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(RESEARCH ARTICLE)

Antibacterial activities of rosemary (*Rosmarinus officinalis L.*) essential oil and ethanol extract

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Abstract

The purpose of this study was to determine the chemical composition and antibacterial potential of rosemary essential oil and ethanol extract to improve its physicochemical and antibacterial properties. the essential oil yield of rosemary leaves was 1.76 % and the predominant compounds were 1,8-cineole (57.55 %), camphor (8.82 %), α-pinene (8.83 %), borneol (5.54 %) and camphene (2.36 %). Rosemary extract had an important amount of total phenolic content with 28.47 mg GAE/g DW. However, flavonoids and condensed tannins were weakly present in rosemary extract having 2.59 mg QE/g and 1.02 mg CE/g, respectively. Rosemary ethanol extract was mainly characterized by the predominance of carnosic acid (58.71 mg /g) and rosmarinic acid (16.51 mg/g). The antibacterial activity of rosemary essential oil and ethanol extract was determined against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Salmonella enterica*, *Bacillus subtilis* and *Enterococcus faecalis*.

Keywords: Rosemary leaves; Ethanol extract; Essential oil; Antibacterial activity

1. Introduction

Antibacterial resistance is a serious threat of growing concern to human, animal and environment health [1]. Bacterial species cause various infections and are speculator pathogens with increasing antibiotic resistance. Finding of new effective antimicrobial agents derived from natural resources has gained high priority among strategies for antibiotic resistance [2].

Plants and their bioactive components are considered as rich sources of antibacterial properties, with different modes of actions [3]. In particular, the antibacterial activity of plant essential oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [4]. Currently, the attention in extracts and essential oils of rosemary has increased due to their richness in bioactive components, as volatile (1,8-cineole, camphor and α-pinene) and phenolic (rosmarinic acid and carnosic acid) components, having a strong antibacterial potential [5]. Rosemary (*Rosmarinus officinalis* L.) is a perennial medicinal and aromatic shrub belonging to the Lamiaceae. Native to the Mediterranean region rosemary is now widely distributed and has been cultivated around the world [6]. Its leaves have been used for thousands of years as a natural food preservative, flavorings, pharmaceuticals, alternative medicine and natural therapies [7].

The aim of this study was to to investigate the chemical compositions of essential oil and ethanol extract from Tunisian rosemary leaves and to evaluate the antibacterial properties against seven bacteria strains, namely *Escherichia coli*,

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Staphylococcus aureus, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Salmonella enterica*, *Bacillus subtilis* and *Enterococcus faecalis*.

2. Material and methods

2.1. Plant material

Wild rosemary (*Rosmarinus officinalis* L.) plants were collected during spring at Djbel Zaghouan region (Tunisian upper semi-arid zone).

2.2. Rosemary essential oil extraction

Rosemary EO was extracted in triplicate from 100 g of dried rosemary leaves using the hydrodistillation by the Clevenger apparatus (Sigma Aldrich) for 180 min [8].

2.3. Gas chromatography analysis (GC-FID)

Rosemary volatile compounds led from the enriched film with EO to the hexane solution was analyzed using the Hewlett- Packard 6890 chromatograph equipped with an electronic pressure control injector, a flame ionization detector, and an HP Innowax (polyethylene glycol capillary) column (30 m × 0.25 mm; 0.25 µ m). Individual peaks corresponding to the volatile components were identified by comparison of their retention indices (RI) relative to (C8-C40) n-alkanes with those of the literature or with those of authentic compounds available in the authors' laboratory.

2.4. Rosemary extract preparation

Rosemary extract was obtained according to Yeddes et al. [9]. Fifteen grams of dried rosemary powder was placed in the filter Whatman cellulose thimble (paper No. 1820- 047 grade) in a Soxhlet apparatus and extracted for 3 hr with 150 ml of absolute ethanol.

2.5. Total phenolic content

The total phenolic contents were assayed using the Folin-Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. [10]. The total extractable phenolic content of ethanolic extract was expressed as milligram gallic acid equivalents per gram (mg GAE/g).

