Chemical Composition and Antibacterial Activity of Essential Oil of Origanum Compactum Against Foodborne Bacteria

Houda Sbayou [1], Nadia Oubrim [2], Brahim Bouchrif [2], Bouchra Ababou,[3]
Khadija Boukachabine [3],Souad Amghar [1]

1: Laboratory of agrofood and health, Faculty of sciences and technologies, University Hassan 1er, Settat Km 3, B.P: 577. Morocco.
2: Laboratory of Microbiology and Hygiene of food and environment. Pasteur Institute. 1, Place Louis Pasteur, Casablanca. Morocco,
3: Laboratory of environmental sciences and development, Faculty of sciences and technologies, University Hassan 1er, Settat Km 3, B.P: 577 Morocco

Abstract

In the present study, chemical composition of essential oil of *Origanum compactum* was identified by gas chromatography coupled to mass spectrometry (GC-MS). Among twenty six constituents, carvacrol (43.97%), p-cymene (17.87%) and thymol (11.56%) were the major components. The antibacterial activity was tested by disc diffusion method and micro-broth dilution. The EO showed strong antibacterial activity against all of the tested *Salmonella* spp. Strains, with minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) ranging from 0.3125 µL/mL to 0.625 µL/mL.

Key words: *Origanum compactum* – GC / MS – foodborne bacteria – antibacterial activity.

1. Introduction

Foodborne diseases comprise a broad spectrum of illnesses and are responsible for substantial morbidity and mortality worldwide. It is a growing public health problem in developing as well as developed countries. Foodborne illnesses result from consumption of food containing pathogens such as bacteria, viruses and parasites [1]. Therefore, there is a need for new methods of reducing or eliminating foodborne pathogens. Among many strategies to inhibit the growth of undesirable microorganisms, is the use of chemical agent exhibiting antimicrobial activity. These chemicals may be either synthetic compounds intentionally added to foods or naturally occurring biologically derived substances [2]. Natural plant extracts are gaining a wide interest in food industry for their antibacterial, antifungal, and antioxidant properties [3, 4, 5, 6].

The genus *Origanum* (Lamiaceae family) is among the most important aromatic plant worldwide. Forty-nine taxa divided into 10 sections belong to this genus, most of them having a very local distribution around the Mediterranean [7]. *Origanum compactum* is an endemic specie growing in the north of Morocco, where it is traditionally used in culinary and medical preparations [8]. *Origanum* species have recently been of great interest, in both academia and the food industry as potential natural additives, to replace synthetic products [9]. Effective antimicrobial doses may exceed organoleptically acceptable levels. Therefore, there is an increasing demand for accurate knowledge of the minimum inhibitory concentration (MIC) of essential oils to establish a balance between the sensory acceptability and antimicrobial efficacy [10].

In the present study, we describe the chemical composition of essential oil obtained from *Origanum compactum* grown in Morocco, and then we investigate the efficacy of the oil against foodborne bacteria.

Material and methods:

1. Essential oil:

Origanum compactum samples were collected during the months of April, May, June and July 2009 in Taounat. The Essential oil was produced by Santis Company, It was extracted by steam distillation from flowers, leaves and stems.

2. Bacterial strains:

A total of five strains of *Salmonella* spp. were included in this study. These strains were obtained from Laboratory of Microbiology and Hygiene of food and environment, Pasteur Institute of Morocco. They were isolated from minced meat and dairy products. *Salmonella* cultures were grown at 37° C for 18 h in Muller Hinton Broth, and then adjusted to 10^6 CFU/mL.

3. Gas chromatography / mass spectrometry (GC/MS):

The chromatographic analysis of essential oils was performed with a gas chromatograph (Trace GC Ultra) coupled to a mass spectrometer (Polaris Q ion trap MS), with a VB-5 capillary column (Methylpolysiloxane with 5% phenyl; 30 m x 0.25 mm; film thickness 0.25µm). Fragmentation was performed by electron impact at 70 eV. Helium (1.4 mL/min) was used as carrier gas. Split-type injector was heated to a temperature of 200 ° C. The volume injected was 1µl. The column was initially maintained at a temperature of 40 ° C for 2 min, increased to 180 ° C at a rate of 4 ° C / min, and finally raised to 300 ° C for 2 min at 20 ° C / min.

