In vitro study of anti-glycation and radical scavenging activities of the essential oils of three plants from Morocco: *Origanum compactum*, *Rosmarinus officinalis* and *Pelargonium asperum*

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ABSTRACT

Introduction: We have carried out our study on the chemical composition; anti-glycation andradical scavenging activities of *Origanum compactum*, *Rosmarinus officinalis* and *Pelargonium asperum* essential oils (EO) that were harvested in the northeast of Morocco. These plants have been traditionally used in medicine as a chief ingredient of many polyherbal formulations for the treatment of several pathologies. **Method:** The phytochemical study was revealed by GC-MS. The protein glycation inhibitory activity of EO extracted from these plant tissues was evaluated *in vitro* using the model system of bovine serum albumin and methylglyoxal. The measure of DPPH• radical reducing power was used to evaluate the antiradical activity. To test each fraction, we used the IC50 value previously obtained for the crude oils. **Results:** The phytochemical study of these essential oils showed that p-Thymol, Eucalyptol and Citronellal were respectively the major components in the three investigated EO of *O.compactum*, *R. officinal is* and *P. asperum*. More than 90% of the total components were detected. The extracts and fractions with glycation inhibitory activity also showed antiradical activity when the DPPH• radical reducing power was measured. **Conclusion:** The glycation inhibitory activity was correlated with the antiradical potency of the extracts. Thus, the positive glycation inhibitory and antiradical activities of these plants might suggest a possible role in targeting aging and diabetic complications.The presence of various bioactive compounds confirms the application of these plants for various diseases by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Key words: Aging, Antioxidant, Antiglycation, Diabetes, Essential oil, Radical scavenger.

INTRODUCTION

Biological age reflects the physiological functioning of our body, the true functional status of people. The measurement of the biological age of diabetics shows that this pathology accelerates aging, and increases 14.65% compared to the is chronological age. Thus, diabetes causes deleterious effects due to the occurrence of oxidative stress and aprotein glycation, which are harmful to the physiological performance of patients.

Glycation is a non-enzymatic browning reaction caused by amino-carbonyl reactions between reducing sugars and amino groups of proteins and lipids, inducing the formation of advanced glycation end products (AGEs). AGEs accumulate in various tissues and play animportant role in the pathogenesis of manydiabetic complications,

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such ashyperlipidemia, hyperinsulinemia, hypertention and atherosclerosis.[1] Several studies have demonstrated that oxidative stress, caused mainly by the formation of AGEs, induces the generation of free radicals that contribute to diabetes development and progression of the secomplications, this lead to cell and tissue damages, and among other causes aging.[2] Thus, agents that inhibit AGEs formation and /or decrease oxidative stress are believed to have the rapeutic potential in patients' with diabetes orage-related diseases.

Research and attempts in pharmacotherapy helped to control diet by the use of various therapeutic agents that inhibit or reverse the progress of glycation (biguanide, metformin …). A representative drug is amino guanidine (AG), a hydrazine compound that prevents AGEs formation by trapping intermediates at the initial glycation stages.[3] This is ensured by the use of medicinal plantain many traditional societies. Recent attention has focused on the benefits of medicinal plants with both antiglycation and antioxidant properties.[4] Never the less, it seems difficult to identify active and effective plants presenting these activities. Thus, in our work, we adopted abio-guided research that focuses on active extracts from three Moroccan plants traditionally used in the treatment of diabetes type2. *Origanum compactum* (Zaâtar) -endemic plantin Morocco -*,Rosmarinus officinalis (Azir) and Pelargonium asperum (*Laâtarcha)are herbs widely used as an infusion for the treatment of several pathologies. For example, *R. officinalis is known for its* diuretic effect, and possesses an antibacterial effect against gram-positive bacteria,[5] but also acts as antioxidant.[6] The antimicrobial and antioxydant activities of *O. compactum* has been largely demonstrated,[7] this plant also showed mutagenic and antimutagenic activities.[8] *P. aspergum* in turn, showed a large antibacterial, antifungal and antioxidant effects.[9] Therefore, these plant species are considered as a source of compounds with promising pharmacological properties. However, their protective power against the glycation of proteins caused by glucoseor its metabolites is little explored.

In the present study, we evaluated the chemical composition of three spontaneous plants: *Origanum compactum, Rosmarinus officinalis and Pelargonium asperum from the area of* Taounate, subsequently we verified their anti-oxydative and anti-glycative effects using oxydation and glycation model systems. We show that these plant extracts and some of their fractions inhibit the formation of glycated proteins and also act as antioxidant. The results reported herein should create new avenues for exploring pharmacological treatments to prevent glycation and related disease conditions. Preventing the accumulation of AGE varieties in diabetic complications and in the aging process, will likely require the combination of several approaches.

MATERIALS AND METHODS

Chemicals

All reagents, unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (Sigma-Aldrich), except (TEMED) that is from Folca.

Plant collection and extract preparation

Plants (*Origanum compactum* (Zaâtar)*, Rosmarinus officinalis* (Azir) *and Pelargonium asperum (*Laâtarcha)) are from the region of Taounate (Northern Morocco), and were collected in March 2013; the botanical identification was made by the Mr.Greche from IMPMA-Taounate. The fresh leaves were selected for the extraction. For this, they were dried at 40 °C in an oven for 15 h. All samples were then ground into a fine powder. The powders were passed through an 80-mesh sieve, collected and sealed in a plastic bag, and then stored at 20 °C until use. The large-scale extraction is performed by vapohydro distillation in the Technological Hall of INPMA-Taounate. The small-scale extraction is done by hydrodistillation (Clevenger). Samples (30 g) were extracted with distilledwater (300 ml) for 45 min. The extracts obtained were stored at 4 °C away from light.

