Brazilian Journal of Microbiology (2010) 41: 1070-1078
ISSN 1517-8382

ROSMARINUS OFFICINALIS ESSENTIAL OIL: ANTIPROLIFERATIVE, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

Abdullah Ijaz Hussain[1,4*], Farooq Anwar[2*], Shahzad Ali Shahid Chatha[1], Abdul Jabbar[1], Shahid Mahboob[3], Poonam Singh Nigam[4]

[1] Department of Chemistry, Government College University, Faisalabad, Pakistan; [2] Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan; [3] Department of Zoology, Government College University, Faisalabad, Pakistan; [4] Centre of Molecular Biosciences, Institute of Biomedical Sciences Research, University of Ulster, Coleraine, BT52 1SA, UK.

Submitted: November 24, 2009; Approved: April 26, 2010.

ABSTRACT

The aim of this work was to investigate and compare the antiproliferative, antioxidant and antibacterial activities of *Rosmarinus officinalis* essential oil, native to Pakistan. The essential oil content from the leaves of *R. officinalis* was 0.93 g $100g^{-1}$. The GC and GC-MS analysis revealed that the major components determined in *R. officinalis* essential oil were 1,8-cineol (38.5%), camphor (17.1%), α-pinene (12.3%), limonene (6.23%), camphene (6.00%) and linalool (5.70%). The antiproliferative activity was tested against two cancer (MCF-7 and LNCaP) and one fibroblast cell line (NIH-3T3) using the MTT assay, while, the antioxidant activity was evaluated by the reduction of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and measuring percent inhibition of peroxidation in linoleic acid system. The disc diffusion and modified resazurin microtitre-plate assays were used to evaluate the inhibition zones (IZ) and minimum inhibitory concentration (MIC) of *R. officinalis* essential oil, respectively. It is concluded from the results that *Rosmarinus officinalis* essential oil exhibited antiproliferative, antioxidant and antibacterial activities.

Key words: Antibacterial, Antioxidant, antiproliferative, 1,8-cienol, Resazurin assay

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is of considerable importance in term of its great an important medicinal and aromatic value. This plant belongs to Lamiaceae family. Rosemary is a perennial evergreen herb with fragrant needle-like leaves (2). Rosemary herbs have been widely used in the traditional medicine and cosmetics. They are also used as flavouring agents in foods (18). *Rosmarinus officinalis* essential oil is also important for its medicinal uses and its powerful antibacterial, cytotoxic, antimutagenic, antioxidant, antiphlogistic and chemopreventive properties (4, 12, 17).

It is now evident that biological activities of the essential oils/extracts are correlated to the presence of specific chemical compounds (5, 4, 8). The ecological conditions of different countries may influence the chemical profile of the plant materials, because some compounds may be accumulated at a particular period in response to environmental conditions (10, 11). Essential oils collected from different countries at different seasons comprise different chemical composition and thus may exhibit different biological activities (8). This prompted us to investigate the chemical profile of *R. officinalis* essential oil native to Pakistan and determine its biological activities.

There is no any detailed report available in the literature

***Corresponding Author. Mailing address:** Department of Chemistry, GC University, Faisalabad-38000, Pakistan.; Tel.: 0092-41-9200066, ext 373.; E-mail: ai.hussain@yahoo.com / fqanwar@yahoo.com

on the antiproliferative, antioxidant and antimicrobial activities of the *R. officinalis* essential oils from sub-continental region and in particular from Pakistan. In the present study, we investigated the chemical composition of the essential oil extracted from the leaves of *R. officinalis* native to Pakistan, and subsequently, their antiproliferative, antioxidant and antimicrobial potentials.

MATERIAL AND METHODS

General

The major equipment used was: GC (model-8700, Perkin-Elmer), GC/MS (6890N, Agilent-Technologies, California, USA), UV-VIS spectrophotometer (U-2001, model 121-0032 Hitachi, Tokyo, Japan) and Clevenger-type hydrodistillation apparatus. Butylated hydroxytoluene (BHT), linoleic acid, 2, 2,-diphenyl-1-picrylhydrazyl, dimethylsulfoxide, 3-[4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide] (MTT), homologous series of C_9-C_{24} *n*-alkanes and various reference chemicals used in this study were obtained from the Sigma Chemical Co. (St Louis, MO, USA). All other chemicals (analytical grade) i.e. ferrous chloride, ammonium thiocyanate, hydrochloric acid, chloroform, ethanol, and methanol used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise. All culture media and standard antibiotic discs were purchased from Oxoid Ltd. (Hampshire, UK).