2.6. Total flavonoid content

The total flavonoid content was measured according to the procedure reported by Dewanto et al [10]. The total flavonoid content of ethanolic extract was expressed as milligram catechin equivalents per gram (mg CE/g).

2.7. Condensed tannin content

In the presence of concentrated H2SO4, condensed tannins transform into anthocyanidols due to their reaction with vanillin [11]. Condensed tannin content of the ethanolic extract was expressed as milligram catechin equivalents per gram (mg CE/g).

2.8. Analysis of rosemary extract by HPLC

The phenolic compounds of rosemary lead extracts were identified and quantified using HPLC system (Agilent1260, Agilent technologies, Germany) provided with avacuum degasser, an auto sampler and a binary pumpwith a maximum pressure of 400 bar. Peaks were identified by congruent retention times compared with standards. HPLC analysis allowed determining the con-tents of carnosic acid and rosmarinic acid.

2.9. Antibacterial activity

The antibacterial activity of rosemary essential oil and ethanol extract was carried out by disc diffusion method according to Gómez-Estaca et al. [12] with a slight modification. Antimicrobial activity was tested against seven bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Salmonella enterica*, *Bacillus subtilis* and *Enterococcus faecalis*. The surface of Mueller- Hinton Agar plates (Merck) was inoculated with 0.1 ml of culture bacterial suspension (105 CFU/ml). Afterwards, the sterile filter paper discs of 6 mm diameter were placed on the plates surface and 10 µ l of rosemary of rosemary essential oil and ethanol extract was added on the discs. In

order to assess the antimicrobial activity of rosemary essential oil and ethanol extract, the 6 mm discs were cut and subjected to decontamination under ultraviolet light for 25 min. Streptomycin was used as positive control (10 µl/disc).

2.10. Statistical analyses

All extractions and determinations were conducted in triplicates and results were expressed based on dry weight (DW). Data are expressed as mean ± standard deviation. The means were compared by the one-way and multivariate analysis of variance followed by Duncan's multiple-range tests. The differences between individual means were deemed to be significant at $p < 0.05$.

3. Results and discussion

3.1. Essential oil composition

As can be shown in Table 1, the essential oil yield of rosemary leaves was 1.76 %. This essential oil yield was higher than that of Saudi Arabia (1.45 %) [13], Algeria (1.07 %) [14] and Spain (1.03 %) [15]. However, the highest essential oil yield was detected in Iranian rosemary reaching up 2.60 % [16]. GC-MS analyses of rosemary essential oil composition allowed the identification of 28 compounds representing 99.47 % of the total essential oils and including oxygenated monoterpenes (78.38 %), monoterpene hydrocarbons (18.38 %), sesquiterpene hydrocarbons (2.50 %) and oxygenated sesquiterpenes (0.20 %). The predominant compounds were 1,8-cineole (57.55 %), camphor (8.82 %), α-pinene (8.83 %), borneol (5.54 %) and camphene (2.36 %). As mentioned in the study of Lakušić et al. [17], rosemary essential oil composition of indigenous and cultivated plants in the Mediterranean area revealed the existence of monodominant and intermediate chemotypes. The most commonly recorded monodominant chemotypes are 1,8-cineole [5,8,13,18-24] and camphor chemotypes [18-21,25], verbenone [22,23,26], α-pinene [27,28], linalool [23] and *p*-cymene [29] chemotypes. Intermediate chemotypes 1,8-cineole/linalool [23], 1,8-cineole/camphor and 1,8-cineole/camphor/borneol [18] were also recorded for only single samples.