The following formula was used to determine the EO yield.[10]

$$R = (P_x / P_y) \times 100$$

R: Oil yield (%), P_x: Oil weight (g) P_y: Plant weight (g)

Phytochemical study

Analysis of the chemical composition

The qualitative and quantitative analysis of the chemical composition of EO was carried out at the Laboratory LBV pam, FST Saint Etienne-France. GC-MS type is Agilent GC 6850 MS 5973. The analytical study is performed by injecting two µl of extract, using hexane as solvent.

Prefractionation

According to the protocol described,[11] each essential oil is fractionated, away from light and air flow at room temperature, into ten fractions by column chromatography

on silica type 60. Each fraction was eluted with 350 ml of the mobile phase. A rotary evaporator type BUCHI Rota vapor R-205 is used to concentrate each eluted fraction.

Evaluationof antiradical activity

The antiradical activity of EO was evaluated by measure of DPPH$^{\bullet}$ radical reducing power as described .[12] We prepared in methanol two dilution series (1/2 and 1/5) for each oil and also for the standard antioxidant BHT used as positive control. After that, these dilutions were mixed (v/v) with a methalonic solution of DPPH (4 mg/100 ml). The negative control is a mixture of the methalonic solution of DPPH$^{\bullet}$ and methanol (v/v). After agitation, the tubes were placed in dark, at room temperature, for 30 min. we used the IC_{50} value previously obtained for the crude oil to test each fraction by the same protocol described above. This step is performed to determine whether any single fraction is able to reduce the absorbance of a DPPH$^{\bullet}$ solution by 50% of DPPH$^{\bullet}$. A spectrophotometer type JENWAY 6800 UV/V measures the optical density at 517 nm. The degree of inhibition of the free radical DPPH$^{\bullet}$ is calculated using the following formula :

$$I = [(A\ blank - A\ sample) / A\ blank] \times 100$$

A blank: Absorbance of negative control (DPPH$^{\bullet}$ in methanol)

A sample: Absorbance of the tested compound.

Evaluation of antiglycation activity

The antiglycation activity of EO and their fractions is determined in the laboratory of biochemistry and molecular Genetics, FST of Tangier.

In vitro glycation of bovine serum albumin[13]

bovine serum albumin (BSA, 5 mg/ml, containing the anti-proteolysis EDTA) was incubated in methylglyoxal (10 mM) and sodium azide (0.02%) in 0.1 M phosphate buffer (pH 7.4). The test compounds were added to the reaction mixture, then incubated for 24h at 50°C protected from light and stirred; individual vials were removed at desired times and stored frozen at 20°C until analyzed.

Electrophoretic migration in native conditions

The mixture solution was applied to PAGE-Native. The samples were separated on a 7 % polyacrylamide gel. After migration, the gels were stained with coomassie blue for 1H. The destaining was also for 1H with a solution of acetic acid 10 % and methanol 45 %.

RESULTS AND DISCUSSION

Phytochemical study

Extraction of EO

The hydro distillation of the aerial part of *Origanumn compactum, Rosmarinus officinalis and Pelargonium asperum* has allowed the extraction of EO with a marked difference in color and smell. Also, *O. compactum has the highest yield compared to R. officinalis* and *P. asperum* Table 1.Comparing our results with other publications shows a slight variation. In fact, the yield of *O.compactum* obtained[14] is2.10±0.07%; for *R. officinalis* it's between 1.74 ± 0.38% to 2.58 ± 0.75%[15] and for the *P. asperum*, the results were on the order of 0.15 to 0.4%.[16] This difference in yield can be attributed to several factors including genotype, age of the plant, maturity, interaction with the environment, mode of culture, period and procedure for harvesting, conservation of plant material, temperature and time of drying, and technique of extraction.[16, 17]

Chemical composition of essential oils

Because of the increased use in folk medicine, the leaves of *O.compactum*, *R. officinalis* and *P.asperum* have been the subject of several phytochemicals studies to identify their active constituents that are either primary or secondary metabolites. In our study, the chemical composition of the three EO are shown in Tables 2, 3 and 4.

The *O. compactum* extract is composed of 28 elements that represent 99.99% of the identified compounds. The major components of this extract are: p-Thymol (63.60 %), γ-Terpinene (17.2504%), p-Cymene (8.4457%), α-Terpinen (2.1893%).These values are, in part, in accordance to those reported in previous studies, with absence of carvacrol

Table 1: Yield and color of EO

Plants	Color	Odor	Yield (%)
Origanum compactum	Yellow orange	Exist	2.1±0.1
Rosmarinus officinalis	Transparent	Exist	1.2±0.1
Pelargonium asperum	Yellow	Exist	0.8±0.1

Table 2: Chemical composition of the essential oil of *O. compactum*

Chemical compounds	Percentage (%)	Retention time (min)
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro derive.	1.0614	5.2806
1S-α-Pinene	0.6103	5.4844
Camphene	0.0863	5.9296
β-Pinene	0.2157	6.6833
3-Octanone	0.0879	6.8281
β-Myrcene	1.4174	6.9595
α-Phellandrene	0.2405	7.5361
3-Carene	0.0747	7.6085
α -Terpinen	2.1893	7.8740
p-Cymene	8.4457	8.1369
D-Limonene	0.2478	8.2844
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)	0.2129	8.3541
1,3,6-Octatriene, 3,7-dimethyl-, (Z)	0.0614	8.8449
γ-Terpinene	17.2504	9.3035
Cyclohexane, 1-methylene-4-(1-methylethenyl)	0.1560	9.7541
Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl	0.1168	10.2878
Benzene, 1-methyl-4-(1-methylethenyl)	0.0779	10.4890
1,6-Octadien-3-ol, 3,7-dimethyl	1.3580	10.8484
Borneol	0.1528	13.7851
2,6-Octadienal, 3,7-dimethyl-, (Z)-	0.0471	14.0399
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	0.4292	14.1257
3-Cyclohexene-1-methanol, α,α4-trimethyl-	0.1651	14.7453
Cyclohexene, 1,6-dimethyl-	0.0773	14.8472
Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)	0.1594	16.5341
p-Thymol	15.7499	18.8782
p-Thymol	47.8524	19.2563
Phenol, 2-methyl-5-(1-methylethyl)-	0.0153	19.5218
Caryophyllene	1.4410	24.0544
Total 28 elements	99,99%	---