Plant materials

The leaves of *Rosmarinus officinalis* L. were collected from the Botanical Garden, University of Agriculture, Faisalabad, Pakistan during winter (November-December), 2007. The mean maximum and minimum temperature (^oC) for the months of November-December, 2007 in the University of Agriculture, Faisalabad region were: 28.6 ± 6.7, 15.0 ± 3.5 (average 21.8), respectively. The average relative humidity and total rainfall for the months of November-December 2007 in the University of Agriculture, Faisalabad region were 49.0 ± 10.4% and 9.8 mm, respectively. The plant specimens were further identified and authenticated by Dr. Mansoor Hameed, Taxonomist of the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The specimen was further authenticated by comparison with an authentic voucher (No. 7087/13.4.34) of *R. officinalis,* deposited in the Herbarium of University of Agriculture, Faisalabad, Pakistan.

Isolation of the essential oil

The air-dried and finely ground (80 mesh) leaves of *R. officinalis* were hydro-distilled for 3 h using a Clevenger-type apparatus. Distillates of essential oil were collected and dried over anhydrous sodium sulfate, filtered and stored at -4 °C until analyzed (8).

Analysis of essential oil

Gas chromatography analysis: The essential oil of *R. officinalis* was analyzed using a Perkin-Elmer gas chromatograph model 8700, fitted with a flame ionization detector (FID) and a HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The temperatures of the injector and detector were set at 220 and 290 ^oC, respectively. The column oven temperature was programmed from 80 ^oC to 220 ^oC at a linear rate of 4 ^oC min^{-1}; initial and final temperatures were held for 3 and 10 min, respectively. Helium was used as a carrier gas at the flow rate of 1.5 mL min^{-1}. A 1.0 µL sample was injected, using the split mode (split ratio, 1:100). All quantification was done by a built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin-Elmer, Norwalk, CT, USA). The composition was reported as a relative percentage of the total peak area. Furthermore, the major components (>10%) were quantified by means of the internal standard addition method (13, 14).

Gas chromatography/mass spectrometry analysis: The essential oil were also analyzed by an Agilent-Technologies (Little Falls, California, USA) 6890N Network gas chromatographic (GC) system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto-injector. Separation of

the essential oil chemical constituents was carried out on HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm; Little Falls, CA, USA). A 1.0 µL sample volume was injected into the column using the split mode (split ratio 1:100). GC/MS detection was performed by an electron ionization system, with ionization energy of 70 eV. The column oven temperature program was the same as used previously in the GC analysis. The helium was used as carrier gas at a flow rate of 1.5 mL min^{-1}. Mass scanning range was 50 –550 *m/z* while the injector and MS transfer line temperatures were set at 220 and 290 ºC, respectively.

Compounds identification: The essential oil components were identified on the basis of comparison of their mass spectra, retention times and retention indices with those of authentic samples and/or the NIST mass spectral library and the literature (1, 13, 14, 20).

Antiproliferative activity

*Rosmarinus officinalis e*ssential oil was solubilized in DMSO and then diluted in culture media for use. The human breast cancer (MCF-7) and fibroblast (NIH-3T3) cell-lines were maintained in Dulbecco's Minimum Essential Medium (DMEM), while hormone dependent prostate carcinoma LNCaP was cultured in RPMI 1640 medium. Both media were supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, 1% penicillin/streptomycin. Cells (10^4/well) were cultivated in 96 well plates for 24h before the test compounds were added. Essential oils dilutions (10-500 µg mL^{-1}) were added to triplicate wells and cells were incubated for further 24 h. DMSO was tested as a solvent control while Doxorubicin as a reference standard. Cell viability was evaluated by the MTT assay and the percent inhibition of cell viability was calculated using cells treated with DMSO as control (15). The IC_{50} values (concentration at which 50% of cells were killed) were calculated.