Table 1 Essential oil composition of *Rosmarinus officinalis* L

Compound	**RI**	**%**
Tricyclene	922	0.06±0.01
α-Thujene	931	0.11±0.03
α Pinene	939	8.83±1.45
Camphene	953	2.36±0.37
Sabinene	977	0.04±0.01
β Pinene	980	2.82±0.66
β Myrcene	986	0.89±0.16
α Phellendrene	1010	0.19±0.03
δ 3 Carene	1011	0.08±0.02
α Terpinene	1018	0.62±0.10
p-Cymene	1025	1.01±0.17
γTerpinene	1057	1.00±0.17
α Terpenolene	1088	0.34±0.06
1.8-Cineole	1034	57.55±3.84
Linalool	1068	0.77±0.18
trans Sabinene Hydrate	1087	0.10±0.04
D-Fenchyl alcohol	1110	0.05±0.01
Camphor	1145	8.82±1.60

Borneol	1167	5.54±1.40
4-Terpineol	1176	1.02±0.21
α Terpeneol	1189	3.68±0.37
Bornyl acetate	1301	0.82±0.08
Caryophyllene oxide	1580	0.20±0.01
α Copaene	1377	0.05±0.01
trans-Caryophyllene	1415	1.99±0.33
α Humulene	1455	0.26±0.03
Aromandendrene	1482	0.06±0.008
δ Cadinene	1526	0.12±0.03
Chemical class		
Monoterpene hydrocarbons		18.38±3.16
Oxygenated Monoterpene		78.38±12.72
Oxygenated Sesquiterpene		0.20±0.04
Sesquiterpene hydrocarbons		2.50±0,53
Total identified		99.47±16.46
Essential oil yeild (%)		1.76±0.30

RI : retention indice

3.2. Phenol contents

Phenolic contents of rosemary leaf extract are summarized in Table 2. Rosemary extract had an important amount of total phenolic content with 28.47 mg GAE/g DW. However, flavonoids and condensed tannins were weakly present in rosemary extract having 2.59 mg QE/g and 1.02 mg CE/g, respectively. Similar total phenol content (28.47 mg GAE/g DW) was obtained in comparison with results of Zaouali et al. (42) in the case of Tunisian rosemary (28.60 mg GAE/g DW). Rosemary total phenol content was only 10.42 mg GAE/g DW in Algeria [30] and 10.83 mg GAE/g DW in Zimbabwe [31]. Yeddes et al. [9] reported that the highest average amounts of Tunisian rosemary extracts were detected in summer for total phenols (38.92 mg GAE/g DW), flavonoids (2.98 mg QE/g DW) and condensed tannins (1.41 mg CE/g DW). In this work, HPLC quantification of the main phenolic compounds of rosemary extract allowed the detection of carnosic acid (58.71 mg /g) and rosmarinic acid (16.51 mg/g). Yesil-Celiktas et al. [32] reported that Turkey rosemary extract was also characterized by the predominance of carnosic acid (27.8-115.8 mg /g DW) and rosmarinic acid (14-30.40 mg/g DW). Yeddes et al. [9] reported that the amount of carnosoc acid was high in summer (138.86 mg/g DW) but it is in winter for rosmarinic acid (19.29 mg/g DW). These variations on phenolic, carnosic acid and rosmarinic acid contents could be due to rosemary plant variety and/or the environmental stress.

Table 2 Phenolic contents of rosemary leaf extracts

	Total phenols	**Flavonoids**	**Condensed tannins**	**Carnosic acid**	**Rosmarinic acid**
Content	28.47±1.03	2.59±0.11	1.02±0.09	58.71±0.25	16.51±1.13

TPC = mg of Gallic acid equivalents dry weigh (mg (GAE) /g DW); TFC = mg of Quercetin equivalents dry weigh (mg (QE) /g DW); TCT = mg of Catechin equivalents dry weigh (mg (CE) /g DW); CAC= mg CA/ mg DW; RAC= mg RA/ mg DW; Ethanol was the best solvent for the extraction of total phenols, flavonoids, condensed tannins and rosmarinic acid while ethyl acetate was the best for carnosic acid.

