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Study of the Antimicrobial Properties of the Essential Oil of Rosemary

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Abstract: The purpose of this study is to identify the antimicrobial activity of the essential oil of Rosemary plant with tetracycline antibiotic and resistant microorganisms. The aerial parts of rosemary were collected from Research Center for Medicinal Plants at Shahid Bahonar University of Kerman prior to the blooming stage. After drying the plant materials in shade, essential oil was obtained by hydro-distillation method. The study of antimicrobial effect of the oil sample was conducted on nine strains of pathogenic bacteria resistant by disk diffusion assay and measuring the diameters of zones of inhibition from growth. The 41 components were identified in the essential oil of Rosemary, the main constituents are α-pinene (15.52%), camphor (11.66%) and verbenone (11.10%) and 1, 8- cineole (10.63%). The results showed that the essential oil of Rosemary has an effective controlling and antimicrobial power against all positive and negative bacteria. The antimicrobial impacts of the essential oil of Rosemary plant under investigation can be related with the high percentage of α-pinene, camphor, verbenone and 1,8-cineole.

Key words: *Rosmarinus officinalis* L. · Antimicrobial activity

INTRODUCTION

Rosemary plant with the scientific name of *Rosmarinus officinalis* L. is of Lamiaceae (Labiatae) family. This type has an evergreen bush which is a local plant of Mediterranean region with pharmacological and decorative value. It is a sustainable plant, so aromatic and has wooden stalks with 50 centimeters up to 2 meters height, growing in Mediterranean region and in particular in the littorals region through Minor Asia areas wildly. Its leaves facing each other are turned down, narrow, long, thick, sharp-pointed and with a tough appearance. The flowers appearing besides the leaves from May to June are in light blue and rarely white. Its fruit is achene and in brown color. [1]. In traditional medicine, Rosemary is used to treat different diseases including: depression, insomniac, gout and arthritic pains [2]. Its dried leaves are also used to prepare soups and sauces. With due attention to their anti-oxidant and anti-bacterial effects and that they give flavor to meat, fish and chicken they are used to keep the quality of fats and meats. Today, this plant or its essential oil is used in different industries including cosmetic and sanitary industries to reinforce the hairs, to fix the hair color and also is utilized in non-alcoholic drinks. [3]. The essential oil of Rosemary plant has been studied in Iran and in the world. For instance, the chemical combinations of the oil of this plant has been studied in lab method and semi-industrial form within the framework of a study compared with different countries by Jaymand and Rezaee, [4].The study has led to the identification of 29 combinations. The main compounds in lab sample have been reported as α-pinene (30.3%) and 1, 8- cineole (15.2 %) and main combinations in semi-industrial essential oil are α-pinene (30%) and and 1, 8- cineole (12.2%) which has differences as compared with Spain, Italy and Hungary. Whereas the combination of Camphor with 0.7% reported in semi-industrial sample in the mentioned countries ranges from 13.4% to 35.3 percent. The studies made by Afzali *et al.* [5] reported the essential oil identified in Kerman as follows: α-pinene(43.9%-46.1%), 1, 8- cineole(11.1%) and Camphor (2.4%-5.3%). Other studies, Emadi *et al.* [6] reported the rate of compounds in the leaves of Rosemary plant being collected in three periods (before, after and during blooming) as α-pinene 20.08, 27.65, 17.82 and 1,8-Cineole, 7.32, 7.55, 9.99 and Camphor as 9.11, 8.84,

