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EVALUATION OF ANTIOXIDANT POTENTIAL OF *CITRUS* PEEL ESSENCE FROM CHLEF REGION (ALGERIA)

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ABSTRACT

The objective of the current study was to evaluate the antioxidant activity of *Citrus* peel essence by using DPPH radical scavenging activity and β- carotene test Methods The essence of four varieties of *Citrus: C. Sinensis, C. paradisi, C. reticulata* and *C. aurantium* were been extracted by cold expression, then the extract was analyzed to determine the chemical composition by Gas Chromatography coupled to Mass spectrophotometry (GC/MS). The antioxidant activity of *Citrus* essences was assessed by DPPH and β- carotene test comparatively to BHT and Quercetin. The results obtained demonstrated that the IC 50 of *Citrus* essences range from : 1,9068 ± 0,0912 µg ml-1 for *Citrus reticulata*, 6,2086 ± 0,0308 µg ml-1 for *Citrus aurantium,* 12,7662 ± 0,0736 µg ml-1 for *Citrus paradisii,* and 19,1567 ± 0,00499 µg ml-1 for *Citrus sinensis*, which were all lower than the IC50 of the synthetic antioxidants : Quercetin (IC50 15,9938 ± 0,01284 µg ml-1) and Butylhydroxyanisole (BHA) (IC50 21,9938 ± 0,01284µg ml-1). In conclusion it has been suggested that *Citrus* essences constituent a real source for natural antioxidant that can be used in several domain such as : pharmaceutics , culinary and food engineering.

KEY WORDS:*Citrus*, Essence, DPPH, Antioxidant potential, Bio-Autography, GC/MS.

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INTRODUCTION

These last years, the focus on the natural antioxidants in relation with their therapeutics proprieties has been considerably increased. Several scientific research were conducted in the aim of developing extraction , identification and quantification of these compounds from many natural substances such as medicinal plants and food agriculture products [1,2,3] In an attempt to find new cures for current ills, the scientific community has recently turned to the constituents of essential oils, for a significant number of volatile compounds. Thus, among the gifted botanical families healing properties it has been found that the Rutaceae which encompasses a wide range of aromatic plants, mainly located in tropical regions [4] *Citrus* essences extracted by cold expression contain natural active compounds which are used in the pharmaceutical and biological field: antimicrobial activity, antioxidant, anti-inflammatory, antispasmodic.....etc[5] Numerous studies have demonstrated that *Citrus* genus was constituted from a number of bioactive secondary metabolites: flavonoids [6], limonoid [7] coumarins, sterols [8].The objective of the current study was to evaluate the antioxidant activity of *Citrus* peel essence by using DPPH radical scavenging activity and β- carotene test.

MATERIALS AND METHODS

Plant Materiel

The four varieties of Citrus fruits were collected locally in Chlef Region in February, 2011. The fruits were cleaned, weighted and peeled. The extraction of essence was done by cold pressing of the fresh peel.

Determination of chemical composition by CG /MS

Quantitative and qualitative analysis to determine the chemical composition of the citrus essence was done by the CG/MS.

Chromatographic conditions

GC: Hewlett Packard Agilent 6890N controlled by Chem Station (NIST 98).The chromatography conditions are as follows:

- Injection of 0.5µl Split mode 1/50
- Injector temperature: 250 ° C
- Capillary Column HP 5 MS (30 mx 0.25 mm x 0.25µm)
- Programming temperature: 35 ° C for 10 min; 4 ° C / min up to 250 ° C for 10 min.
- Flow of carrier gas: Helium (1ml/min)
- Mass spectrum: model Agilent 5973
- Temperatures: interface (280°C), source (230°C), quadrupole (150°C)
- The ionization energy of 70 eV.

Estimation of Antioxidant potential

The antioxidant activity was assessed by plate CCM Bio-Autography (qualitative test) and by spectrophotometric dosage (quantitative test).

Bio-Autography

The antioxidant potential of *Citrus* essence was determined following the method of Khartal et *al*., (2007) [9] by measuring the inhibition of the oxidative degradation of β carotene (Discoloration) by the products of acid linoleic oxidation .

