Research Article

Chemical Composition and Biological Activity of Essential Oil of Mandarin (*Citrus reticulata)* Cultivated in Algeria.

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ABSTRACT

In the present study, the volatile compounds of *Citrus reticulata* were detected and identified by GC–MS and FTIR analysis. GC–MS allowed us to identify 24 volatile compounds and indicated that the main compounds constituting the volatile oil were mainly Limonene (67.04 %), γ-Terpinene (15.50 %) and α-Pinene (2.75 %), this compounds were also identified by FTIR analysis. The essential oil was also subjected to a biological screening for its possible antioxidant effect by means of DPPH radical scavenging test; the sample tested showed slight antioxidant activity in comparison with the positive control (Ascorbic acid). *Citrus reticulata* essential oil was examined also against a panel of 16 bacterial strains using the agar diffusion method. The obtained results showed that the essential oil exhibited moderate to strong antimicrobial activity against the tested microorganisms. These results suggested that the *Citrus reticulata* essential oil possesses a good antimicrobial and antioxidant properties.

Keywords: *Citrus reticulata,* Essential oil, Antimicrobial Activity, Antioxidant Activity.

INTRODUCTION

Citrus plants are well-known crops all over the world with potential socio-economic influence. They are well-known for their flavor, nutritional value and medicinal features. The medicinal activities for this genus are attributed to the presence of many medicinally active secondary metabolites such as essential oils[1].

Citrus essential oils have been applied in many products, such as cosmetics, medical formulation, beverages and foods, as flavoring agents as well as for aromatherapy. They are also used for their germicidal, antioxidant and anticarcinogenic properties[2].

Mandarins are a diverse group of thin-skinned, easy-peeling fruit that includes popular citrus types such as Satsumas, Clementines and Tangerines. Mandarins are becoming increasingly popular with consumers, largely due to the ease with which they can be eaten as compared to other types of citrus that are more difficult to peel[3].

The purpose of the present work was to evaluate the antioxidant and antimicrobial activities of *Citrus reticulata* essential oil cultivated in Algeria. And relate them with their chemical composition, for further application in food and pharmaceutical industries as natural valuable products.

MATERIALS AND METHODS

Plant Material

1500 g of the tested sample; ripe fruit peel, was obtained from a private farm located in the region in Azzaba (Skikda city, North-East of Algeria). Plant harvesting was carried in November 2016. The identity of the plant was confirmed by Nadia Amoura Prof. of Pharmacognosie, Department of pharmacy, Faculty of medicine, University of Badji Mokhtar, Annaba, Algeria.

The upper part of the pericarp was harvested from fresh fruit, this choice was justified by the richness of zest in essential oil compared to other parts of the fruit[4].

Isolation of the Essential Oil

Obtaining essential oil was carried out by cold expression (physical process), it's the simplest processes applied only to citrus fruit[5], this extraction does not change the composition of the oil[6]. The product obtained is called gasoline, because it does not undergone any chemical modification [5,7]. The obtained essential oil was stocked at 4 °C until further analyses.

Chromatography Analysis

The GC-MS analysis was performed using a Hewlett Packard 5973-6800 system operating in EI mode (70 eV) equipped with a split/splitless injector (250°C), a split ratio 1/50, using a fused silica HP-5 MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 µm. The temperature program for the HP-5 MS column was from 60°C to 280°C at a rate of 2°C/min. Helium was used as a carrier gas at a flow rate of 0.5 ml/min. Injection volume of the sample was 0.2 µl. The identification of the components was conducted in an IS system managing a library of spectrum wiley7n.l.

FTIR Analysis

The Fourier transformed infrared spectroscopy (FTIR) is realized using a Perkin Elmer apparatus (Universal ATR Sampling Accessory), the range of analysis was 4000-600 cm^{-1}. Results are compared with the internal Euclidean bibliography of the device.

Antioxidant Activity

The antioxidant activity of Mandarin essential oil was measured in terms of hydrogen-donating or radical scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent Burits and Bucar (2000)[8]. Fifty microliters of each concentration (200, 400, 600, 800 and 1000 µg/ml) of each sample was diluted in methanol and were added to 5 ml of methanolic solution of DPPH (0,004 %). The inhibition of the DPPH by the ascorbic acid was even analyzed at the same concentration for comparison. Absorbance measurements were read at 517 nm, after 20 min of incubation time at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. All determinations were performed in triplicate. The inhibition percentage of the DPPH radical by the samples was calculated according to the formula presented by Sharififar et *al*., (2007)[9]:

% inhibition = ($[A_{White} - A_{Sample}]/A_{White}$) where:

A_{White}: the absorption of the blank sample (t = 0 min).