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15.68%, respectively. The main and important ingredient studied in different regions of the world include: Flavonoids, phenolic acids, volatile oils and diterpenoids each of which includes some materials. For example phenoloic acids includes Caffeic acid, Cryogenic acid and Rosemarinic acid. Rosemarinic and Caffeic acids have anti-oxidant activities [7]. of other and main compounds studied is Carnosyic acid which along with Carnosol are pre-fabricator of Phenolic Diterpenoids being used in the structure of Lactons [8]. Antimicrobial properties of Rosemary plant, the combinations of phenolic diterpenoids have been reported which have impacts on both positive and negative bacteria mass [9]. The anti-bacterial effects of Rosemary plant on Pseudomonas aeroginosa has been studied and proved [10]. Other study, the chemical compounds, genetic differences, anti-microbial and anti-fungal impacts of Rosemary plant have been studied [11]. In one more research, the anti-microbial impact of the essential oil of Rosemary on Staphylococcus aureus bacteria in comparison with Erythromycin antibiotic has been studied and proved [12]. Also, the impact of Rosemary essential oil on 5 bacteria (including 2 negative bacteria, Escherichia coli and Pseudomonas aeroginosa and 3 positive bacteria, Staphylococus aureus, Staphylococcus epidermidis and Micro coccus loteous) were studied and it has been reported that it has had the highest rate of effect on *Escherichia coli* bacteria [13]. Concerning the anti-microbial effects of α-Pinene combinations, 1,8- Cineole, Camphor and Verbenone which are the main ingredients of essential oil of Rosemary Plant, it was decided that in addition to the identification of the chemical compounds of the essential oil of sample and compare it with other samples to study its anti-microbial impacts as well. [14].

MATERIALS AND METHODS

Plant Material: The leaves of Rosemary plant were obtained from the Research Center for Pharmacological Plants, Kerman University in May before blooming. The samples were cleaned in shade condition to prevent hydrolyze of the existing materials and to keep the natural color of the sample fixed. Then they were dried in the temperature of the environment and were powdered and kept at appropriate conditions from the viewpoint of temperature and light until the essential oil taking stage. Afterwards, essential oil was taken from 150 grams of the powdered sample in hydro distillation method with the help of Clevenger set for three hours. Following the sample oils were dried with anhydrous sodium sulfate.

Identification: In order to analyze and identify the combinations forming the essential oil, the Chromatograph Gas set attached to a Mass Spectrometry, Model Shimadzu-QP5050A was used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column DB5-MS in 40 meters length, internal diameter of 0.18 mm and layer thickness of 0.18 micro meter, thermal program of oven (5 minutes) in 60 centigrade, then 60-275 centigrade with a 5 centigrade slope per minute, then 10 minutes in 275 centigrade, the temperature of place of injection 280 centigrade, gas conveying Helium, the speed of gas move 0.9 milliliter per minute, the ratio of fission 1 to 43, the rate of injection 0.1 micro liter, temperature of the reservoir of ionization 230 centigrade, ionization mode EI, Ionization energy 70eV. The series of normal Alckans C8-C28 were also injected to the set under the same condition with that of essential oil injection to calculate Restrictive Index (RI) of components of essential oil. The Restrictive Index of components of the sample was calculated by using a computerized program. Finally, the components of essential oil was identified by comparing the mass spectrums obtained with the existing standard mass spectrums at electronic library of Wiley 2000 existing in Labsolution software of GC/Ms set and calculation of standard Restrictive Index in accordance with C_8-C_{28} Alckans and comparing them with the existing standard figurers in references [15].

Antimicrobial Effects: The antimicrobial activity of Rosemary essential oil on 9 pathogenic bacterial strains resistant was determined, namely: 2 positive bacteria, *Staphylococcus aureus* (ATCC=25922), *Staphylococcus epidermidis* (ATCC=1435) and 7 negative bacteria, *Pseudomonas aeroginosa* (ATCC=1074), *Shigella flexneri* (ATCC=1234), Kellebsiella pnuomonae (ATCC=1053), *Salmonella typhi* (ATCC=1634), *Serratia marcescens* (ATCC=1111) and two strains of *Escherichia coli* (ATCC=157, 25923). The bacteria under experiment were obtained from the Center for Fungi and Bacteria of Iranian Scientific and Industrial Researches Organization. For this purpose, the disk diffusion assay was used. Of the cultured bacteria for 24 hours on the media of Muller Hinton Agar, a suspension with a dilution of 0.5 Mac Farland at the culture media of Muller Hinton Broth was prepared. Then 1 milliliter of suspension of each of bacteria was cultured in pure plate method (0.5 milliliter of suspension was mixed with 25 milliliter of the media of culture of Muller Hinton Agar). Then the blank sterile disks containing 30 microliter of 1.5 dilution of essential oil being diluted with DMSO were placed on culture media. After that, the diameters of zones of inhibition from growth after 24 hours from incubation of plates in 37 centigrade was measured. At that time the antimicrobial impacts of essential oil of Rosemary compared with tetracycline antibiotic (8 milligram per milliliter) as evidence was studied.