as major compound identified in other publications. A study on EO extracted from O. compactum collected from several regions, showed the presence of three major components with variable rates: carvacrol (3.8 - 71%), thymol (0 - 43.4%) and p-cymene (0 - 25.4%).[14,18] this extract also contained these three major components: Carvacrol (36.46%), thymol (29.74%), p-cymene (24.31%).

The R. *Officinalis extract is composed of* 37 elements that represent 91.40% of the identified compounds. The major components of this extract are: Eucalyptol (35.20 %), 1R-α-Pinene (19.51 %), (1R)-Camphor (7.32 %), Verbenone (5.65%). These values are different to reported in previous studies,[15] Eucaliptol (18.9 à 22.1%), Camphor (15.0 à 24.0%), Camphene (6.5 à 8.0%), Borneol (4.1 à 7.1%), Verbenone (4.0 à 5.4%).

The *P. asperum extract* is composed of 18 elements that represent 73.94% of the identified compounds. The major components of this extract are: Citronellol (25.0708 %), Citronellyl formate (10.5261 %), Geraniol (10.4604 %), β-Maaliene (5.9391 %), 2-isopropyl-5-methyl-cyclohexanone (5.8805 %). These values are also different to reported in previous studies,[9] Citronellol (29.98%), Géraniol (14.12%), formiate de Citronellyle (9.09%), Isomenthone (7.80%), Linalol (5.97%), (Z)-β-farnezène

Table 3: Chemical composition of the essential oil of *R. officinalis*

Chemical compounds	Percentage (%)	Retention time (min)
Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-, (1S)-	0.1129	5.2350
1R-α-Pinene	19.5096	5.4898
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	2.8712	5.9296
Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)-	0.3512	6.0155
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	0.6343	6.6940
3-Octanone	0.1462	6.8308
β-Pinene	1.0380	6.9595
(+)-4-Carene	0.1975	7.8741
Benzene, 1-methyl-4-(1-methylethyl)-	3.3604	8.1369
D-Limonene	3.5273	8.2898
Eucalyptol	35.2038	8.4105
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.1989	9.2955
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.1700	10.2905
Benzene, 1-methyl-4-(1-methylethenyl)-	0.1822	10.4863
1,6-Octadien-3-ol, 3,7-dimethyl-	2.8668	10.8537
1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-	0.4107	11.6959
1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol	0.1281	11.9158
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1α,3α,5α)]-	0.0783	12.4924
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1α,3α,5α)]-	0.1495	12.5032
(1R)-Camphor	7.3225	12.7499
Bicyclo[2.2.1]heptan-2-ol, 2,3,3-trimethyl-	0.1279	13.0878
Bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene-	0.8015	13.3533
2-methyl-6-methylene-7-octen-2-ol	0.4659	13.7208
Borneol	4.6807	13.7878
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	1.5234	14.1230
Benzenemethanol,α,α,4-trimethyl-	0.2400	14.4154
3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-	4.7384	14.7506
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	0.3645	15.0671
Verbenone	5.6493	15.2119
6-Octen-1-ol, 3,7-dimethyl-, (R)-	0.2807	16.0675
Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	0.1456	16.6414
Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	0.2241	16.9230
2,6-Octadien-1-ol, 3,7-dimethyl-, (E)-	1.1567	17.0518
Bornyl acetate	0.6086	18.4625
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	0.1869	20.7046
Caryophyllene	0.2072	24.0571
1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	0.1393	25.5188
Total 37 elements	91,40%	---

Table 4: Chemical composition of the essential oil of *P. asperum*

Chemical compounds	Percentage (%)	Retention time (min)
1R-.α.-Pinene	1.2236	5.4846
β-Pinene	0.2669	6.9597
α -Phellandrene	0.1251	7.5390
Benzene, 1-methyl-3-(1-methylethyl)-	0.5503	8.1344
D-Limonene	0.3506	8.2873
β-Phellandrene	0.1436	8.3570
1,3,6-Octatriene, 3,7-dimethyl-, (E)-	0.2452	8.4723
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	0.2452	8.8451
1,6-Octadien-3-ol, 3,7-dimethyl-	3.6166	10.8512
Rosenoxide	1.5522	11.2401
2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	0.5851	11.8811
2-isopropyl-5-methyl-cyclohexanone	5.8805	13.4688
Citronellol	25.0708	16.0945
Geraniol	10.4604	17.0653
Citronellyl formate	10.5261	18.0255
2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (E)-	4.0983	19.0741
(+)-Ledene	3.0623	26.9484
β-Maaliene	5.9391	32.0361
Total 18 elements	73,94%	---