A_{Sample}: the absorption of the tested oil or substance solution (t = 20 min).

The kinetic reactions of the essential oil and the ascorbic acid with DPPH, were mentioned for every concentration tested[8]. The essential oil and the ascorbic acid of the inhibited DPPH, were recognized at the end of the reaction in order to reach the index IC_{50}. This parameter is defined as the antioxidant concentration required to reduce the concentration of the initial DPPH of 50 %.

Time balance Determination of TEC_{50}

TEC_{50} parameter was defined when time reached balance with an antioxidant concentration equal to IC_{50}. This time is graphically calculated.

Determination of the Antiradical Efficiency (AE)

The two factors IC_{50} and TEC_{50} were combined in order to get the Anti-radical efficiency:

$AE = AA/IC_{50} x TEC_{50}$ Statistics Analysis

The classical methods of statistics were used to calculate the average and the standards deviations. All measurements were performed in triplicate, and results were presented as an average ± standard deviation. Analyses of variation were realized by ANOVA with the software «SPSS». The probability of p inferior to 0.05 was admitted as a criterion of a significant difference.

Antimicrobial Activity

In this study 16 different bacteria were used. The bacterial group included 10 Gram-negative bacteria, namely *Klebsiell pneumanae carbapinémase* (+): KPC+, *Klebsiell pneumanae carbapinémase* (-): KPC -, *Salmonella* S 226, *Acinetobacter* S 239, *Proteus mirabilis* S 120, *Shigella spp* S 241, *Citrobacter freundii* CF S 208, *Serratia sp, klebsiell oxytoca* S 113, *Entérobacter aerogenes* S 214 and 06 Gram-positive bacteria namely *Streptococcus faecalis* SFL S 156, *Staphylococcus sp* SCN 128, *Staphylococcus aureus* ATTC 23, *Staphylococcus aureus* S 178, *Entérobacter faecalis* EFS 154, *Entérobacter faecalis* ATTC 29. All microorganisms were obtained from the Bacteriology Laboratoy, Faculty of Medicine, HCU of Dorban in Annaba (Algeria).

The antibacterial activities were carried out using the disc diffusion method on solid medium; the strains were reactivated using a 20 h culture growth at 37 °C and adjusted to 10^8 CFU/mL. The bacterial strains are sowed on the surface of the agar in radial spots form by means of swab and suspensions of young bacterial cultures prepared according to the CLSI (committee for laboratory standards institute[10]). The application is made by sterile filters paper disks (6 mm diameter, 06/limp) which were placed on the inoculated agar surfaces and impregnated with 10 µL of the solution; the plates were incubated during 24 h at 37 °C[11]. The reading of the results is made by the measurement of the inhibition diameter around the disk.

RESULTS AND DISCUSSION

Essential Oil Yield and Chemical Composition

The obtained essential oil yielded 0,88% (v/w), the same yield was noticed in the sample collected from Guelma city (Algeria), by Bouguerra et *al*., (2014)[12], where it was reported that essential oil *Citrus reticulata* yielded from 0,88±0,8 %.

The physico-chemical properties have been measured as follows optical density: 0.850[13] and refractive index: 1.475[14].

Literature survey on the chemical constituents of *Citrus reticulata* essential oil revealed a great variability, which may have been due to several factors, among the geographical location, season and environmental factors, as well as the part of the plant used and extraction method.

The chromatographic analyses resulted in the identification of 24 compounds, representing (95.41 %) of the essential oil, Limonene (67.04 %), γ-Terpinene (15.50%) and α-Pinene (2.75 %) were the major components. These results were not in agreement with previous research reported for the same plant.

The chemical constituents of peel and leaf essential oils of 15 species of mandarins among 41 varieties of *Citrus reticulata* were investigated (Lota et *al*., 2000, 2001)[15,16].

The mandarin peel essential oil was reported to have two major chemotypes, limonene and limonene/γ-terpinene. The leaf oil showed variation in components and distinguished for peel oils with three major chemotypes: sabinene/linalool, linalool/γ-terpinene and methyl Nmethylanthranilate (Lota et *al*., 2000)[15]. Sesqui terpenes were hardly spotted in these species (Sawamura et *al*., 2004)[17].

