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Full Length Research Paper

Chemical composition and evaluation of antimicrobial properties of *Rosmarinus officinalis* L. essential oil

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Preservatives used in the food industry are undergoing increasing scrutiny and reappraisal. There is therefore a renewed interest in the antimicrobial properties of herbs and spices. Rosemary (*Rosmarinus officinalis* L.) belonging to the Lamiaceae family, is a pleasant-smelling perennial herb. The antimicrobial activities of the *R. officinalis* oil against *Leuconostoc mesenteroides* (PTCC1591), *Lactobacillus delbruekii* (PTCC1333), *Saccharomyces cerevisia* (PTCC5269) *and Candida krusei* (PTCC 5295) were determined. The results indicate that among the tested microbes, the essential oil had a stronger inhibitory effect on the bacteria as compared to yeasts. Minimum inhibitory concentration (MIC) values for bacteria *L. mesenteroides*, *L. delbruekii*, *S. cerevisia* and *C. krusei* ranged between 0.5 and 1.5 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, chemical composition, antimicrobial properties.

INTRODUCTION

Even though artificial additives have been widely used in processed food products to extend their shelf lives, the tendency is to decrease their use because of the growing apprehension among consumers about these types of products (Tsigarida et al., 2000). Currently, there is a growing interest on the use of natural additives, such as herb and spice extracts for the preservation of foods due to their special flavor and antioxidant as well as antimicrobial activities (Fernandez-lopez et al., 2004). Several researches have established the antimicrobial activity of essential oils (EOs) in both model and real food systems (Koutsoumanis et al., 1998; Tsigarida et al., 2000). Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food. Since EOs are generally regarded as safe (GRAS) (Kabara, 1991), there is possibility of strengthening their natural antimicrobial effects by the addition of small amounts to achieve an equilibrium between their sensorial attributes and antimicrobial efficiency (Rasoli et al., 2008). Rosemary (*Rosmarinus officinalis* L.), belonging to the Lamiaceae family, is a pleasant-smelling perennial shrub that grows in several regions all over the world (Özcan and Chalchat, 2008). 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 useful for urinary ailments (Haloui et al., 2000; Mahmoud et al., 2005). The 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 (Pinto et al., 2002). It is used either in the form of dried herb or oil as spice and flavoring agents in several food formula for its desirable flavor, high antioxidant activity and recently as antimicrobial agent (Lo et al., 2002; Ouattara et al., 1997). *R. officinalis* contains a great amount of essential oil (up to 1%). Moreno et al. (2006) reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and

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negative bacteria. Its strong antimicrobial activity may be attributed to the presence of carnosic acid and carnosol. The aim of our study was to investigate the antimicrobial activity of rosemary essential oil in concentrations that can be used as natural additives in foods.

MATERIALS AND METHODS

Yeasts

The organisms used were *Saccharomyces cerevisia* PTCC 5269, *Candida krusei (I. orientalis)* PTCC 5295. They were maintained on Sabouraud Dextrose Agar (Merck, Germany) at 4°C.

Bacteria

The organisms used were *Leuconostoc mesenteroides* PTCC 1591*, Lactobacillus delbruekii* PTCC 1333. They were maintained on MRS Agar (Merck, Germany) at 4°C.

Essential oil

The essential oil was prepared from IRANIAN Zardband company (Tehran, Iran).

Antibacterial analysis

The amount of essential oil was estimated using the Broth Dilution Susceptibility test (Barnon and Fineg old, 1990). The purpose of this method was to evaluate the effect of the decree of accentuations of the antimicrobial agent(s), which usually are prepared in serial two 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 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 was incubated to serve as a negative growth control. After overnight incubation, the tubes were cultured on the plates containing MRS agar and were incubated at 37°C (*L. delbruekii*) and 26°C (*L. mesenteroides*) overnight (Barnon and Fineg old, 1990).