Antioxidant activity

DPPH radical scavenging assay: The antioxidant activity of the essential oil was assessed by their ability to scavenging 2, 2′-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The DPPH assay was performed as described by Mimica-Dukic *et al.* (14). The samples (from 10 to 500 µg mL^{-1}) were mixed with 1 mL of 90 µM DPPH solution and made up with 95% methanol, to a final volume of 4 mL. Synthetic antioxidant, BHT was used as control. After 1h incubation period at room temperature, the absorbance was recorded at 515 nm. Percent radical scavenging concentration was calculated using the following formula:

$$\text{Radical Scavenging (\%)} = 100 \times (A_{blank} - A_{sample}/A_{blank})$$

Where A_{blank} is the absorbance of the control (containing all reagents except the test essential oil/compounds), and A_{sample} is the absorbance of the test essential oil/compounds. IC_{50} values, which represented the concentration of essential oil that caused 50% scavenging, were calculated from the plot of inhibition percentage against concentration.

Percent inhibition in linoleic acid system: The assessment of antioxidant activity of essential oil was also made in terms of percent inhibition of peroxidation in linoleic acid system following a reported method with slight modification (9). Essential oil (5 mg) were added to a solution mixture of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M sodium phosphate buffer (pH 7). Total mixture was diluted to 25 mL with distilled water. The solution was incubated at 40 °C for 175 h. The extent of oxidation was measured by peroxide value colorimetrically. To 0.2 mL sample solution, 10 ml of ethanol (75%), 0.2 mL of an aqueous solution of ammonium thiocyanate (30%) and 0.2 mL of ferrous chloride solution (20 mM in 3.5% HCl) were added sequentially. The mixture was stirred for 3 min and then absorbance recorded at 500nm. A control was performed with linoleic acid but without essential oil. Butylated hydroxytoluene (BHT) was used as a positive control. Percent inhibition of linoleic acid oxidation was calculated using the following equation:

Percent inhibition of linoleic acid oxidation = 100 – [(Abs. increase of sample at 175h / Abs. increase of control at 175h) × 100]

Antibacterial activity

The essential oil were tested against eight strains of bacteria: *Staphylococcus aureus* (NCTC 6571), *Bacillus cereus* (ATCC 11778), *B. subtilis* (NCTC 10400), *Bacillus pumilis* (wild type), *Pseudomonas aeruginosa* (NCTC 1662), *Salmonella poona* (NCTC 4840), *Escherichia coli* (ATCC 8739) and amphicilin resistant *Escherichia coli* (NCTC 10418). The bacterial strains were obtained from the Microbiology Laboratory, School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, UK. The strains were cultured overnight at 37 ºC in nutrient agar (NA, Oxoid).

Disc diffusion method: The antibacterial activity of the *Rosmarinus officinalis* essential oil was assessed by disc diffusion method (16). Briefly, 100 µL of the suspension containing 10^8 colony-forming units (CFU)/mL of bacteria cells were spread on nutrient agar medium. The paper discs (6 mm in diameter) were separately impregnated with 15 µL of essential oil or main components and placed on the agar which had previously been inoculated with the selected test microorganism. Ciprofloxacin (25 µg/dish) was used as a positive reference while the discs without samples were used as a negative control. Plates were kept at 4 ºC for 1 h and then incubated at 37 ºC for 24 h. Antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms comparing to the controls.

Resazurin microtitre-plate assay: The minimum inhibitory concentration (MIC) of the essential oil was evaluated by a modified resazurin microtitre-plate assay as reported by Sarker *et al.* (19) with modification. Briefly, a volume of 100 µL of 5.0 mg mL^{-1} (w/v) essential oil solutions in 10% dimethyl sulfoxide (DMSO, v/v) and 1 mg mL^{-1} of standard antibiotic in 10% DMSO was transferred into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth were added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. Thirty microliter of 3.3 time stronger isosensitised broth (3.3x) and 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 mL of sterile distilled water) were added to each well. Finally, 10 µL of bacterial suspension were added to each well to achieve a concentration of approx 5×10^5 cfu/mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a Ciprofloxacin as positive control, a column with all solutions with the exception of the test compound, a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead and a column with 10% DMSO (v/v) solution as a negative control. The plates were prepared in triplicate, and incubated at 37 °C for 24 h. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change appeared was taken as the MIC value.