DPPH Test

Citrus essences were diluted in absolute methanol (1%) to get different concentrations. (20%, 40%, 60%, 80% and 100%) [10]

Procedure

DPPH ((2,2'-diphenyl-1-picrylhydrazyl)) is used as a stable radical. In this test, the antioxidants reduces the DPPH with a purple color in yellow product , when the intensity of the color is inversely proportional to the antioxidants potential present in the reaction mixture . Briefly; 50 µl of sample were mixed with 1950 µl of methanolic solution of DPPH (6.10^{-5} M). The mixture is allowed for 30 minutes in dark. The measurement of the Optic density is done at 517 nm. The positive control is represented by reference antioxidants: Butylhydroxyanisole (BHA) andQuercetin . The percentage of Inhibition of DPPH is calculated by the following formula [11]:

$$\% \text{ DPPH inhibition} = ((\text{Abs control} - \text{Abs Sample}) / \text{Abs Control}) * 100$$

When

Abs control : The optic density of the control (DPPH solution in methanol)

Abs Sample : The optic density of the sample (*Citrus* essence + DPPH solution in methanol.

The IC 50 is value that represents the concentration of antioxidant which can inhibit 50 % of signal reference. Hence this value allows the comparison between the differences antioxidants tested.

Statistical Analysis

All results were expressed as mean ±S.E.M The values were analyzed for statistical significance by T-Test. For all analysis, a difference was considered significant at $p< 0, 05$.

RESULTS AND DISCUSSION

Analyze of Citrus essence composition by CG/SM

Chemical analysis showed a determined number of components for both species: 29 compounds for the essence of *C. aurantium* (99.92%) (Table 1), 17 compounds for the essence of *C. sinensis* (99.55%) (Table 2), 08 compounds for the essence of *C. reticulate* (99.92 %) (Table 3), and 31compounds for the essence of *C. paradisi*(99.55%) (Table 4). This analysis showed that these essences were constituted from a major component which is the "limonene" with different percentages (87.38% for *C. aurantium* essence, 86.29% for the essence of *C. sinensis*, 94,75% for the essence of *C. reticulata*and 82,98% for the essence of *C.paradisi*). Moreover minor compound in essence of *C. aurantium* has minor compounds: β-pinene (3.59%), α-pinene (1.47%) and Furrancarboxaldehyde (1.13%), and in traces: phellandene (0.31%) Cyclohexane and (0.7%) (Table 1). In addition to limonene essence of *C. sinensis* are represented by β-pinene (2.33%), bicycloheptene (2.43%), acetic acid (2.94%), and in trace α-pinene (0.75%) and Octanol (0.16%) (Table 2).Moreover minor compounds essence of *C. reticulata*are represented by

β - pinene(2.44%) ,1-5 dimethyl 1venyl (0.72 %),αpinène (0.91 %), β-phéllandrene(0.61 %) (Table3). In addition to limonene, the essence of *C. paradisi*has minor compounds:β-myrcene (2.66%), 2H-1-benzopyranone (2.07 %) and in trace:α-pinène (0.31 %) etl'acide n-hexadecanoique (0.88 %) (Table4).The results found through this experiment are in agreement with the study of Moufida and Marzouk (2003) [12] which confirmed that limonene is the major compound in Citrus essential oil. Hence , it has been found through the chemical analysis of the Citrus composition that acyclic compounds like Nerol and Geraniol are absent in the essence of *C. aurantium* and *C. sinensis*.In fact , Gancel et *al*. (2005) [13] reported that these two compounds are present only in the essential oil of C. limonum. Several studies [3,12,14] have demonstrated that generally, essential oil of Citrus were constituents mainly of monoterpene compounds (97%) , and other compounds such as : alcohol , aldehyde and esters were presents in weak amounts (1,8 et 2,2 %). In the study of the chemical composition of Citrus essential oil by Nogata et *al*., (2006) [15] they found that flavonoïdes represent the non-volatile fraction of essential oil and are used to make differentiation between Citrus varieties . So the results of the chemical analysis by GC/MS was conformed to other studies and to the international norms which confirmed that the extraction procedure , treatment of peel and technique of recuperation of *Citrus* essence were done in the right conditions.

Table 1
Components (%) of C. aurantium Essence analyzed by GC /MS.