The essential oil isolated by hydro-distillation from the peel of fully matured ripen fruits of *Citrus reticulata* Blanco were analyzed by GC and GC–MS. Thirty-seven different components were identified constituting approximately 99% of the oil. The major components were limonene (46.7%), geranial (19.0 %), neral (14.5 %), geranyl acetate (1.1 %) (Chutia et *al*., 2009)[18].

Limonene contributes to the aromatic odor of the oil and hence the plant belongs to the limonene chemotype. Some other compounds were linalool (0.7 %), 6-methyl-5 hepton-2 one (0.7 %), decanol (0.6 %), b-bisobolene (0.6 %) etc. These compositions of *Citrus reticulata* significantly vary from the other studies reported earlier (Sawamura et *al*., 2004)[17].

Limonene, neral and geranial were the major oil components of four different varieties of *Citrus sinensis* essential oils (Sawamura et *al*., 2005)[19]; while b-pinene and gterpinene were completely absent in *Citrus reticulata*. Minh Tu *et al*. (2002)[20] observed maximum limonene (95.1%) content in *Citrus reticulata* Blanco var.

Table 1: Chemical composition of *Citrus reticulata* essential oil.

No.	Compounds	Retention time (min)	%
1.	α-Thuyene	6.046	0.04
2.	**α-Pinene**	7.686	**2.75**
3.	Sabinene	9.606	1.49
4.	Myrcene	9.874	0.44
5.	α-Terpinene	11.128	0.26
6.	**Limonene**	12.547	**67.04**
7.	β -phellandrene	12.658	0.40
8.	**γ-Terpinene**	13.919	**15.50**
9.	p- Cymene	14.584	0.74
10.	Octanal	15.040	0.75
11.	Decanal	23.109	0.27
12.	Linalol	24.802	0.31
13.	Terpinene-4-ol	27.021	0.24
14.	l-Caryophyllene	27.183	0.27
15.	α- Terpinenol	30.501	0.35
16.	N-N-butylpyrrole	41.026	0.72
17.	l-Caryophyllene	43.136	0.77
18.	DimethylAnthranilate	43.332	0.42
19.	Germacrene-d	43.725	0.47
20.	Thymol	46.666	0.23
21.	δ-muurolen	46.751	0.67
22.	β-Cubebene	47.048	0.65
23.	Copaen	47.764	0.43
24.	2-isopropyl-5metylphenol	48.105	0.20
	Total		**95.41**

Antioxidant Activity

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogendonating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[21]. The scavenging ability of the essential oil and positive control are presented in Table 2.

Table 2: DPPH scavenging activity (%) of *Citrus reticulata* essential oil and standard antioxidant.

Concentrations (µg/ ml)	*Citrus reticulata* essential oil	Ascorbic acid
200	14,64±0 ,040	26,58±0 ,077
400	15,51±0 ,044	32,27±0 ,097
600	17,39±0 ,048	51,89±0 ,155
800	25,03±0 ,084	71,77±0 ,217
1000	27,65±0 ,092	94,93±0 ,286

Kinetic Reaction

The kinetic reactions of the free radical DPPH obtained for each concentration of the ascorbic acid and of the essential oil are mentioned in Figure 1 and 2.

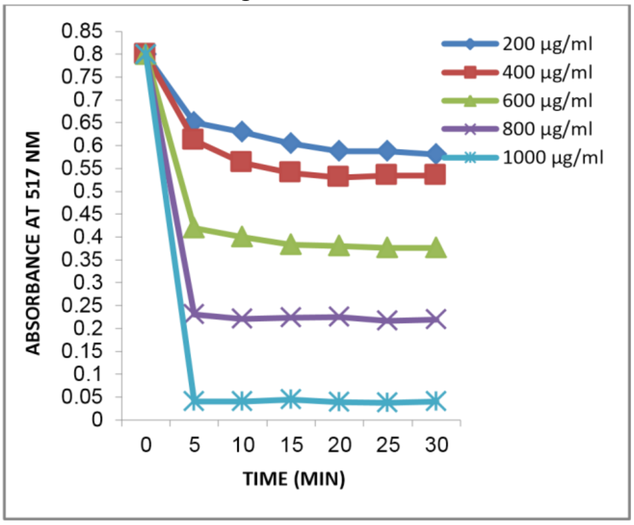

Figure 1: Kinetic reduction of DPPH of ascorbic acid.