4. Disc diffusion method:

The determination of the inhibitory effect of EO on test bacteria was carried out by the disc diffusion method [11]. Sterile Paper disk (6 mm in diameter) were impregnated with 10µL of essential oil and transferred into the Luria Bertoni Agar present in Petri dishes, which had previously seeded by spreading 1mL of bacterial suspension adjusted to 10^6 CFU/mL. Standard antibiotics amoxicillin (25 µg) were used as positive control. After incubation at 37°C during 24h, the diameters of inhibition zones were measured in millimeters. Tests were carried out in triplicate.

5. Determination of MIC and MBC:

MIC was determined in this work by the method of micro-broth dilution [12]. A serial of dilution of essential oil ranging from 20 µL/mL to 0.15 µL/mL were prepared in test tubes containing Broth Luria Bertoni medium with 0.15% Agar. Each tube was inoculated with the same volume of bacterial suspension adjusted to 10^6 CFU/mL. The tubes were then incubated at 37° C for 18 h. The MIC was defined as the lowest concentration of the essential oil at wich the microorganism did not show visible growth. The microorganism growth was indicated by the turbidity of the culture. To determine the minimum bactericidal concentration (MBC), broth was taken from each tube, spread on Muller Hinton Agar (MHA) and incubated at 37°C for 24h. The MBC was defined as the lowest concentration of essential oil at wich the incubated microorganism was completely killed. Each assay was repeated thrice.

Results and Discussion:

1. Chemical composition:

The percentage composition of the oil of *Origanum compactum* is presented in table 1. Twenty six components were identified, accounting for 99.37% of the oil.

Table1: Chemical composition of EO of *Origanum compactum*

Pic	Constituents	Retention time	Area %
1	α-Phellandrene	7.63	0.46
2	3-Carene	7.81	0.80
3	Camphene	8.26	0.17
4	1R-α-Pinene	9.22	0.11
		9.91	0.21
5	3-Carene		
6	α-Phellandrene	10.26	0.12
7	α-Terpinene	10.69	1.15
8	p-cymène	10.96	17.87
9	Bicyclo[3.1.1]hept-3-ene, 2-formylmethyl-4,6,6-trimethyl-	11.13	1.80
10	ç-Terpinene	12.20	8.43
11	3-Carene	13.73	0.45
12	α-CAMPHOLENE ALDEHYDE	15.08	0.17
13	Nopol	15.91	0.23
14	trans-Sabinene hydrate	16.37	0.29
15	3-Carene	16.88	0.23
16	2-Isopropyl-1-methoxy-4-methylbenzene	18.76	0.24
17	Thymol	20.45	11.56
18	Carvacrol	20.78	43.97
19	Caryophyllene	24.38	1.85
20	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	27.25	0.12

21	α-Guaiene	27.58	0.15
22	Cholic acid	29.23	0.15
23	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	32.09	0.16
24	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	43.66	4.80
25	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	43.84	2.22
26	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	44.16	1.66
Total			99.37%

The analysis showed that carvacrol (43.97%) was the main component in the oil of *Origanum compactum*. Other major components were identified as p-cymene (17.78%) and thymol (11.56%). Several studies have shown the predominance of carvacrol as major component of the *Origanum compactum* essential oil. In fact, samples from Morocco analyzed by Charai and Chebli contain carvacrol at concentrations of 49.5% and 58.1% respectively [13, 14, 15]. In 2003, Jeannot studied hydrolat of *Origanum compactum* from Morocco, and after ether extraction shows predominance of carvacrol (55-76%) [16]. Recently, Nhu-Trang quantified in essential oil from Morocco significant levels of carvacrol (60%) [17].

1. Antibacterial activity:

The antibacterial activity of the EO of *Origanum compactum* against *Salmonella* spp. Strains examined in the present study and their potency was assessed by the inhibition zone diameter and MIC values. The results are in Table 2.