3.3. Antibacterial activity of rosemary essential oil

The evaluation of the antibacterial activity showed that rosemary essential oil possessed a significant bactericidal power. In fact, there was an inhibition of the growth of bacteria C. jejuni (ID = 16.75 mm), S. enterica (ID = 18.65 mm),

P. aeruginosa (ID = 19.50 mm), E. aerogenes (ID = 21.33 mm), and E. coli (ID = 26.00 mm) (Figure 1). These diameters were higher than those of the positive control (streptomycin). In contrast, the ID of B. subtilis (12.66 mm, S. aureus14.16 mm, and E. faecalis (10.16 mm) were lesser than streptomycin. According to Abd El Mageid et al. [33], rosemary essential oil from Egypt exhibited lower antibacterial activity than that obtained in our study for E. coli (ID = 13.76 mm). In addition, Bosnić et al. [34] reported that Bosnian rosemary essential oil has significant antibacterial activity against E. coli (ID = 13 mm) and *P. aeruginosa* (ID = 9 mm). Likewise, recent studies have shown that this antimicrobial activity was mainly due to the presence of high contents of 1,8-cineole [35] and camphor [36]. The antibacterial properties of essential oils from the leaves of *R. officinallis* is primarily determined by content of α-pinene [37].

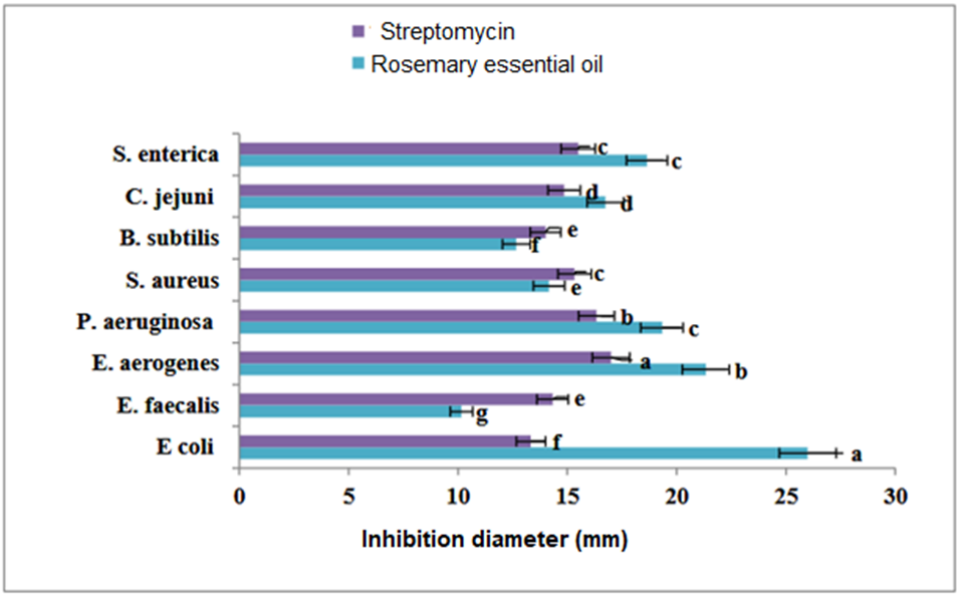

Figure 1 Antibacterial activity of rosemary essential oil The data marked with the different small letter share significant differences at *P*< 0.05 (Duncan test).