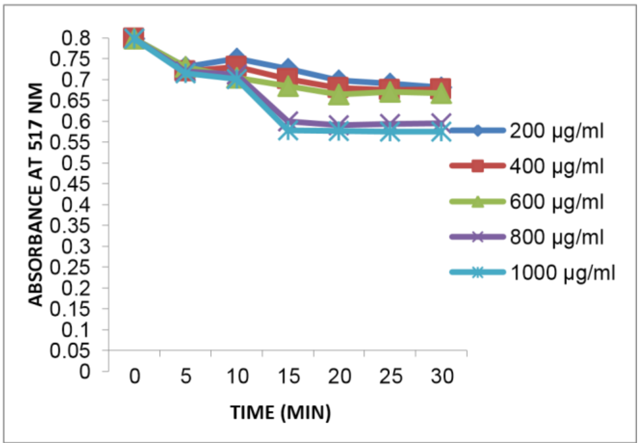

Figure 2: Kinetic reduction of DPPH of *Citrus reticulata* essential oil.

From the results obtained, we found that the biphasic reaction has a quick weakening of absorbance during the first minutes, followed by a slow step till balance is reached, so we can distinguish two areas: area of strong kinetic of DPPH radical scavenging with radical scavenging absorbed during the first 5 minutes, as for the ascorbic acid for all concentrations during ten minutes for a concentration of 1000 µg/ml. This area is observed during the first fifteen minutes for the essential oil. A second area with a slow kinetic of DPPH radical scavenging a tendency zone towards a recognized balance after five minutes for all concentrations of the ascorbic acid except the concentration 1000 µg/ml. For the essential oil of this area, it is recognized after fifteen minutes.

While making the reaction between DPPH and the ascorbic acid with hydrogen, we can recognize in this reaction that balance is reached in a short period of time compared to the essential oils. The antioxidant activity is dependent to the hydrogen atom movement of the hydroxyl group of the phenolic components of the essential oil. In presence of the free radical DPPH, the H atom is transferred to DPPH stable molecule. This induces a diminution in the concentration of the free radical and the absorbance during reaction till the weakening of the antioxidant capacity as a hydrogen donor.

The inhibition percentage results of the radical DPPH are mentioned in Figure 3.

We observe that the inhibition percentage of the free radical of the essential oil is low to those of the ascorbic acid for all concentrations used.

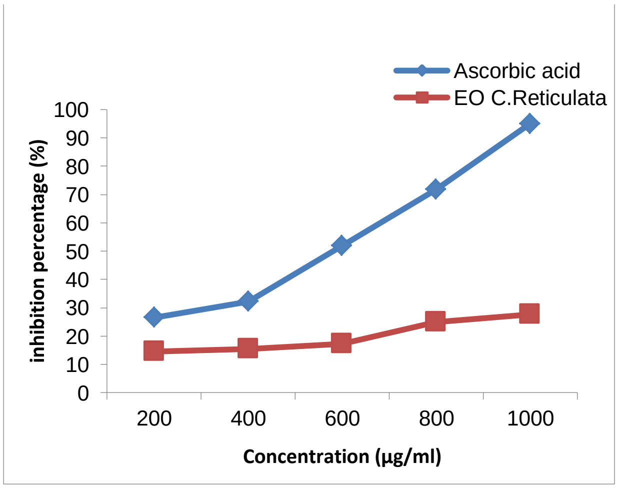

Figure 3: The inhibition percentage of essential oil and ascorbic acid.

We observe that the inhibition percentage of the free radical of the essential oil is low in comparison to those of the ascorbic acid for all concentrations used. For the highest concentration (1000 µg/ml), the essential oil revealed an inhibition percentage of 27,65 %, while the ascorbic acid is inhibited with 94,93 % of DPPH[22].

IC_{50} Determination

The IC_{50} is the quantity of the antioxidant needed to reduce the concentration of the free radical DPPH to 50 %. We have chosen the state of balance as a period of measurement where growth reaction can't go further. Timing of balance state depends on the reactivity of the essential oil and the concentrations used. We recognize that the ascorbic acid reacts rapidly with DPPH. The IC_{50} for essential oil studied was 15±1 min, so the ascorbic acid needs just 5±0,66 min to reduce the concentration of the radical free to 50 %. It`s well known that not only the main components of the essential oil are responsible for the antioxidant activity, this activity may be attributed also to the minor components that may interfere in synergy and antagony to create this system against the free radicals[23,24].