Statistical Analysis

All the experiments were conducted in triplicate and the data are presented as mean values ± standard deviation. Statistical analysis of the data was performed by Analysis of Variance (ANOVA) using STATISTICA 5.5 (Stat Soft Inc, Tulsa, OK, USA) software and a probability value of $p \leq 0.05$ was considered to denote a statistical significance difference among mean values.

RESULTS AND DISCUSSION

Yield and composition of *R. officinalis* essential oil

The oil content of *Rosmarinus officinalis* was found to be 0.93 g/100g (Table 1). The chemical components determined in the essential oils are given in Table 1. Eighteen compounds in the essential oil of *R. officinalis,* representing 97.42% of the oil, were identified (Figure 1). The major constituents (> 5%) in the essential oil were found to be 1,8-cineol (38.5%), camphor (17.1%), α-pinene (12.3%), limonene (6.23%), camphene (6.00%), and linalool (5.70%), respectively. In addition, the tested essential oil also contained considerable amounts of various minor constituents (Table 1). The amounts,

calculated using calibrated curves with pure standards compounds, of the three main components, 1,8-cineol, camphor and α-pinene, in the essential oil were found to be 37.1, 15.8 and 11.4 g/100g of oil (Table 1). As far as the groups of chemical constituents are concerned, the *R. officinalis* essential oil mainly consisted of oxygenated monoterpenes (67.0%) followed by monoterpene hydrocarbons (26.0%).

There are no previous data available in the literature on the quantitative (g/100g) analysis of *R. officinalis* essential oil components with which our present results could be compared. However, there are some reports in the literature on the chemical composition of the different chemotypes of *R. officinalis* essential oil from different countries (2, 4, 20). Little variation in the chemical compositions of *R. officinalis* essential oil across countries might be due to different ecological conditions. Our results are in agreement with the findings of Celiktas et al. (4), who also identified 1,8 cineol and α-pinene as a major components of *R. officinalis* essential oil.

Table 1. Yield and chemical composition of *Rosmarinus officinalis* essential oil as identified by GC/MS analysis. [a]

Components[b]	**RI**[c]	**Essential oil composition**		**Mode of identification**[d]
		%	**g/100g**[e]	
Monoterpene hydrocarbons		26.0		
α-pinene	939	12.3 ± 0.7	11.4 ± 0.3	RT, RI, MS
camphene	954	6.00 ± 0.20	---	RI, MS
β-pinene	979	0.20 ± 0.27	---	RT, RI, MS
β-myrcene	991	0.70 ± 0.33	---	RI, MS
α-phellandrene	1003	0.15 ± 0.03	---	RI, MS
limonene	1029	6.23 ± 0.31	---	RT, RI, MS
γ-terpinene	1060	0.40 ± 0.08	---	RI, MS
Oxygenated monoterpenes		67.0		
1,8-cineol	1031	38.5 ± 1.1	37.1 ± 1.1	RT, RI, MS
linalool	1097	5.70 ± 0.22	---	RT, RI, MS
(+)-camphor	1144	17.1 ± 1.0	15.8 ± 0.5	RT, RI, MS
isoborneol	1156	0.20 ± 0.02	---	RI, MS
borneol	1169	3.25 ± 0.15	---	RT, RI, MS
α-terpineol	1189	2.30 ± 0.13	---	RT, RI, MS
Sesquiterpene hydrocarbons		3.56		
verbenone	1207	1.11 ± 0.04	---	RI, MS
β-caryophyllene	1421	1.12 ± 0.08	---	RT, RI, MS
β-farnesene	1458	1.13 ± 0.09	---	RI, MS
γ-muurolene	1480	0.20 ± 0.06	---	RI, MS
Oxygenated sesquiterpenes		0.20		
caryophyllene oxide	1583	0.20 ± 0.03	---	RI, MS
Others		0.63		
tricyclene	927	0.63 ± 0.02	---	RI, MS
3-octanone	984	---		RI, MS
Total		97.42		
Yield (g/100g)		0.93		

[a] Values are mean ± standard deviation of three different samples of *Rosmarinus officinalis*, analyzed individually in triplicate.
[b] Compounds, listed in order of elution from a HP-5MS column.
[c] Retention indices relative to C9-C24 *n*-alkanes on the HP-5MS column.
[d] RT, identification based on comparison of retention time with standard compounds; RI, Identification based on retention index; MS, identification based on comparison of mass spectra.
[e] The amounts, calculated using calibrated curves with pure standards compounds.