3.4. Antibacterial activity of rosemary extract

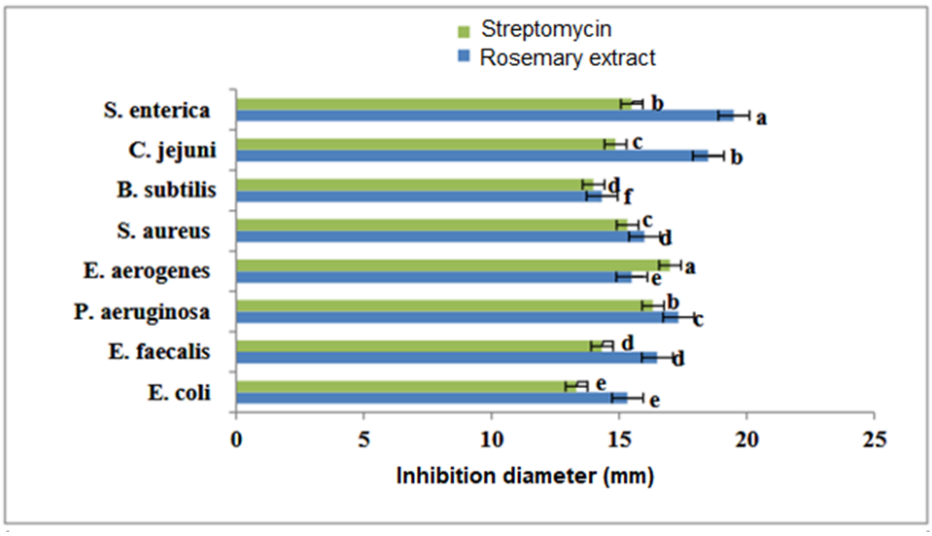

Figure 2 Antibacterial activity of rosemary ethanol extract The data marked with the different small letter share significant differences at P< 0.05 (Duncan test)

The antibacterial activity of rosemary ethanolic extract through the measurement of the inhibition diameters of bacterial growth showed that this extract exhibited significant antibacterial activity against C. jejuni (ID = 18.50 mm), S. enterica (ID = 19.50 mm), B. subtilis (ID = 14.33 mm), S. aureus (ID = 16.00 mm), P. aeruginosa (ID = 17.33 mm), and E. faecalis (ID = 16.50 mm) and E. coli (ID = 15.33 mm). Results showed that these ID of ethanolic extracts were higher

than streptomycin (ID = 17.00 mm) with the exception of E. aerogenes (ID = 15.50 mm) (Figure 3). According to Nieto et al. [38] and Bernardes et al. [39], the inhibitory effect of rosemary extract results from the action of rosmarinic acid, carnosic acid and their derivatives such as carnosol, rosmanol and isorosmanol. These compounds interact with the cell membrane of microorganisms by causing changes in genetic material and nutrients by modifying the transport of electrons, also causing leakage of cellular components and changes in the production of fatty acids hence the deterioration of the cell membrane. Likewise, Vegara et al. [40] reported that the efficacy of carnosic acid against pathogenic bacteria is superior to that of other major extract components, including rosmarinic acid.

4. Conclusion

The observed antibacterial activity of rosemary ethanolic extract and essential oil against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Campylobacter jejuni, Salmonella enterica, Bacillus subtilis* and *Enterococcus faecalis* considered as valuable steps toward doing clinical trials to use this plant in treating human infection.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare no conflict of interest.

References

[1] Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010; 74(3): 417–433.

[2] World Health Organization (WHO). WHO global strategy for containment of antimicrobial resistance. WHO Swizerland. 2001; 1-96.

[3] Hili P, Evans CS, Veness RG. Antimicrobial action of essential oils: the effect of dimethylsulfoxide on the activity of cinnamon oil. Lett Appl Microbiol. 1997; 24: 269-275.

[4] Lis-Balchin M, Deans SG Bioactivity of selected plant essential oils against Listeria monocytogenes. J Appl Bacteriol. 1997; 82: 759-762.

[5] Yeddes W, Nowacka M, Rybak K, Boruszewski P, Ostrowska-Ligeza E, Betlej I, Aidi Wannes W, Hammami M, Jallouli S, Horchani-Naifer K, Witrowa-Rajchert D, Saidani-Tounsi M. Effect of rosemary essential oil and ethanol extract on physicochemical and antibacterial properties of optimized gelatin-chitosan film using mixture design. J Food Process Preserv. 2021; 00: e16059.

[6] Rafie H, Soheila H, Grant E. Rosmarinus officinalis (rosemary): A novel therapeutic agent for antioxidant, antimicrobial, anticancer, antidiabetic, antidepressant, neuroprotective, anti-inflammatory and anti-obesity treatment. Biomed J Sci & Tech Res. 2017; 03(02): 1-6.