RESULTS AND DISCUSSION

The results showed that the output of essential oil resulting from Rosemary plant being collected from the Research Center for Pharmacological Plants at Kerman University is 3.2 percent. The identified combinations in essential oil, restrictive index (RI) and quantitative percentage of the compounds are presented in Table 1. Of the 41 compounds being identified in the essential oil of this plant with 99.74 percent, the combinations of α-pinene, Camphor, Verbenone and 1,8-cineol with 48.91 percent constitute the highest percentage of essential oil. The results of studying the antibacterial impacts of the Rosemary essential oil shows that the oil of this plant has a 34 and 30 millimeter diameter of zone of inhibition from growth on positive bacteria of *Staphylococcus aureus* and *Staphylococcus epidermidis*,respectively and has a diameter of zone of inhibition from growth of 29,23,24,25,28,32 and 36 millimeter on negative bacteria of *Pseudomonas aeroginosa, Shigella flexneri, Kellebsiella penomonae, Salmonella typhi, Serratia marcescens, Escherichia coli* (25923) and *Escherichia coli*(157), respectively.

The study of the analysis of Rosemary plant essential oil under investigation showed that the output of essential oil is 3.2 percent. The reviews show that of the 41 identified combinations (99.74%) existing in the essential oil of Rosmary as compared with the region of Lalehzar in Kerkman, there are some differences and similarities [5]. It is such that α-Peinen with 15.52 percent is the main compound but the same compound in Lalehzar regin, with 43.9 percent is of considerable quantity. The highest rate of the mentioned combination which is of monoterpens combinations in Lalezar region in Kerman is due to the coldness of the mentioned region. Jaymand and Rezaee [4] has reported the percentage of α-pinene from the Rosemary plants being collected from the Research Field of Pharmacological Plants at the Higher Education Center of Agriculture Jihad, Semnan province in a lab sample to be 30.3 percent and in the essential oil of a semi-industrial sample as equal to 30 percent. It has been reported in Spain, Italy and Hungary to be equal to

Table 1: Combinations identified in the essential oil of Rosmarinus Officinalis L. plant

Percentage (%)	Restrictive Index (RI)	Compound Name	Compound No.
0.22	925	Tricyclene	1
15.52	937	α-pinene	2
5.00	954	Camphene	3
0.35	956	Thuja-2,4(10)-diene	4
0.47	979	1-octen-3-ol	5
0.95	981	B-pinene	6
5.09	985	3-octanone	7
2.50	989	β-myrcene	8
0.45	996	3-octanol	9
0.20	1019	α-terpinene	10
1.08	1027	ρ-cymene	11
3.45	1032	Limonene	12
10.63	1037	1,8-cineole	13
0.33	1061	γ-terpinene	14
0.34	1088	Terpinolene	15
0.19	1092	ρ-cymenene	16
3.38	1098	Linalool	17
0.45	1103	Filifolone	18
0.27	1124	endo-fenchol	19
0.16	1131	α-campholenal	20
0.36	1152	trans-verbenol	21
11.66	1156	Camphor	22
1.03	1167	trans-pinocamphone	23
0.24	1169	Pinocarvone	24
0.21	1176	delta-terpineol	25
7.29	1180	Borneol	26
1.08	1184	cis-pinocamphone	27
1.34	1186	terpinene-4-ol	28
0.26	1192	ρ-cymen-8-ol	29
2.27	1201	α-terpineol	30
0.21	1203	Myrtenol	31
1.20	1209	borneol,formate	32
11.10	1217	Verbenone	33
1.94	1248	cis-myrtanol	34
1.63	1256	trans-myrtanol	35
5.41	1290	bornyl acetate	36
0.19	1346	Piperitenone	37
0.19	1368	cis-myrtanol acetate	38
0.34	1376	trans-myrtanol acetate	39
0.45	1432	E-caryophyllene	40
0.28	1598	Caryophyllene oxide	41
0.26	1769	Unknown	42