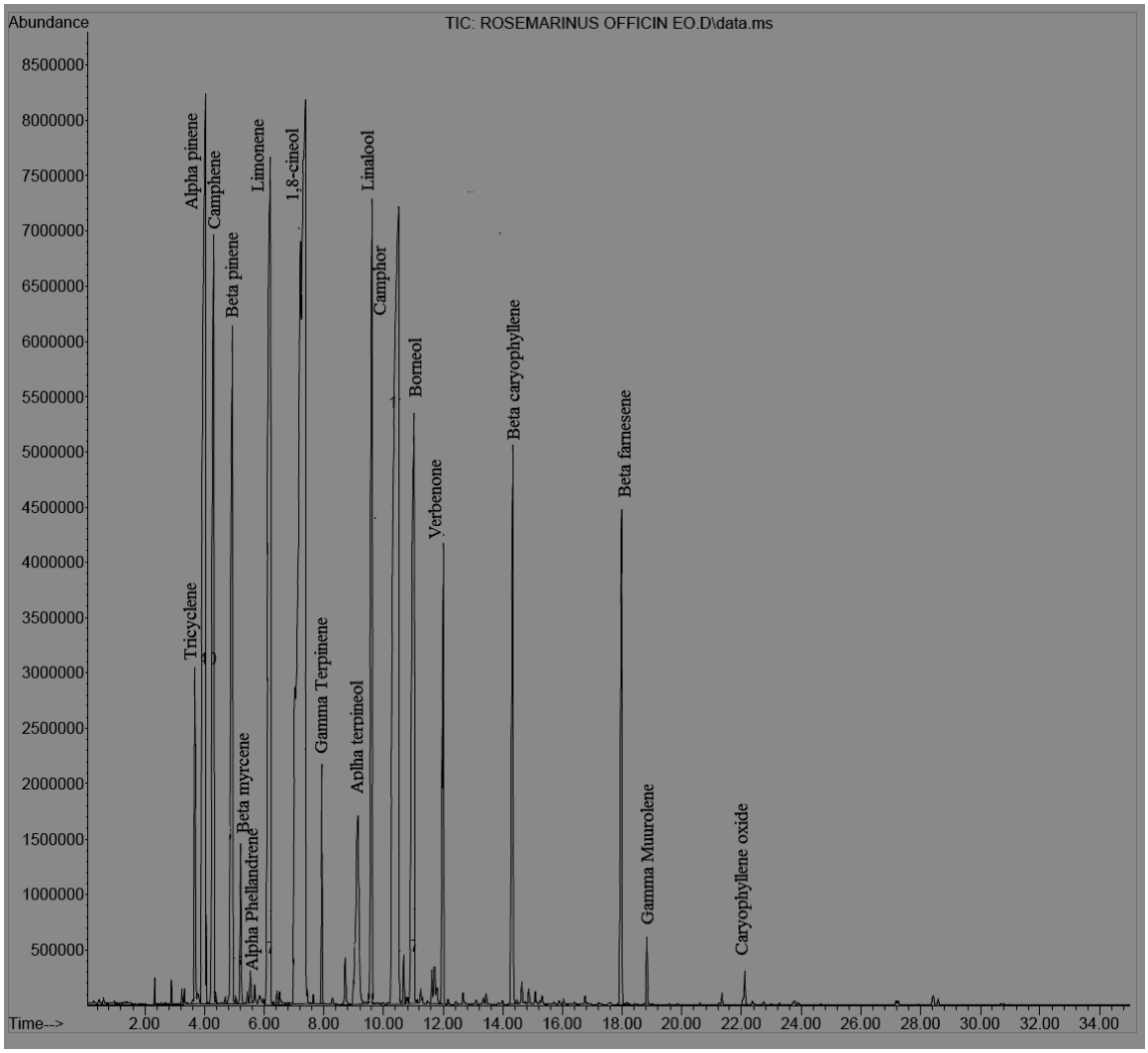

Figure 1. Typical GC-MS chromatograms of *Rosmarinus officinalis* essential oil showing the separations of chemical components.