Antimicrobial Activity

Data on the antimicrobial activity of the different dilutions of *Citrus reticulata* essential oil against several bacteria are summarized in Table 3. Limonene is the major component of the essential oil (67.04 %) showed a lower antimicrobial efficacy. Based on these observations, it is clear that limonene has no influence on the antibacterial potential of essential oil tested.

Our observations are similar to those which were made by Inouye et *al*., (2001)[25], that essential oil containing phenol or alcohol as major compounds were more active than those containing limonene (mono terpene hydrocarbon) as the major compound.

These same observations were also shared by Dorman and Deans (2000)[24], which showed that monoterpene hydrocarbons, abundant in essential oil Citrus containing limonene as the main component is weakly inhibitory against a variety of microorganisms.

Table 3: Results of the antibacterial activity of the essential oil of *Citrus reticulata.*

Microorganisms	Halo of inhibition	Sensitivity*
Klebsiell pneumanae carbapinémase (+): KPC+	>6	-
Klebsiell pneumanae carbapinémase (-): KPC -	>6	-
Streptococcus faecalis SFL S 156	>6	-
Salmonella S 226	>6	-
Acinetobacter S239	>6	-
Staphylococcus sp SCN 128	11	+
Proteus mirabilis S 120	>6	-
Shigellaspp S 241	8	-
Citrobacter freundii CF S 208	>6	-
Serratia sp	>6	-
Staphylococcus aureus ATTC 23	>6	-
Staphylococcus aureus S 178	>6	-
Enterobacter faecalis EFS 154	26	+++
Enterobacter faecalis ATTC 29	>6	-
Klebsiell oxytoca S113	21	+++
Enterobacter aerogenes S 214	>6	-

Each value represents the mean of two replicates ± standard deviation; *The sensitivity to the different strains was classified by the diameter of the inhibition zone as follows[26]:

-: diameter less than 8 mm, not sensitive; +: sensitive, diameter 9-14 mm; ++: very sensitive, diameter 15-19 mm; +++: extremely sensitive for diameter larger than 20 mm.

CONCLUSION

In the present work, we have characterized the chemical composition of *Citrus reticulata* essential oil collected from Azzaba, Skikda city (Algeria). The identification of the chemical constituents was realized on the basis of FTIR and GC-MS analysis.

The FTIR analysis permitted the qualitative identification of ten volatile components, in the other hand the chromatographic analysis permitted the qualitative and the quantitative identification of 24 components with the co-dominance of the Limonene (71.14 %), γ-Terpinene (19.55 %) and α-Pinene (2.74 %). The antimicrobial potential of *Citrus reticulata* oil of Algeria was evaluated against pathogenic bacteria stains. The use of the volatile formulations on the basis of medicinal and aromatic plants may present several advantages for today synthesis products.

In fact; the essential oils are less toxic for the environment and may have a high biocide activity. Our interest was at the same time based on the antioxidant activity of the essence of the *Citrus reticulata* for the purpose to find new natural antioxidant in order to avoid the use of synthetically ones which may some of them be toxic or carcinogenic.

The Results obtained confirm that the medium antioxidant potential of the essential oil of this plant according to others. These results keep an open perspective for research of formulations on the basis of essences of the *Citrus reticulate* in place of other synthesis preservatives or antioxidant on the basis of plant used in the field of food industry, pharmaceutical and cosmetics industry.

REFERENCES

1. Caccioni DR, Guizzardi M, Biondi DM, Agatino R, Ruberto G, Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*, *Int. J. Food Microbiol*, 43, 1998, 73-79.
2. Rushdy Mohamed AH. Chemical and biological evaluation of deterpenated orange and mandarin oils. Al-Azhar University, 3, 2015.
3. Obenland D, Collin S, Mackey B, Sievert J, Arpaia ML, Storage temperature and time influences sensory quality of mandarins by altering soluble solids acidity and aroma volatile composition, *Postharvest Biol, Technol*, 59, 2011, 187-193.

