.J.D., A. Peltoketo, R. Hiltunen and M.J. Tikkanen, 2003. Characterisation of the antioxidant properties o f de-odourised aqueous extracts from selected Lamiaceae herbs. J. Food Chem., 83: 255-262.
18. Santoyo, S., S. Cavero, L. Jaime, E. Ibanez, F.J. Senorans and G. Reglero, 2005. Chemical composition and antimicrobial activity of Rosmarinus officinalis L. essential oil obtained via supercritical fluid extraction. J. Food Protect., 68(4): 790-795.
19. Gill, A.O., P. Delaquis, P. Russo and R.A. Holley, 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. J. Food Microbiol., 73: 83-92.
20. Proestos, C., N. Chorianopoulos, G.-J.E. Nychas and M. Komaitis, 2005. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. J. Agricul and Food Chem., 53: 1190- 1195.