Biological activities

Anti-proliferative activity: MTT assay, a sensitive, simple and reliable practice, which measured cell viability, was used to evaluate the anti-proliferative activity of *R. officinalis* essential oil. The effect of increasing amounts of *R. officinalis* essential oil on the cell proliferation of two human cancer (MCF-7 and LNCaP) and one fibroblast (NIH-3T3) cell lines was determined. The inhibitory effect of *R. officinalis* essential oil on cell viability ranged from 81-89% at 500 µg mL^{-1} (data not shown). The IC_{50} values, calculated from the graphs, are presented in Table 2, indicating that the essential oil showed the prominent anti-proliferative activity against both the cancer cell lines (IC_{50} 190.1 and 180.9 µg mL^{-1}, respectively). According to the published guidelines, the IC_{50} < 10 µg mL^{-1} represents potentially very toxic; IC_{50} 10-100 µg/ml represents potentially toxic; IC_{50} 100-1000 µg/ml represents potentially harmful, and IC_{50} > 1000 µg mL^{-1} represents potentially non-toxic (6).

Antioxidant activity: The antioxidant activity of *R. officinalis* essential oil and the most abundant component were assessed by different *in-vitro* tests. In DPPH assay, the radical scavenging capacity of the tested essential oil increased in a concentration dependent manner. The values for 50%

scavenging (IC_{50}) are given in Table 2. The essential oil showed better radical scavenging (IC_{50}: 20.9 µg mL^{-1}) than the main component of *R. officinalis* essential oil. Table 2 shows the level of percent inhibition of linoleic acid oxidation as exhibited by the tested essential oil. Again, the maximum inhibition was observed from *R. officinalis* essential oil (59.1 %) while the main component of the oil showed poor activity (33.6%). This might be due to the synergistic effect of some minor components present in the essential oil of *R. officinalis*.

Lipid peroxidation is a big issue in food industries and while comparing our results with the literature, we could not find a single report showing the percent inhibition of peroxidation by *R. officinalis* essential oil. However, there are some reports available in the recent literature on the radical scavenging activity of *R. officinalis* essential oil (3, 21). Our results are in close agreement with the findings of these reports.

Antibacterial activity: *Rosmarinus officinalis* essential oil exhibited varying antibacterial activity as is shown by the inhibition zones (IZ) and MIC values (Table 3). The results from the disc diffusion assay followed by modified resazurin assay indicated that the tested essential oil showed higher antibacterial activity against Gram-positive bacteria (IZ 18.0-24.2; MIC 0.20-0.48 mg mL^{-1}) than against Gram-negative bacteria (IZ 12.8-17.5; MIC 1.16-1.72 mg mL^{-1}). It can be seen from Table 3 that *R. officinalis* essential oil exhibited better antibacterial activity than 1,8-cineol, a principal component of *R. officinalis* essential oil. There are also some reports in the literature on the antimicrobial activities of *R. officinalis* essential oil (2, 4, 7) but the *R. officinalis* essential oil collected from Pakistan depicted better antibacterial activity as compare with these published reports. This might be due to little variation in the chemical profile of *R. officinalis* essential oil, native to Pakistan.

Table 2. Antiproliferative and antioxidant activities of *Rosmarinus officinalis* essential oil.[a]

Parameters	Essential oil	1,8-cineol	BHT	Doxorubicin
Antiproliferative activity				
MCF-7, IC_{50} (µg mL^{-1})	190.1 ± 6.0[b]	---	---	28.8 ± 1.2[a]
LNCaP, IC_{50} (µg mL^{-1})	180.9 ± 4.5[b]	---	---	33.3 ± 1.1[a]
Fibroblast (NIH-3T3)	212.5 ± 6.7			
Antioxidant activity				
DPPH, IC_{50}, (µg mL^{-1})	20.9 ± 0.9[b]	45.7 ± 1.5[c]	7.7 ± 0.3[a]	---
Inhibition in linoleic acid system (%)	59.1 ± 3.1[b]	33.6 ± 1.2[a]	92.9 ± 2.7[c]	---

[a] Values are mean ± standard deviation of three different samples of each *Rosmarinus officinalis*, analyzed individually in triplicate. Means followed by different superscript letters in same row represent significant difference ($p < 0.05$).