[7] Couto RO, Conceicao EC, Chaul LT, Oliveira EMS, Martins FS, Bara MTF, Rezende KR, Alves SF, Paula JR. Spray-dried rosemary extracts: Physicochemical and antioxidant properties. Food Chem. 2012; 131: 99-105.

[8] Yeddes W, Aidi Wannes W, Hammami M, Smida M, Chebbi A, Marzouk B, Saidani Tounsi M. Effect of environmental conditions on the chemical composition and antioxidant activity of essential oils from Rosmarinus officinalis L. growing wild in Tunisia. J Essent Oil Bear Pl. 2018; 21(4): 972-986.

[9] Yeddes W, Chalghoum A, Aidi-Wannes W, Ksouri R, Saidani Tounsi M. Effect of bioclimatic area and season on phenolics and antioxidant activities of rosemary (Rosmarinus officinalis L.) leaves. J Essent Oil Res. 2019; 31: 432-443.

[10] Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity J Agric Food Chem. 2002; 50: 3010-3014.

[11] Sun B, Richardo-da-Silvia JM, Spranger I. Critical factors of vanillin assay for catechin and proanthocyanidins. J Agric Food Chem. 1998; 46: 4267-4274.

[12] Gómez- Estaca J, López de Lacey A, López- Caballero ME, Gómez-Guillén MC, Montero P. Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiol. 2010; 27: 889-896.

[13] Guetat A, Al-Ghamdi FA, Osman AK. 1,8-Cineole, α-pinene and verbenone chemotype of essential oil of species Rosmarinus officinalis L. from Saudi Arabia. Int J Herb Med. 2014; 2: 137-141.

[14] Boutekedjiret C, Belabbes R, Bentahar F, Bessiere J. Study of Rosmarinus officinalis L. essential oil yield and composition as a function of the plant life cycle. J Essent Oil Res. 1999; 11: 238-240.

[15] Salido S, Altarejos J, Nogueras M, Saánchez A, Luque P. Chemical composition and seasonal variations of rosemary oil from southern Spain. J Essent Oil Res. 2003; 15: 10-14.

[16] Malekydozzadeh M, Khadiv-Parsi P, Rezazadeh S, Abolghasemi H, Salehi Z, Li Q. Application of multistage steam distillation column for extraction of essential oil of Rosmarinus officinialis L. Ir J Chem Eng. 2012; 9: 54-65.

[17] Lakušić D., Ristić M, Slavkovska V, Lakušić B. Seasonal variations in the composition of the essential oils of rosemary (Rosmarinus officinalis, Lamiaceae). Nat Pro communicat. 2013; 8(1): 131-134.

[18] Lakušić D, Ristić M, Slavkovska V, Šinžar-Sekulić J, Lakušić B. Environment-related variations of the composition of the essential oils of rosemary (Rosmarinus officinalis L.) in the Balkan Peninsula. Chem Biodivers. 2012; 9: 1286-1302.

[19] Zaouali Y, Messaoud C, Ben Salah A, Boussaid M. Oil composition variability among populations in relationship with their ecological areas in Tunisian Rosmarinus officinalis L. Flav Fragr J. 2005; 20: 512-520.

[20] Zaouali Y, Bouzaine T, Boussaid M. Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem Toxicol. 2010; 48: 3144-3152.

[21] Celiktas OY, Kocabas EEH, Bedir E, Sukan FV, Ozek T, Baser KHC. Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chem. 2007; 100: 553-559.

[22] Papageorgiou V, Gardeli C, Mallouchos A, Papaioannou M, Komaitis M. Variation of the chemical profile and antioxidant behavior of Rosmarinus officinalis L. and Salvia fruticosa Miller grown in Greece. J Agricult Food Chem. 2008; 56: 7254-7264.

[23] Varela F, Navarrete P, Cristobal R, Fanlo M, Melero R, Sotomayor AJ, Jordan JM, Cabot P, Sanchez de Ron D, Calvo R, Cases A. Varibility in the chemical composition of wild Rosmarinus officinalis L. Acta Horticult. 2009; 826: 167-174.