Pics number	Retention time (min)	Chemical composition	% relative	Reconnaissance level
1	17,85	**α-pinène**	**1,47**	**95**
2	20,03	β-phellandrene	0,31	91
3	21,04	**β-pinène**	**3,59**	**94**
4	23,52	**D-limonène**	**86,29**	**94**
5	23,83	1, 3, 7-octatriene	0,36	95
6	24,84	Formicacid	0,19	91
7	26,05	1,6-octadien-3-ol	0,83	94
8	29,81	α-terpineol	0,10	91
9	30,24	Decanal	0,20	91
10	31,81	β-myrcene	0,26	90
11	34,84	Cyclohexene	0,07	95
12	36,23	2,6-octadien-1-ol	0,15	91
13	37,68	Caryophyllene	0,14	99
14	39,63	Germacrene D	0,24	96
15	41,99	1,6, 10-dodecatrien-3-ol,	0,33	91
16	48,83	2(3H)-Naphthalenone	0, 55	99
17	52,81	n-Hexadecanoicacid	0,78	99
18	54,87	7H-Furo(3, 2-g) (1) benzopyran-7-ol	0,28	93
19	56,59	Osthole	0,67	96
20	56,83	9,12-octadecadienoic acid	0,78	99
21	56,94	(z) 6, (z) 9-pentadecadien-1-ol	0,36	95
22	57,04	9-octadecenoic acid,	0,15	90
23	58,20	Cobalt	0,08	50
24	58,69	**2-Furancarboxaldehyde**	**1,13**	**49**
25	58,85	N.I.	0,16	35
26	59,64	Auraptenol	0,10	72
27	63,23	N.I.	0,07	27
28	65,03	1H-indole, 5-methyl-2-phenyl	0,21	62
29	65,11	Bis (2-ethylhexyl) phthalate	0,15	90
Total	/	/	99.92	/

Table 2
Chemical composition (%) of C. sinensis Essence analyzed by GC/SM.

Pics number	Retention time (min)	Chemical composition	% relative	Reconnaissance level
1	2.50	Hexane	0, 11	90
2	5.99	**Aceticacid**	**2,94**	**91**
3	7.65	2-Butanone	1,24	86
4	17.82	α-pinéne	0,75	97
5	20.02	**Bicyclo (3, 1,1) heptane**	**2,43**	**91**
6	21.01	**β-pinène**	**2,33**	**91**
7	23.21	**Limonène**	**87,38**	**93**
8	24.81	1-Octanol	0.16	90
9	26.03	1-6-octadien-3-ol	0.81	91
10	29.50	4H-pyran-4one	1.35	81
11	30.24	Decanal	0.40	91
12	30.97	2-Furancarboxaldehyde	0.72	93
13	33.94	2-methoxy-4-vinylphenol	0.19	91
14	35.22	1,2-Cyclohexanediol	0.44	53
15	39.98	Naphthalene	0.25	91
16	52.70	n-Hexadecanoicacid	0.28	98
17	56.69	9,12-octadecadienoic acid	0.20	99
Total	/	/	99.55	/

Table 3
Chemical composition (%) of C. reticulate essence analyzed by GC/SM.

Number of Pics	Retention time (min)	Chemical composition	% relative	Reconnaissancelevel
1	1.727	Acideformique	0.24	4
2	2.012	Ether éthyle	0.13	91
3	17.907	α pinène	0.70	97
4	20.099	β-phéllandrene	0.61	91
5	21.079	β-pinene	2.44	94
6	23.169	**D-limonene**	94.75	94
7	26.086	1-5 dimethyl 1venyl	0.72	49
8	40.103	Naphtalene	0.42	99

Table 4
Chemical composition (%) of C. paradisii Essence analyzed by GC/SM.

Number of Pics	Retention time (min)	Chemical composition	% relative	Reconnaissancelevel
1	1.870	Ethanol	0.61	90
2	17.937	α pinene	0.91	96
3	20.116	β-phellandrene	0.49	90
4	21.114	β-myrcene	2.66	86
5	23.443	**Limonene**	82.98	93
6	23.840	Octariene	0.38	96
7	24.898	Acideformique	0.20	91
8	26.103	Octadien-3-ol	0.19	72
9	29.905	Cyclohexane	0.10	72
10	30.315	Decanal	0.29	86
11	36.331	α-cubebene	0.53	96
12	36.723	1,6cyclodecadiene	0.42	95
13	37,804	Caryophullene	1.25	99
14	38.945	Cycloundecatriene	0.17	98
15	39.111	Cycloheptasiloxane	0.08	90
16	39.741	1,6cyclodecadiene	0.31	96
17	40.863	Naphthalene	0.56	94
18	43.958	Sylane	0.19	55
19	48.139	Cyclononasiloxane	0.10	55
20	48.947	Naphthalenone	0.82	95
21	51.863	Cycloheptasiloxane	0.09	52
22	52.927	Acide n-hexadecanoique	0.88	98
23	55.267	Acidebenzeneacetique	0.09	50
24	55.962	Acide 9,12octadecadienoique	0.09	99
25	56.674	Osthole	0.21	99
26	58.361	7 chloro-10-ethyl	0.17	46
27	58.741	2-naphthaldehyde	0.32	50
28	63.933	Cyclononasiloxane	0.16	52
29	65.263	Acide 1-2 benzenedicarboxylique	0.27	80
30	67.003	Acidebenzenesulforique	0.22	56
31	67.538	2H-1-benzopyranone	2.07	46