The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C_8-C_{28}) to DB-5 column

Table 2: The results of the antimicrobial effects of the essential oil of *Rosmarinus officinalis* L. plant

Tetracycline (8 mg/mm)	The zone diameter of inhibition of growth (mm)	Bacteria
27	34	*Staphylococcus aureus*
23	30	*Staphylococcus epidermidis*
18	29	*Pseudomonas aeroginosa*
20	23	*Shigella flexneri*
21	24	*Kellebsiella pnuomonae*
19	25	*Salmonella typhi*
22	28	*Serratia marcescens*
25	32	*Escherichia coli (25923)*
28	36	*Escherichia coli(157)*

0.9, 12.8 and 20 percents, respectively. Other study reported by Emadi *et al.* [6] on Rosemary plants collected from the Herbarium of Pharmacology Faculty of Tehran University of Medical Science in three periods of before, after and during blooming, the percentages are 20.08, 27.65 and 17.82, respectively. The combination of Camphor in the present study is the second main combination with 11.66 percent. The study conducted by Jaymand and Rezaee [4] indicated that in the essential oil of semi-industrial sample, the Camphor combination is of least rate with 0.7 percent and maximum of 35.3 percent in the sample of Spain and in Lalehzar region, it is 2.4 percent, while Emadi *et al.* [6] reported that it ranges from 8.84 to 15.68 percent. The combination of 1,8- Cineol in the present study is 10.63 percent that in comparison with the study carried out by Jaymand and Rezaee[4], it has been reported 15.2 percent and in the semi-industrial sample as 12.2 percent and in Spain samples (24%), Italy (23.5%) and Hungary (15.3%). Whereas in the Afzali's sample it has been reported 11.1 percent and Emadi's sample has reported from 7.32 percent to 9.99 percent. Other main combination with anti-microbial properties is the combination of Verbenone. In the present study, it is 11.10 percent which compared with the results of Emadi from 4.39 percent to 15.5 percent is a considerable rate. Whereas in other studies the mentioned combinations have not been reported. The other identified combinations include Borneol (7.29%), Bornyl acetate (5.41%), 3-octanone (5.09%) and Camphene (5%) with differences are presented in Table 1 along with other studies.

The quality and quantity of the materials forming Rosemary essential oil had some differences and similarities with the cases reported in other regions but it has much similarities with the cases reported by Emadi *et al.* [6] on Rosemary essential oil both from the viewpoint of main combinations and also that of percentage of these combinations. The reason can be the fact that they belong to a rather similar climate and geography. The studies of the ingredients of the essential oil of botanical populations with ecological and genetic differences can be of great importance in identifying the variety of essential oil inside the population of specie. [16]. The results of the study of the antimicrobial impacts of the essential oil of Rosemary plant on all positive bacteria of *Staphylococcus aureus* and *Staphylococcus epidermidis* (With diameter of zone of inhibition from growth of 34 and 30 millimeter respectively) and on negative bacteria of *Pseudomonas aeroginosa, Shigella flexneri, Kellebsiella pnuomonae, Salmonella typhi, Serratia marcescens* and strains of *Escherichia coli* with numbers 25923 and 157 (with diameter of zone of inhibition from growth of 29, 23, 24, 25, 28, 32 and 36 millimeter respectively) have a considerable antimicrobial impacts. The results show the highest controlling and antimicrobial power of Rosemary essential oil under investigation. The antimicrobial effects of Rosemary essential oil can be attributed to the Monoterpens combination and in particular α-Pinene whose antimicrobial effects of this combination has been proved [11,17]. Other combination with anti-microbial properties, the combinations of Camphor and Verbenone and 1,8-Cineole can be mentioned. [13,17]. So with regard to the antimicrobial effects of Rosemary essential oil under investigation as compared with tetracycline antibiotic, this essential oil can be used as a combination with antimicrobial effects and natural origin.

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