Anti yeast activity

The described method with some modification was also used for the evaluation of anti yeast activity. The amount of essential oil was estimated using the Broth Dilution susceptibility test (Barnon and Fineg old, 1990). The purpose of this method was to evaluate the effect of decree of accentuations of the antimicrobial agent(s), which usually are prepared in serial two 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 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 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 was 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 (*S. cerevisia*) and 25°C (*C. krusei*) overnight (Barnon and Fineg old, 1990).

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 x 0.25 mm, film thickness 0.25 µm). The operating conditions were as follows: injector and detector temperature, 290 and 220°C, respectively; 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; electron multiplier energy 2000 V. The identities of the oil components were established from their GC retention indices, relative to C7-C88 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.

RESULTS AND DISCUSSION

Antimicrobial analysis

Generally, the essential oils of *R. officinalis* exhibited wide spectra of activity against the tested micro-organisms. Santoyo et al. (2005) attributed the anti-microbial property of the essential oil of *R. officinalis* to the presence of a-pinene, 1, 8-cineole, camphor and borneol. The quantities of these compounds were very high in our oils. The volatile oils of *R. officinalis* were screened against two bacteria strains (*L. mesenteroides* and *L. delbruekii*) and two yeast (*S. cerevisia and C. krusei*) strains. The results of the effect of the essential oil from *R. officinalis* on tested microbial strains are shown in Tables 1 and 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}. *L. mesenteroides* was more susceptible to essential oil (0.5 mg mL^{-1}) than the *L. delbruekii*. The essential oil caused the ruin of both bacteria and yeast at MBC (minimum bactericidal concentration) values ranging between 1.0 and 2.0 $mgmL^{-1}$. Generally, these results show that *R. officinalis* was strongly active against bacteria as compared to yeast.

Chemical composition

Chemical analysis of the components of oil led to the identification of 20 components in *R. officinalis* (Table 3).

Table 1. Results of MIC of various concentration of rosemary essential oil against bacteria and yeasts.

Species of bacteria or yeast	Concentration of essential oil (mg mL^{-1})						
	0 (Control)	0.125	0.25	0.5	1.0	1.5	2.0
L. mesenteroides	++	++	+	-	-	-	-
L. delbruekii	++	++	+	+	-	-	-
S. cerevisia	++	++	++	+	+	-	-
C. krusei (S. orientalis)	++	++	++	++	+	+	-

-: No growth, ++: extreme growth, +: fair growth.

Table 2. MBC value (mg mL^{-1}) of *R. officinalis* L. essential oil against two bacteria and two yeasts.

MBC	Species of bacteria or yeast
L. mesenteroides	1. 0
L. delbruekii	1.5
S. cerevisia	1.75
C. krusei (S. orientalis)	2.0

Table 3. Composition percentage of *R. officinalis* L. essential oil.

S/N	RT(min)	Compound	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	γ-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	Unknown	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	Unknown	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

Table 3. continues

31	25.36	Unknown	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	Unknown	6.40
49	74.59	Cis-jasmone	0.39
50	74.80	Unknown	0.16

The oil components, their retention times and the percentage are presented in Table 3. 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%), respectively. 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 the essential oil of *R. officinalis* analyzed in this study. The 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 probably be responsible for the antimicrobial activity of some essential oils (Rasooli et al., 2008). Davidson and Naidu (2000) identified a group of terpenes (borneol, camphore, 1, 8 cineole, α-pinene, camphene, verbenone and bornyl acetate) in rosemary which are responsible for its antimicrobial activity. However, the role of other minor compounds should not be neglected. Gill et al. (2002) 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 (Mourey and Canillac, 2002). The presence of these compounds as the major components of oil used in this study indicates their major role in the inhibition of microbial growth. These results indicate the latency of essential oil of *R. officinalis* as natural preservatives in food against *L. mesenteroides*, *L. delbruekii*, *S. cerevisia* and *C. krusei*.

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