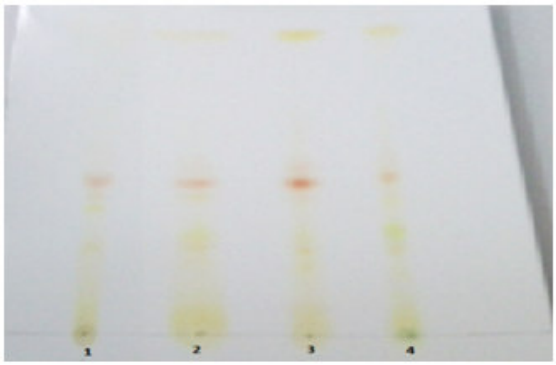

Figure 1
Results of autobiography
For CCM, the system is: Hexane / Chloroforme / Acétone (70 : 27: 3 ; v/v)
1, E. Citrus aurantium, 2, E. Citrus paradisii, 3 E. Citrus reticulate, 4 E. Citrus sinensis

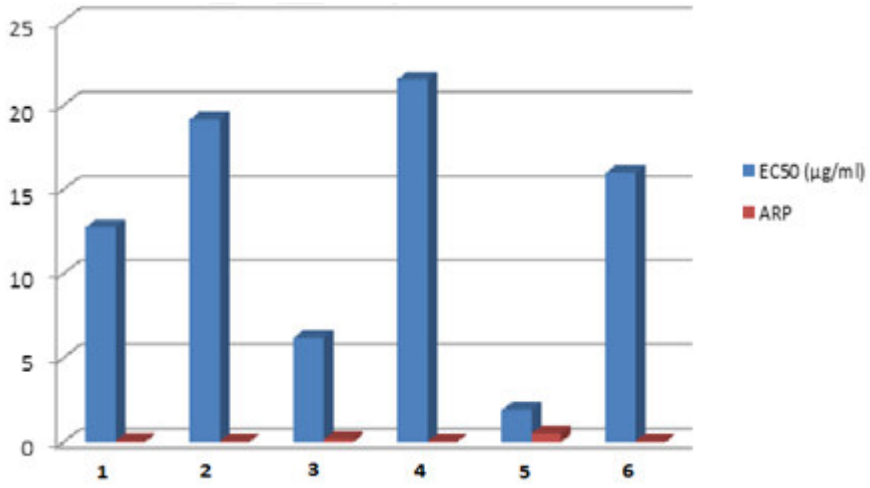

Figure 2
Histogram represent the IC 50 values and ARP of the essences of the Four varieties of Citrus , Quercetine and BHA.
1,E. Citrus paradisii, 2, E. Citrus sinensis, 3, E. Citrus aurantium, 4, BHA, 5, E. Citrus reticulata, 6: Quercetine

The presence of antioxidant can neutralize the free radicals derived from linoleic acid , so as to prevent the oxidation [16, 17.] As it can be seen in the table , the value of IC 50 of the tested essences , ranged from1,9068 ± 0,0912 µg ml-1 for *Citrus reticulata*, 6,2086 ± 0,0308 µg ml-1 for *Citrus aurantium,* 12,7662 ± 0,0736 µg ml-1 for *Citrus paradisii,* and 19,1567 ± 0,00499 µg ml-1 for *Citrus sinensis* . In fact , all the IC 50 were lower than these of the synthetics antioxidants : Quercetine (IC50 15,9938 ± 0,01284 µg ml-1) and BHA (IC50 21,9938 ± 0,01284µg ml-1). This effect is probably do to the mobility of hydrogen atom of hydroxyl group of certain citrus essences compounds .In the presence of DPPH radical , the atom of hydrogen is transferred on this last which transformed DPPH to a stable molecule and caused a diminution of the concentration of free radicals thus absorbance in the time of reaction until the exhaustion of the donor antioxidant capacity of hydrogen [18]. In part , This activity may be due to certain compounds considered as major in the studied citrus essences such as : carotenoids and flavonoids which are present in amount of de 0.05-0.12 mg/g et 5 mg/g for Citrus Sinensis, vitamin C with 1.6-2.3 mg/g for de *Citrus* Limon [19] which have a strong antioxidant capacity [20]. In another part , the minor constituents presents in the Citrus essence play a role in the antioxidant potential of the studied plants with a synergic or / and antagonist mechanism for developing an efficient system against free radicals.