Table 3. Antibacterial activity of the *Rosmarinus officinalis* essential oil.[a]

Microorganisms	Essential oil		1,8-cineol		Ciprofloxacin	
	IZ [b]	MIC [c]	IZ	MIC [c]	IZ [b]	MIC [c]
Staphylococcus aureus	22.0 ± 1.0[a]	0.30 ± 0.01$_b$	22.0 ± 0.8[a]	0.83 ± 0.02$_c$	27.3 ± 1.0[b]	6.0 x 10^{-3} $_a$
Bacillus cereus	24.2 ± 1.1[b]	0.26 ± 0.01$_b$	21.5 ± 0.9[a]	0.91 ± 0.03$_c$	34.0 ± 1.6[c]	8.0 x 10^{-3} $_a$
Bacillus subtilis	23.0 ± 0.9[a]	0.20 ± 0.01$_b$	23.4 ± 1.0[a]	0.60 ± 0.02$_c$	32.1 ± 1.0[b]	4.4 x 10^{-3} $_a$
Bacillus pumilis	18.0 ± 0.7[a]	0.48 ± 0.02$_b$	19.6 ± 0.7[a]	0.98 ± 0.03$_c$	22.6 ± 1.0[b]	6.2 x 10^{-2} $_a$
Pseudomonas aeruginosa	17.0 ± 1.0[b]	1.26 ± 0.03$_b$	9.6 ± 0.5[a]	1.59 ± 0.05$_c$	30.1 ± 1.8[c]	3.0 x 10^{-2} $_a$
Salmonella poona	17.5 ± 0.9[b]	1.16 ± 0.03$_b$	12.3 ± 0.4[a]	1.31 ± 0.06$_c$	24.7 ± 1.1[c]	2.5 x 10^{-3} $_a$
Escherichia coli	14.3 ± 0.7[b]	1.52 ± 0.04$_b$	12.0 ± 0.5[a]	1.47 ± 0.04$_b$	31.0 ± 1.7[c]	4.8 x 10^{-3} $_a$
Escherichia coli [d]	12.8 ± 0.5[b]	1.72 ± 0.04$_b$	9.0 ± 0.3[a]	1.80 ± 0.08$_b$	33.0 ± 1.5[c]	5.5 x 10^{-3} $_a$

[a] Values are mean ± standard deviation of three different samples of each *Rosmarinus officinalis*, analyzed individually in triplicate.
[b] IZ, diameter of inhibition zones (mm) including disc diameter of 6 mm.
[c] MIC, minimum inhibitory concentration (mg mL^{-1}).
[d] Amphicilin resistant *Escherichia coli* strain.
Different alphabetic letters in superscript indicate significant differences in IZ among essential oil, 1,8-cineol and control while alphabetic letters in subscript indicate significant differences in MIC among essential oil, 1,8-cineol and control.

CONCLUSION

The results of the present study indicate that *R. officinalis* essential oil collected from Pakistan possessed very good antimicrobial and antiproliferative potentials as well as considerable antioxidant activity. A further study under the *in-vivo* conditions is recommended to further elaborate the antimicrobial and antioxidant principles of *Rosmarinus officinalis* essential oil for various useful applications. The investigated essential oil may be used for the preservation of processed foods as well as pharmaceutical and natural therapies for the treatment of infectious diseases in humans and plants.

ACKNOWLEDGEMENTS

We would like to extend our special gratitude to Professor M.I. Bhanger, Director, National Center of Excellence in Analytical Chemistry (NCEAC), University of Sindh, Jamshoro, Pakistan, for allowing us to use GC–MS instrumental facility. This work is a part of PhD project funded by the Higher Education Commision, Pakistan under the scheme of International Research Support Initiative Program (IRSIP) and Indigenous Ph.D. Fellowship Program.