Table 2: Diameter of inhibition zone (mm), MIC and MBC values:

Microorganism species	**Inhibition zone diameter in (mm)**		**MIC (µL/mL)**	**MBC (µL/mL)**
	Origanum compactum	**Positive control (AMX)**		
Salmonella spp. 1	30 ± 0	21 ± 0	0.3125	0.3125
Salmonella spp. 2	30 ± 0	0 ± 0	0.3125	0.625
Salmonella spp. 3	30 ± 0	18 ± 0	0.625	0.625
Salmonella spp. 4	32.5 ± 2.12	18 ± 0	0.3125	0.3125
Salmonella spp. 5	34.5 ± 2.12	18 ± 0	0.3125	0.3125

The inhibition zones and MIC values for *Salmonella* spp. Strains were in the range 30-34.5 mm and 0.3125-0.625 µL/mL respectively. The largest inhibition zones and the highest inhibitory activity produced by the EO were observed with strain *Salmonella* spp.5. Mostly, the MBC values were equivalent to the MIC values, confirming the bactericidal effect of EO of *Origanum compactum*.

The high antibacterial activity of EO tested can be attributed to the presence of high concentration of carvacrol, which is widely reported to possess high levels of antimicrobial activity [10, 18, 19, 20, 21, 22].

Approved as a safe food additive in the USA and Europe [23, 24], carvacrol has attracted considerable attention as a result of its wide spectrum antimicrobial activity. However, the compounds present in the greatest proportions are not necessarily responsible for the total activity; the involvement of less abundant constituents should be also considered [25]. Therefore, the activity could be attributed to the presence of other components such as p-cymene and thymol also known to possess an antibacterial activity [26]. Moreover, possible antagonistic and synergistic effects may occur between the oil components depending on the microorganism tested [27].

Acknowledgment:

We thank Santis Company for the essential oil.

Conclusion:

In this study, the chemical composition and antibacterial activity of the EO of *Origanum compactum* from Taounat (Morocco) were investigated. The quantitative data from GC-MS showed that the major components presented in EO were carvacrol (43.97%), p-cymene (17.87%) and thymol (11.56%). The EO has a significant antibacterial activity against all strains of *Salmonella* spp. tested. Our results suggest that the EO of *Origanum compactum* may act as an alternative to synthetic bactericides for use in food industry, where bacterial pathogens like *Salmonella* cause several destructions. However, further investigations are necessary to determine the critical toxicity level of this oil.

References:

[1]: World Health Organization [WHO]. (2011c). Initiative to estimate the Global Burden of Foodborne Diseases: Information and publications. Retrieved June 26, 2011, from http://www.who.int/foodsafety/foodborne_disease/ferg/en/index7.html

[2]: Mahassine Amensour, Samira Bouhdid, Juana Fernández-López, Mohamed Idaomar, Nadia Skali Senhaji & Jamal Abrini, Antibacterial Activity of Extracts of *Myrtus communis* Against Food-Borne Pathogenic and Spoilage Bacteria, International Journal of Food Properties, (2010), 13:6, 1215-1224.

[3]: Klein G, Rüben C, Upmann M, Antimicrobial activity of essential oil components against potential food spoilage microorganisms, Curr Microbiol, (2013), 67 (2), 200-208.

[4]: Cakir, A., Kordali, S., Zengin, H., Izumi, S. and Hirata, T, Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. Flavour Fragr. J., (2004), 19: 62–68. doi: 10.1002/ffj.1279.

[5]: Snu-Yao Tsai, Shih-Jeng Huang, Charng-Cherng Chyau, Ching-Hsuan Tsai, Chu-Chun Weng and Jeng-Leun Mau Composition and Antioxidant Properties of Essential Oils from *Curcuma* rhizome Asian Journal of Arts and Sciences, 2011, *Vol. 2, No. 1, pp. 57-66,*.

[6]: Belma Aslim, Nihal Yucel, *In vitro* antimicrobial activity of essential oil from endemic *Origanum minutiflorum* on ciprofloxacin-resistant *Campylobacter* spp., Food chemistry, (2008) 107 (2), 602-606.

[7]: S. Kokkini, in: Proceedings of the IPGRI InternationalWorkshop on Oregano, 1996, pp. 2–12.

[8]: Bellakhdar, J., La Pharmacopée marocaine traditionnelle. Editions Le Fennec, Casablanca, Maroc, (1997), 348–349.

[9]: Tepe, B., Daferera, D., Sokmen, M., Polissiou, M., & Sokmen, A, The in vitro antioxidant and antimicrobial activities of the essential oil and various extracts of Origanum syriacum L var bavanii, Journal of the Science of Food Agriculture, (2004), 84, 1389–1396.

[10]: Lambert, R. J. W., Skandamis, P. N., Coote, P. J. & Nychas, G. J. E, A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol, (2001), 91, 453–462.