Table 5: Yield and density of fractions

		Origanum Compactum			*Rosmarinus officinalis*			*Pelargonium asperum*		
Fractions	Chemical familieseluted	weight (mg)	%	Density (g/cm³)	weight (mg)	%	Density (g/cm³)	weight (mg)	%	Density (g/cm³)
1	Hydrocarbons	5193.6	16.4	0.84	7049.3	22.5	0.86	3213.6	10.4	1.1
2	Esters + Ethers	1167.9	3.7	0.94	3607.3	11.5	0.88	747.5	2.4	0.92
3	Aldehydes + Ketones	12110.6	38.3	0.7	12947.0	41.3	0.8	7125.7	23.1	0.82
4	Aldehydes + Ketones + Alcohols	7867.6	24.9	0.7	2130.4	6.8	0.76	8431.6	27.3	0.78
5	Aldehydes + Ketones + Alcohols	1339.2	4.2	0.86	728.5	2.3	0.72	2450.2	7.9	0.8
6	Alcohols	921.2	2.9	0,84	305.6	1.0	0.84	426.5	1.4	0.7
7	Alcohols	367.8	1.2	0,84	110.4	0.4	0.82	96.1	0.3	0.8
8	Alcohols	43.2	0.1	0,84	130.2	0.4	0.78	44.6	0.1	0.76
9	Alcohols	1995.6	6.3	1	2393.0	7.6	0.86	7769.5	25.2	0.92
10	Acids	600.4	1.9	0,92	1934.2	6.2	0.92	544.9	1.8	1.3
Total	-	31607	100	-	31336	100	-	30850.1	100	-

(4.27%), formiate de Géranyle (4.07%).

The qualitative and quantitative changes in the chemical composition of these EO, are related to several parameters, including the genotype, the age of the plant, the degree of maturity and the interaction with the environment.[16, 17] In addition, degradation and transformation of some compounds may occur depending on the extraction method adopted.[17] The structure of chemical compounds can also be changed and others not detected during analysis by GCMS.[19,20] the exact composition of the oils is notal ways specified in articles and generally, EO have different chemical compositions depending on their origin.[21] This variability may affect their physicochemical properties and bioactivity.[9] A recent studies work on parameters of standardization in order to optimize the quality and quantity of the chemical composition of EO.[17]

Table 6: Essential Oils Density

	Density (g/cm^3) *Origanum Compactum*	*Rosmarinus officinalis*	*Pelargonium asperum*
Crude extract	0.9	0.96	0.78

Prefractionation by liquid chromatography

The result of fractionation by liquid chromatography shows that each fraction has a defined smell and color, but some fractions (F1 and F2 of each EO) are transparent. The yield and density of each fraction are shown in Table 5.

On the grounds of the above-mentioned results, we infer that there is a difference in yield between the fractions obtained from the same extract; it may be proportional to a difference in polarity between the molecules. For each extract, the total mass after splitting is greater than the initial mass (30000 mg) weighed before fractionation; the excess observed is probably due to the presence of residues of the elution solvent that remain even after the concentration of the fractions by Rota vapor. Therefore, it will be interesting to perform an analysis using the GC-MS in order to determine the percentage of residues remained in each fraction.

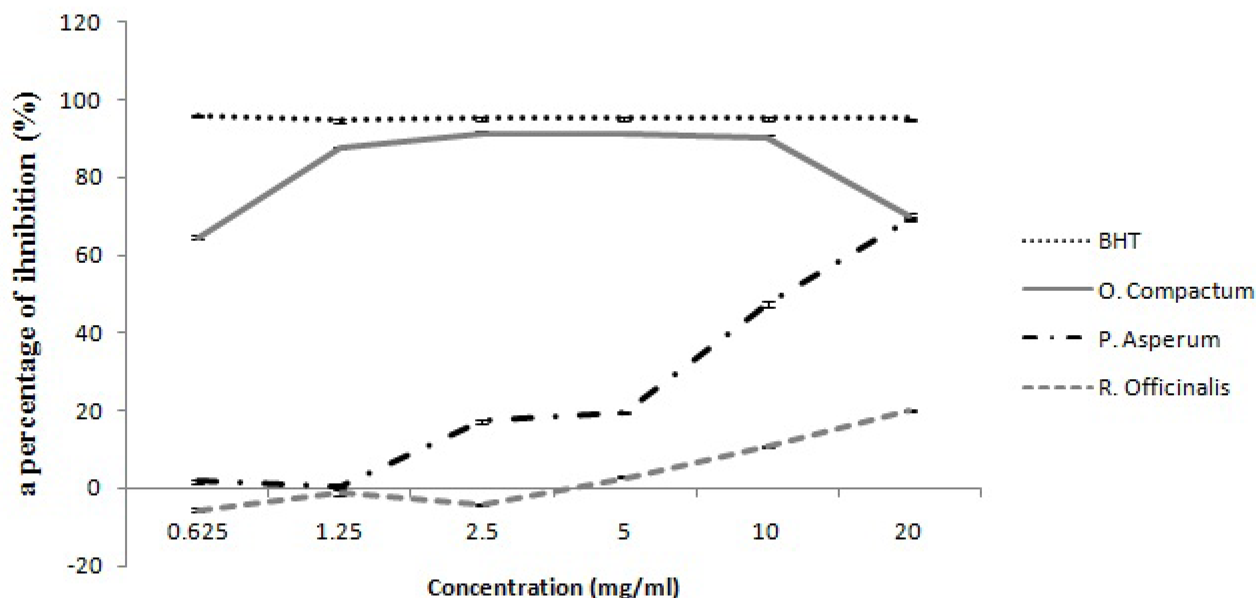
For all the fractions of R.officinalis, the density is less than that of crude extract and water Table 6. While for *O.compactum*, 30% of the fractions obtained are denser than the raw extract and only F9 has an equal density to that of water. Concerning the fractions of *P.asperum, there is a contradiction*; because 70% are denser than the crude extract and only F1 and F10 have a density higher than that water. This difference of elementary and total densities is probably related to the chemical composition, which characterizes each fraction. Therefore, a comparison between the chemical composition of EO and that of their fractions is necessary.[22]

the density tells us about the quality of the chemical composition. A density less than 0.9 g/cm 3 indicates that both compounds aliphatic and terpenic are highly concentrated in the oil; however, a density greater than1 indicates the presence of polycyclicterpenic compounds.