[24] Napoli EM, Curcuruto G, Ruberto G. Screening of the essential oil composition of wild Sicilian rosemary. Biochem Syst Ecol. 2010; 38: 659-670.

[25] Zaouali Y, Boussaid M. Isozyme markers and volatiles in Tunisian Rosmarinus officinalis L. (Lamiaceae): A comparative analysis of population structure. Biochem Syst Ecol. 2008; 36: 11-21.

[26] Mata AT, Proença C, Ferreira AR, Serralheiro MLM, Nogueira JMF, Araujo MEM. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. Food Chem. 2007; 103: 778-786.

[27] Napoli EM, Curcuruto G, Ruberto G. Screening of the essential oil composition of wild Sicilian rosemary. Biochem Syst Ecol. 2010; 38: 659-670.

[28] Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M, Dessi S, Coroneo V, Cabras P. Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of Rosmarinus officinalis L. J Agricult Food Chem. 2004; 52: 3530-3535.

[29] Özcan MM, Chalchat JC. Chemical composition and antifungal activity of rosemary (Rosmarinus officinalis L.) oil from Turkey. Int J Food Sci Nutr. 2008; 59: 691-698.

[30] Zeghad N., Merghem R. Antioxidant activity offlavo-noids isolated from Rosmarinus officinalis L. J Plant Sci Res. 2016; 3: 2349-2805.

[31] Muchuweti M, Kativu E, Mupure CH, Chidewe C, Ndhlala AR, Benhura MAN. Phenolic composition and antioxidant propreties of some species. Am. J. Food Technol. 2007; 2: 414-420.

[32] Yesil-Celiktas O, Girgin G, Orhan H, Wichers HJ, Bedir E, Vardar-Sukan F. Screening of free radicals cavenging capacity and antioxidant activities of Rosmarinus officinalis extracts with focus on location and harvesting times. Eur Food Res Technol. 2007; 224: 443-451.

[33] Abd El Mageid MM, Helmy SA, Mohamed NS. Evaluation of rosemary essential oil for its chemical constituents, antimicrobial, antioxidant and antidiabetic activities. Food Sci Technol. 2010; 1-20.

[34] Bosnić T, Softić D, Grujić-Vasić J. Antimicrobial activity of some essential oils and major constituents of essential oils. Acta Med Acad. 2006; 35: 19-22.

[35] Kifer D, Mužinić V, Klarić MŠ. Antimicrobial potency of single and combined mupirocin and monoterpenes, thymol, menthol and 1, 8-cineole against Staphylococcus aureus planktonic and biofilm growth. J Antibiot. 2016; 69(9): 689.

[36] Zainudin Z, Mohd Azim MK. Study on the antibacterial and antifungal effects of camphor oil; Cinnamomum camphora with emphasis on the control of overpopulation of tilapia. Int J Fish Aquat Stud. 2015; 2(5): 337-340.

[37] Ngân TTK, Hường NC, Le X, Long P, Toan TQ, Vo DMH, Danh VT, Trung LNY, Trieu TA. Physico-chemical characteristics of Rosmarinus officinalis L. essential oils grown in Lam Dong Province, Vietnam. Asian J Chem. 2019; 31(12): 1-4.

[38] Nieto G, Ros G, Castillo J. Antioxidant and antimicrobial properties of rosemary (Rosmarinus officinalis L.): A review. Med. 2018; 5(3): 98.

[39] Bernardes WA, Lucarini R, Tozatti MG, Souza MG, Silva ML, Filho AA, Martins CH, Crotti AE, Pauletti PM, Groppo M, Cunha WR. Antimicrobial activity of Rosmarinus officinalis against oral pathogens: relevance of carnosic acid and carnosol. Chem Biodivers. 2010; 7(7): 1835-1840.

[40] Vegara S, Funes L, Martí N, Saura D, Micol V, Valero M. Bactericidal activities against pathogenic bacteria by selected constituents of plant extracts in carrot broth. Food Chem. 2011; 128: 872-877.