4. Robert A et Lobstein A, Plantes aromatiques : épices, aromates, condiments et huiles essentielles. *Ed: Tec et Doc*, Lavoisier, Paris, 2005, 522 p.

5. Roux D, Conseil en aromathérapie, 2 ème édition, proofficina, 2008, 187 p.

6. Chiej R, Guide vert, Les plantes médicinales, Edition Solar, 1982,331 p.

7. Basil A, Jimenez-carmonna MM, Clifford AA, Extraction of rosemary by super-heated water, *Journal of food chemistry*,46, 1998, 5205-5209.

8. Burits M and Bucar F, Antioxidant activity of *Nigella sativa* essential oil, *Phototherapy Research*, 14, 2000, 323-328.

9. Sharififar F, Moshafi MH, Mansouri SH, Khodashenas M, Khoshnoodi M, In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multiflora Boiss*, *Food Control*, 18, 2007, 800-805.

10. Kiehlbauch JA, Hannet GE, Salfinger M, Archinal W, Monserrat C, Carlyn C, Use of the national committee, in: L. Duraffourd (Ed.), Traité de Phytothérapie Chimique (Chemical Treaty of Phytotherapy), Edition Masson, 1987.

11. Duraffourd L, Traité de Phytothérapie Chimique (Chemical Treaty of Phytotherapy), Edition Masson, 1987.

12. Bouguerra A, Himed L, Barkat M, Antimicrobial activity of essential oils extracted from *Citrus reticulate* peels, *Nutr. Santé*, Vol: 3 N°1, 2014,32-39.

13. AFNOR, Huiles essentielles, Paris, France, NF T-75, 2000, 111, 112 et 202.

14. AFNOR, Recueil des normes : les huiles essentielles, Echantillonnage et méthodes d'analyse, Tome 1, Paris, 2000, 440 p.

15. Lota ML, de Rocca Serra D, Tomi F, Casanova J, Chemical variability of peel and leaf essential oils of 15 species of mandarins, *Biochem. Syst. Ecol*, 29,2001,77-104.

16. Lota ML, de Rocca Serra D, Tomi F, Joseph C, Chemical variability of peel and leaf essential oils of mandarins from *Citrus reticulata* Blanco, *Biochem. Syst. Ecol*, 28,2000,61-78.

17. Sawamura M, Tu NTM, Onishi Y, Ogawa E, Choi HK, Characteristic odor components of *Citrus reticulata* Blanco (Ponkan) cold pressed oil, *Bioscience, Biotechnology and Biochemistry*, 68(8), 2004, 1690-1697.

18. Chutia M P, Deka Bhuyan MG, Pathak TC, Sarma P, Boruah A, Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India, LWT - *Food Science and Technology*, 42, 2009, 777-780.

19. Sawamura M, Tu NTM, Yu X, Xu B, Volatile constituents of the peel oils of several sweet oranges in China, *Journal of Essential Oil Research*, 17(3), 2005, 2-6.

20. Minh Tu NT, Thanh LX, Une A, Ukeda H, Sawamura M, Volatile constituents of Vietnamese pummelo, orange, tangerine and lime peel oils, *Flavour Fragrance Journal*, 17, 2002, 169-174.

21. Lu F and Foo LY, Antioxidant activity of polyphenols from sage (*Salvia officinalis*), *Food Chemistry*, 75, 2001, 197-202.

22. Soares JR, Dins TCP, Cunha AP, Almeida LM, Antioxidant activity of some extracts of *Thymus zygis*, *Free Radical Research*, 26,1997, 469-478.

23. Sing, R, Marimuthu P, De Heluani CS, Catalan Ceser AN, Antioxidant and biocidal Activities of *Carumnigrum* (seed) Essential oil, Oleoresin, and Their Selected Components, *Journal of Agricultural and Food Chemistry*, 54,2006,174-181.

24. Dorman HJD and Deans SG, Antimicrobial agents from plants: antibacterial activity of plant volatile oils, *Journal of Applied Microbiology*, 88 (2), 2000, 308-316.

25. Inouye S, Takizawa T, Yamaguchi H, Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *Journal of Antimicrobial Chemotherapy*, 47, 2001, 565-573.

26. Ponce AG, Fritz R, Del Valle C, Roura SI, Antimicrobial Activity of Essential Oils on the Native Microflora of Organic Swiss Chard, *Lebensmittel-Wissenschaft und-Technology*, 36, 2003, 679-684.

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