Antiradical activity

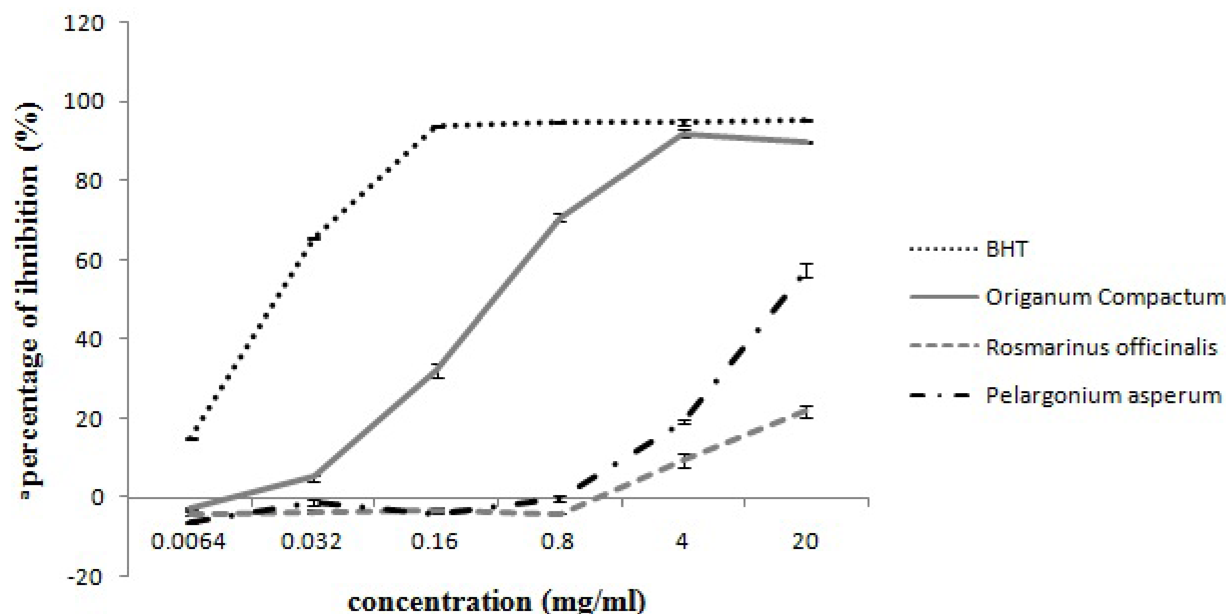
In this study, the antiradical activity of the EO is evaluated by DPPH test, considered as a standard and an easy colorimetric technique.[23] The BHT was used as positive control. DPPH (diphenylpicryl-hydrayl), is commonly used as a reagent assessing the activity of scavenging free radicals of natural antioxidants. A product is an antiradical, when the DPPH solution lose the characteristic deep purple color and becomes yellow. The intensity of the yellow color is inversely proportional to the ability of antiradicals in the medium to provide protons.[24,25] the action of antioxidants on DPPH is due to the irability to give hydrogen.

The graphs 1 and 2 show the results of DPPH inhibition



Graph 1: Variation of DPPH• reduction percentage of the EO compared to that of BHT (dilution 1/2)

[a]Average percent inhibition of three repetitions.



Graph 2: Variation of DPPH• reduction percentage of the EO compared to that of BHT (dilution 1/5)

[a]Average percent inhibition of three repetitions.

Table 7: IC_{50} of BHT and essential oils

	IC_{50} (mg/ml)	Equations of calcul	R^2
BHT	0.036	Y = 0,0004X+ 0,0089 (Y=1/y et X=1/x)	0.9874
Origanum compactum	0.52	y = 85,495x + 4,9655	0.9193
Rosmarinus officinalis	42.08	y = 1,3012x - 4,7577	0.954
Pelargonium asperum	16.99	y = 3,0493x - 1,793	0.9643

degree. A significant decrease of the concentration of DPPH radicalis due to the scavenging activity of the extract and the standard molecule. Also, the inhibition of the free radical increases with increasing concentration either for the BHT or the three extracts tested. For both concentrations, antiradical activity of *O. compactum* was significantly higher than that of *R.officinalis* and *P. asperum*, but this activity is still lower than that of BHT.

IC_{50} determination

IC_{50} is the concentration of a compound decreasing the absorbance of a DPPH solution by 50 %. LowIC_{50} value indicates a high anti radical activity.[26] This value is calculated from trend lines equations that have a correlation coefficient (R^2) close to 1. TheIC_{50}value of BHT is calculated by the logarithmic equation 1/y=a/x+b using the values obtained from the dilutions ½ (Y = 2E-05X + 0.0105 and R^2 = 0.0675) and 1/5 (Y = 0,0004X+ 0.0089 and R^2 = 0.9874).

Several EO from *Origanum* have been reported for their antioxidant activity.[27] In this study Table 7, the essential oil of *O. Compactum* has a strong scavenging activity correlated with the presence of a large quantity of thymol (representing 63.60% of the constituents of this oil) known for their radical scavengingproperty.[28] This activity can also be due to thep-Cymene[29] representing 17.25%of the total composition.[14] using the DPPH method, found that the IC_{50}=60.1 ± 3.3 mg/l.