[11]: National Committee for Clinical Laboratory. Standards methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Wayne Pa., (1995) vol 15, p. 15 (approved stansards no. M7 –A3).

[12]: H. Ismaili, L. Milella, S. Fkih – Tetouani, A. Ilidrissi, A. Camporese, S. Sosa, G. Altinier, R.D. Loggia, and R. AAquino, In vivo topical anti-inflamatory and in vitro antioxidant activities of two extracts of thymus satureioides leaves, J. Ethnopharmacol, (2004) 91, 31 – 36.

[13]: CHARAI M., MOSADDAK M. and FAID M., Chemical composition and antimicrobial activities of two aromatic plants : Origanum majorana L. and

O. compactum Benth., J. Essent. Oil Res., (1996) 8, pp. 657-664.

[14]: CHARAI M., FAID M., and CHAOUCH A ., , Essential oils from aromatic plants (Thymus broussonetti Boiss., O. compactum Benth. and Citrus limon (L.) N.L. Burm.) as natural antioxidants for Olive oil, J. Essent. Oil Res., (1999) 11, pp. 517-521.

[15]: BOUCHRA C., ACHOURI M., IDRISSI HASSANI L.M. and HMAMOUCHI M., Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against Botrytis cinerea Pers: Fr., Journal of Ethnopharmacology, (2003), 89, pp. 165-169.

[16]: JEANNOT V., Origanum compactum BENTHAM: Composition of the Hydrolat Aromatic Fraction, Comparaison with the Essential Oil and its Interest in Aromatherapy, The International Journal of Aromatherapy, (2003), 13, pp. 90-94.

[17]: NHU-TRANG T-T, CASABIANCA H and GRENIER-LOUSTALOT M.F., Deuterium/hydrogen ratio analysis of thymol, carvacrol, gamma-terpinene and p-cymene in thyme, savory and oregano essential oils by gas chromatography-pyrolysis-isotope ratio mass spectrometry, Journal of Chromatography A, (2006), 1132, pp. 219-227.

[18]: Ben Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N. & Chalier, P, Antimicrobial activity of carvacrol related to its chemical structure, Lett Appl Microbiol, (2006), 43, 149–154.

[19]: Chami, F., Chami, N., Tennis, S., Trouillas, J. & Remmal, A., Evaluation of carvacrol and eugenol as prophylaxis and treatment of vaginal candidiasis in an immunosuppressed rat model, J Antimicrob Chemother (2004), 54, 909–914.

[20]: Helander, I. M., Alakomi, H.-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., Gorris, L. G. M. & von Wright, A., Characterization of the action of selected essential oil components on Gram-negative bacteria, J Agric Food Chem, (1998), 46, 3590–3595.

[21]: Ultee, A., Gorris, L. G. & Smid, E. J., Bactericidal activity of carvacrol towards the food-borne pathogen Bacillus cereus, J Appl Microbiol, (1998), 85, 211–218.

[22]: Veldhuizen, E. J. A., Tjeerdsma-van Bokhoven, J. L. M., Zweijtzer, C., Burt, S. A. & Haagsman, H. P., Structural requirements for the antimicrobial activity of carvacrol, J Agric Food Chem, (2006), 54, 1874–1879.

[23]: Center for Food Safety and Applied Nutrition (2006). EAFUS: a Food Additive Database. Washington, DC: US Food and Drug Administration, Center for Food Safety and Applied Nutrition.

[24]: Commission of the European Communities (1999). Commission Decision of 23 February 1999 Adopting a Register of Flavouring Substances Used in or on Foodstuffs Drawn Up in Application of Regulation (EC) No. 2232–96 of the European Parliament and of the Council of 28 October 1996 (Notified under Number C(1999) 399) (Text with EEA Relevance) (1999/217/EC). Official Journal of the European Communities L084, pp. 1–137. Brussels, Belgium: Commission of the European Communities.

[25]: Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ, Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo, J. Ethnopharmacol, (2002), 79: 213-220.

[26]: Bagamboula CF, Uyttendaele M, Debevere J., Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*, Food Microbiol, (2004) 21: 33-42.

[27]: Cox, S.D., Mann, C.M. and Markham, J.L., Interactions between components of the essential oil of Melaleuca alternifolia, J Appl Microbiol, (2001), 91, 492–497.