CONCLUSION

The study was conducted in the aim of valorization of *Citrus* peel which contains a certain amount of bioactive substances. The chromatographic (GC/MS) analysis of Citrus essence demonstrated an abundance of monoterpene compounds mainly limonene (94.75 % for the essence of *C. reticulata* , 82.98% for the essence of *C. paradisi*, 87.38 % for the essence of *C. aurantium* and 86.29% for the essence of *C. sinensis*). The study allows us to determine the antioxidant activity of Citrus essence which justify the traditional use of this fruit in the treatment of numerous affections due mainly to the oxidative stress.

REFERENCES

1. Huang, D, Ou, B, Prior, RL. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 2005. 53: 1841-1856.
2. Marc, Fr, Davin, A, DeglèneBenbrahim L, Ferrand, C. Méthodes d'évaluation du potentiel antioxydant dans les aliments. Erudit, M/S: médecine sciences, 2004. 20(4), 458-463.
3. Rehman, S, Hussain S, Nawaz H. Inhibitory effect of citrus peel essential oils on the microbial growth of bread, Pak J Nutr, 2007. 6: 558-61.
4. Parray, SA, Bhut, JU, Ahmed, G, Jahan, N, Sofi, G, Iqbal, SM. Ruta graveolens from traditional system of medicine to modern pharmacology- an overview. Am J Pharm Tech Res. 2012. (2):239–52.
5. Watson, RR, Gerald, JK, Preedy, VR. Nutrients dietary supplements and Nutraceuticals: cost analysis versus chemical benefits – édition: Hamana Press, (New York, USA), 2011. (1): 477-528.
6. Spiegel-Roy P Goldschmidt EE. Biology of *Citrus*. Ed: Cambridge University Press. 1996. 230.
7. Manners, GD. *Citrus* limonoids: analysis, bioactivity, and biomedical prospects. J. Agric. Food Chem. 2007. 55:8285-8294.
8. Ladaniya, MS. *Citrus* Fruit Biology, technology and evaluation, Academic press, London, UK. 2008. 1-11.

9. Khartal, N, Sokmen, M, Tepe B ., Daferera, D., Polissiou, M, Sokmen A.Investigation of the antioxidant properties of Ferula orientalisL.using a suitable extraction procedure .*Food Chemistry,* 2007. 100:584-589
10. Brand-Williams, W, M.E Cuvelier and C. Benset, . Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol., 1995. 28: 25-30.
11. Yildirim, A, Mavi A and Kara, A. Determination of antioxidant and antimicrobial activities of *Rumes crispus L*. extract; Journal of Agricultural and Food Chemistry. 2001. 49: 4083-4089.
12. Moufida, S and Marzouk, B. Biochemical characterisation of blood orange, sweet orange, lemon, bergamot and bitter orange. Phytochemistry, 2003. 62 8, 1283-1289.
13. Gancel, AL, Ollitrauet, P, Froelicher, Y, Tomi, F, Jacquemond C, Luro F and Brillouet JM. Leaf volatile compounds of six *Citrus* somatic allotetraploidhybribs originating from various combinations of lime, lemon, citron, sweet orange and grapefruit. Journal of agricultorol and food Chemistry. 2005. 53: 2224-2230.
14. Bellti, N, Nidagijimana, M, Sisto, C, Guerzoni, ME, Lanciotti, R and Gardini, F. Evaluation of the antimicrobial activity of *Citrus* essences on Saccharomycescerevisiae.Journal agricultural food chemistry. 2004. 52 23: 6932 – 6938.
15. Nogata, Y, Sakamoto, K, Shiratsuchi, H, Ishii, T, Yano, M, Ohta, H. Flavonoid composition of fruit tissues of citrus species. Biosci. Biotech. Biochem., 2006. 70 1: 178–192.
16. Villano, D, Fernandez-Pachon MS, Moya ML, Troncoso AM Garcia-Parrilla MC Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta 2007. 71: 230–235.
17. Yanishilieva, NVI and Marinova, EM. . Effects of antioxidants on the stability of triacylglycerols and methyl esters of fatty acids of sunflower oil. *Food Chemistry*, 1995. 54:337-382.
18. Tripoli, E, Guardia ML, Giammanco S, Majo DD, Giammanco M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chem. 2007. 104: 466-479.
19. Sanchez-Moreno, C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *International Journal of Foods Science and Technology,* 2002. 8: 121-137
20. Causse, C. Les secrets de santé des antioxydants. Ed : Alpen Editions s.a.m. 2005. 8: pp: 95.