The scavenging activity of theses ential oil of *R.officinalis* is probably also related to its major compounds: eucalyptol (35.20%), 1R-α-Pinene (19.51%) and (1R)-Camphor (7.32%) which constitute 62.03%of the total composition. In contrast, other publications have revealeda strong activity of this plant extract.[30]

IC_{50} =36.78±0.38to111.94±2.56 μg/ml,[31] IC_{50} =3.82μg/ml,[32] IC_{50} =62.45±3.42μg/ml.

For *P. asperum*, the scavenging activity can be mainly attributed to the terpenic alcohols representing its major compounds: Citronellol (25.07%), Geraniol (10.46%). Moreover, several publications have shown that the scavenging activity of the EO of this plant is primarily

Table 8: Experimental Percentage of DPPH In hibition at the IC_{50} calculated for each fraction

	Origanum Compactum **IC_{50}= 0,51 mg/ml**	***Rosmarinus officinalis*** **IC_{50}=42.08 mg/ml**	***Pelargonium asperum*** **IC_{50}=16,96 mg/ml**
Crude extract	57.39	18.94	60.51
F1	-0.16	32.70	91.29
F2	-4.21	4.83	18.90
F3	69.70	20.87	46.98
F4	28.83	87.40	33.90
F5	91.27	58.47	26.09
F6	35.78	53.78	60.11
F7	7.57	0.00	0.00
F8	0.00	21.01	0.00
F9	3.96	-2.56	-1.86
F10	-2.00	-4.81	-9.91

related to terpene compounds such ascitronellol and geraniol.[33, 9] This activity can also be related to the action of other compounds such as Citronellyl formate (10.52%), β-Maaliene (5.93%) and 2-isopropyl-5-methyl-cyclohexanone (5.88%). The value of the IC_{50} found by other researchers indicates a high activity of this oil:

IC_{50} = 6.675 µg /ml,[9] IC_{50} = 14.49 µg/ml,[34] IC_{50} = 66.5 µg/ml.[33]

The difference observed between the IC_{50}values is probably due to a difference in chemical composition between these EO due to the influence of geographical location, the extraction and analysis protocols. Generally, the anti-radical activity is greater in the presence of phenols and terpenicalcohols[35] than ketones orsesquiterpenes.[33,9] However, the total activity is not attributed only to the major compounds, because inter actions between different compounds can exist in a synergistic or antagonistic way to reduce free radicals.[36]

Experimental evaluation of the fractions

Experimental evaluation revealed that some fractions have an antiradical effect higher than the IC_{50}, as F5 of *O. compactum*, F4 of *R. officinalis* and F1 of *P. asperum* Table 8. The IC_{50} of BHT gave a percentage inhibition of 82%.

Antiglycation activity

The results obtained in this test are shown respectively in figures 1, 2 and 3. Comparingthemigration profile of each band to that of positive and negative control, we can determine if the EO or the fraction have an inhibitory effect on protein glycation. The ethanol was used to dissolve EO and to determine if it has an effect on the migration of crude extracts bands.

The EO of *O.compactum* has a weak inhibitory effect on glycation (Figure. 1), despite this low activity,[37] reported that the methanolic extract of *Origanum majorana* was effective in inhibiting AGEs formation at concentrations of 5-10 mg/ml. These activities have been attributed to their antioxidant activity and ability to scavenge reactive carbonyls. In fact, the *O. Majorana* reduces oxidative stress in diabetic conditions by inhibiting lipid per oxidation. Therefore, the absence of the antiglycation effect of *O.compactum* oil at the concentration of 1.25 mg/ml, face to an interesting antiradical power effect (57.39%), may be due to the complexity of its components. Fractionation of oil to test the activity of each fraction allowed us to verify that some of them have, in pure state, an interesting effect against glycation. It's the case of the fractions F2, F3, F6, F9, F10 and slightly F4, representing 78% of the total mass of the essential oil (Figure. 1). This suggests an antagonistic interaction between the compounds making the crude extract unable to protect BSA against glycation.

The EO of *R. officinalis* and eight of these fractions (F2, F3, F5, F6, F7, F8, F9, and slightly F10, representing 70.67% of the total mass of the extract) have the antiglycation power (Fig. 2), it allows us to deduce that these essential oil molecules interact in asynergistic way more dominant than their antagonist effect. This activity, at the concentration of1.25mg/ml, face to an average antiradical power (18.94%), could be due to the complexity of its components and therefore probably related to these major compounds: eucalyptol(35.20%),1R-α-pinene (19.51%) and the(1R)-Camphor (7.32%) whichconstitute62.03% of the total composition.

Figure 1 : Anti-glycation activity of *Pelargonium asperum* essential oil.

Electrophoretic migration profile under native conditions (7%) of BSA (5 mg/ml) incubated for 5 days with methylglyoxal (MG) (10 mM) ; C-: negative control (BSA alone), C+: positive control (BSA + MG), P: *P.asperum*, R/E: *P.asperum* + 3% of ethanol, from F1 to F10: fraction (1.25 mg/ml) + BSA + MG. The red line indicates the migration of the positive control as a reference.

Figure 2 : Anti-glycation activity of *Orignanum Compactum* essential oil.

Electrophoretic migration profile under native conditions (7%) of BSA (5 mg/ml) incubated for 5 days with methylglyoxal (MG) (10 mM) ; C-: negative control (BSA alone), C+: positive control (BSA + MG), O: *O.compactum*, O/E: *O. compactum* + 3% of ethanol, from F1 to F10: fraction (1.25 mg/ml) + BSA + MG. The red line indicates the migration of the positive control as a reference

Figure 3 : Anti-glycation activity of *Rosamrinus officinalis* essential oil.