REFERENCES

1. Adam, R.P. (2001). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy.* Allured: Carol Stream, IL.
2. Bousbia, N.; Vian, M.A.; Ferhat, M.A.; Petitcolas, E.; Meklati, B.Y.; Chemat, F. (2008). Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. *Food Chem.* 14, 355-362.
3. Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Jovin, E. (2007). Antimicrobial and antioxidant properties of rosmary and sage (*Rosmarinus officinalis* and *Salvia officinalis*, Lamiaceae) essential oils. *J. Agric. Food Chem.* 55, 7879-7885.
4. Celiktas, O.Y.; Kocabas, E.E.H.; Bedir, E.; Sukan, F.V.; Ozek, T.; Baser, K.H.C. (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus oficinalis*, depending on location and seasonal variations. *Food Chem.* 100, 553-559.
5. Silva, A.B.; Silva, T.; Franco, E.S.; Rabelo, S.A.; Lima, E.R.; Mota, R.A.; da Camara, C.A.G. Pontes-Filho, N.T.; Lima-Filho, J.V. (2010). Antibacterial activity, chemical composition, and cytotoxicity of leaf's essential oil from Brazilian pepper tree (*Schinus terebinthifolius,* Raddi) *Braz. J. Microbiol.* 41, 158–163.
6. Gad-Shayne, C. (1999). Alternatives to in vivo studies in toxicology. *In*: Balantyne B., Marrs T., Syversen T. (eds). *General and applied toxicology,* Vol (1). Grove's Dictionari's Inc., USA, p. 178.
7. Gachkar, L.; Yadegari, D.; Rezaei, M.B.; Taghizadeh, M.; Astaneh, S.A.; Rasooli, I. (2007). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. *Food Chem.* 102, 898-904.
8. Hussain, A.I.; Anwar, F.; Sherazi, S.T.H.; Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem.* 108, 986-995.
9. Iqbal, S.; Bhanger, M.I.; Anwar, F. (2005). Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chem*. 93, 265–272.
10. Kamatou, G.P.P.; Van-Zyl, R.L.; Van-Vuuren, S.F.; Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Viljoen, A.M. (2008). Seasonal variation in essential oil composition, oil toxicity and the biological activity of solvent extracts of three South African *Salvia* species. *South African J. Bot*. 74, 230-237.
11. Koenen, E.V. (2001). *Medicinal poisonous and edible plants in Namibia*. Klaus Hess Publishers, Verlag, Berlin.
12. Koschier, E.H.; Sedy, K.A. (2003). Labiate essential oils affecting host selection and acceptance of Thrips, *Tabaci lindeman*. *Crop Prot.* 22, 929–934.
13. Massada, Y. (1976). *Analysis of essential oils by gas chromatography and mass spectrometery.* John Wiley & Sons, New York.
14. Mimica-Dukic N.; Bozin, B.; Sokovic, M.; Mihajlovic, B.; Matavulj, M. (2003). Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Medica* 69, 413-419.
15. Mosmann, T. (1983). Rapid colorimetric assay or cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol Meth.* 65, 55-63.
16. NCCLS (National Committee for Clinical laboratory Standards). (1997). *Performance standards for antimicrobial disc susceptibility test* (6th ed.) approved Standard. M2-A6, Wayne, PA.
17. Ohno, T.; Kita, M.; Yamaoka, Y.; Imamura, S.; Yamamoto, T.; Mitsufuji, S.; Kodama, T.; Kashima, K.; Imanishi, J. (2003). Antimicrobial activity of essential oils against *Helicobacter pylori*. Helicobacter 8, 207-215.
18. Pintore, G.; Usai, M.; Bradesi, P.; Juliano, C.; Boatto, G.; Tomi, F.; Chessa, M.; Cerri, R.; Casanova, J. (2002). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. *Flav. Frag. J.* 17, 15–19.
19. Sarker, S.D.; Nahar, L.; Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth,

and its application in the in vitro antibacterial screening of phytochemicals. *Methods* 42, 321-324.

20. Vagionas, K.; Graikou, K.; Ngassapa, O.; Runyoro, D.; Chinou, I. (2007). Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania. *Food Chem.* 103, 319-324.
21. Wang, W.; Wu, N.; Zu, Y.G.; Fu, Y.J. (2008). Antioxidant activity of *Rosmarinus officinalis* L. essential oil compared to its main components. *Food Chem.* 108, 1019-1022.