Electrophoretic migration profile under native conditions (7%) of BSA (5 mg/ml) incubated for 5 days with methylglyoxal (MG) (10 mM) ; C-: negative control (BSA alone), C+: positive control (BSA + MG), R: *R.officinalis*, R/E: *R. officinalis* + 3% of ethanol, from F1 to F10: fraction (1.25 mg/ml) + BSA + MG. The red line indicates the migration of the positive control as a reference

Concerning the EO of *P.asperum*, 50%of the fractions (F2, F3, F4, F5 and F9) inhibit the glycation (Figure 3).These fractions represent 85.98% of the total mass of this oil. This gives an idea about the presence of an antagonistic effect that dominates lightly the synergistic effect between molecules, because this oil inhibits slightly the BSA glycation (Figure. 3). Thus, the small antiglycation effect of this essential oil, at a concentration of 1.25mg/ml, facing an interesting antiradical power (60.51%) could be due to the complexity of its components. The activity of the EO of P. asperum can be attributed to terpenic alcoholsre presenting its major compounds: Citronellol (25.07%), Geraniol (10.46%), Citronellyl formate (10.52%), β-Maaliene (5.93%), 2-isopropyl-5-methyl-cyclohexanone (5.88%), which constitute 57% of the total composition.

The extracts of all the spices tested displayed good antiglycation ability, though *R.officinalis* extract showed the highest potential. In addition, we founded that the ethanol has no effect on the migration of all crude extract bands, so we can use it us solvent for EO. Glycation is known to be associated with increased free radical production. Therefore, agents that possess good antioxidant activity by mopping up free radicals can simultaneously inhibit the formation of advanced glycation end products.[38] As such, some fractions from these spices can effectively serve as an antioxidant and antiglycation agent in the diets of diabetics.

Many plants have shown anti-glycation activities due to their high phenolic content.[37] The dicarbonyl intermediates, such as methylglyoxal, are mediators of formation of advanced glycation end products and are known to react with lysine residues, arginine and cysteine to form glycosylamine cross linked proteins.[39] According to various studies, glycation is the major source of ROS and reactive dicarbonyl intermediates that are generated by oxidative and non-oxidative pathways of glycation. Thus, acorrelation exists between the potential of scavenging free radicals and *in vitro* inhibition of AGEs formation.[40]

The *in vivo* accumulation of AGEs is known for its role in atherosclerosis, the pathophysiology of aging and chronic diabetic complications. Hyperglycemia, which is the origin of all the vascular complications associated with diabetes, promotes glycation and may cause intra or intermolecular protein level.[41] Free radicals attack biological macro molecules in the direct environment of their place of production. Thus, the formed lipid hydro peroxides are degraded mainly to malondialdehyde (MDA)[42] and 4-hydroxynonenal (4-HNE)[43] covalently reacting with proteins and inactivate their activity. These products are highly toxic because they can also modify the DNA and are involved in apoptotic mechanisms.[44]

CONCLUSION

The work of this paper showed some differences in the chemical composition of the three EO original from Taounat, Morocco. The discrepencies with other works[7,9,15]

are probably attributed to several factors: genetics, environmental and technological, which may influence these oils biological activity. These EO and especially their fractions, havean interesting antioxidant power, coupled with anti-glycation activity. These results orient us to study the effects of a wide variety of medicinal plant extracts from Morocco, that are not studied to date, in the protection against alteration of proteinin order to select those that can prevent diabetic complications related toglycation and oxidative stress. Thus, the use of plants could represent a good alternative to expensive drugs for the rural population of Morocco. This work could lead to the discovery of new molecules presenting interesting activities to fight diabetes; so, future work will aim to assess the therapeutic value of these medicinal plants, their active principles and their toxicities. In addition, this study will continue by the search for other biological properties of these plants, such as antibacterial, anti-inflammatory and antiviral activities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Sepici A, Gurbuz I, Cevik C, Yesilada E. Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits. J Ethnopharmacol. 2004; 93(2): 311-8.
2. Rahimi R, Nikfar S, Larijani B, *et al.* A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacothe. 2005; 59(7): 365–73.
3. Brownlee M, Vlassara H, Cerami A. Inhibition of heparin-catalyzed human antithrombin III activity by nonenzymatic glycosylation. Possible role in fibrin deposition in diabetes. Diabetes 1986; 33(6): 532-5.
4. Osawa T, Kato Y. Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann NY Acad Sci. 2005; 1043(1): 440–51.
5. Ayadi S, Jerribi C, Abderrabba M. Extraction et étude des huiles essentielles de *Rosmarinus Officinalis* cueillie dans trois régions différentes de la Tunisie. Journal de la Société Algérienne de Chimie J. Soc. Alger. Chim. 2011; 21(1): 25-33.
6. Inatani Reiko, Nakatani Nobuji, Fuwa Hidetsugu. Antioxidative Effect of the Constituents of Rosemary (*Rosmarinus officinalis* L.) and Their Derivatives. Agricultural and Biological Chemistry 1983; 47(3) : 521-8.
7. Bouhdid S, Skali SN, Idaomar M, Zhiri A, Baudoux D, Amensour M, *et al.* Antibacterial and antioxidant activities of *Origanum compactum* essential oil African Journal of Biotechnology 2008 ; 7(10) : 1563-70.
8. Mezzoug N, Elhadri A, Dallouh A, Amkiss S, Skali NS, Abrini J, *et al.* Investigation of the mutagenic and antimutagenic effects of *Origanum compactum* essential oil and some of its constituents. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 2007; 629(2): 100–10.
9. Said Omar, Said Hassane, Mohamed Ghanmi, Badr Satrani, Nazik Mansouri, Hayria Mohamed, *et al* . Composition chimique et activités antibactériennes, antifongiques et antioxydante de l'huile essentielle de *Pelargonium asperum* Ehrh. ex Wilde des Comores. Acta Botanica Gallica. 2011; 158(2): 225-37.
10. Carré J. Le poivre. *Bull. Madagascar* 1953; 74(75): 48-82.
11. Greche H, Mrabet N, Ismaili-Alaoui M, Hajjaji N, Boukir A, Benjilali B. Etude phytochimique de *Tanacetum annuum* L. dite Camomille bleue du Maroc. Recherche sur les plantes médicinales et aromatiques, Acte du Congrès International, Hassan Greche & Abdeslam Ennabili (Ed.) ; 2009. P 102-13.
12. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. Journal of Ethnopharmacology 2002; 79(3): 379-81.
13. Yam Faguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from Garcinia indica fruit rind. J. Agric. Food Chem. 2000; 48(2): 180–5.
14. El Babili F, Bouajila J, Souchard JP, Bertrand C, Bellvert F, *et al.* Oregano., chemical analysis and evaluation of its antimalarial, antioxidant, and cytotoxic activities. J Food Sci. Apr; 2011 ; 76(3): C512-8.
15. María Jordána J, Vanesa Lax, María Rota C, Susana Lorán, José A. Sotomayor Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. Food Control 2013; 30(2): 463-8.
16. Pibiri P. Assainissement microbiologique de l'air et du système de ventilation au moyen d'huiles essen-tielles. Thèse de doctorat École polytechnique fédérale. Lausanne 2005 ; 158(2): 161.
17. Greche H, Mrabet N, Ismaili-Alaoui M, Hajjaji N, Benjilali B. Détermination des paramètres de la standardisation de l'huile essentielle de *Tanacetum annuum* L. Recherche sur les plantes médicinales et aromatiques, Acte du Congrès International, Hassan Greche & Abdeslam Ennabili (Ed.); 2009. P 114-5.
18. Van Den Broucke CO, Lemli JA. Chemical investigation of essential oil of *Origanum compactum*. Planta Med. 1980; 41(38): 264-6.
19. Figueredo Gilles. Étude chimique et statistique de la composition d'huiles essentielles d›origans (Lamiaceae) cultivés issus de graines d'origine méditerranéenne. Thèse de doctorat. Université Blaise Pascal; 2007.
20. Degryse AC, Delpla I, Voinier M.A. Risques et bénéfices possibles des huiles essentielles. *Atelier santé environnement* -IGS- EHESP, 2008.p.87.
21. Greche H, Belkheir K, Hajjaji N, Boukir A, Loukili A, Les huiles essentielles « bio » du Maroc. Recherche sur les plantes médicinales et aromatiques, Acte du Congrès International, Hassan Greche & Abdeslam Ennabili (Ed.); 2009. 130-40.
22. Garnéro J. Huiles essentielles. Dossier : K345. Base documentaire : Constantes physico -chimiques. vol. papier n° : K2; 1996.
23. Mishra K, Ojha H, Chaudhury NK. Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. *Food Chemistry*. 2012; 130(4): 1036 –43.
24. Sanchez-Moreno C. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Technol Int. 2002; 8(3): 121–37.
25. Baumann J, Wurn G, Bruchlausen FV. Prostaglandin synthetase inhibiting O2− radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche pharmakologische; 1979. 27-32.
26. Simionatto E, Stüker CZ, Porto C, Dalcol II, Da Silva UF, Morel AF, et al. Essential oil of Pluchea quitoc Dc. (Asteraceae). J Essent Oil Res. 2007; 19: 494–7.
27. Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food

Chem. 2004 ; 85(4) 633–40.

28. Bouhdid S, Skali SN, Idaomar M, Zhiri A, Baudoux D, Amensour M, Abrini et al. Antibacterial and antioxidant activities of *Origanum compactum* essential oil African Journal of Biotechnology 2008; 7(10): 1563-70.
29. Dorman DHJ, Deans SG, Noble RC. Evaluation *in vitro* of plant essential oils as natural anti-oxidants. J. Essent. Oil Res. 1995; 7(6): 645-51.
30. Beretta G, ArtaliR, Maffei Facino R, Gelmini F. An analytical and theoretical approach for the profiling of the antioxidant activity of essential oils : The case of *Rosmarinus officinalis* L. Journal of Pharmaceutical and Biomedical Analysis. 2011; 55(5): 1255–64
31. Bozin Biljana, Neda Mimica-Dukic, Isidora Samojlik, Emilija Jovin. Antimicrobial and Antioxidant Properties of Rosemary and Sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) Essential Oils. J. Agric. Food Chem. 2007; 55 (19): 7879–85.
32. Wang WN. Wu, YG. Zu YJ, Fu. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chemistry 2008; 108(3): 1019–22.
33. Sayed A, Fayed M. Antioxidant and anticancer activities of Citrus reticulate (Petitgrain Mandarin) and *Pelargonium* graveolens (Geranium) essential oils. Research J. Agric. Biol. Sci. 2009; 5(5): 740-7.
34. Lalli JY, Van Zyl RL, Vuuren, Viljoen AM. *In vitro* biological activities of South African Pelargonium (Geraniaceae) species. South African J. Bot. 2008; 74(1): 153-7.
35. Chun SS, Vattem DA, Lin YT, Shetty K. Phenolic antioxidants from clonal oregano (Origanum vulgare) with antimicrobial activity against Helicobacter pylori. *Process* Biochem. 2005; 40(2): 809-16.
36. Safaei-Ghomi J, Ebrahimabadi AH, Djafari-Bidgoli Z, Batooli H. GC/MS analysis and *in vitro* antioxi-dant activity of essential oil and methanol extracts of Thymus caramanicusJalas and its main constituent carvacrol. Food Chemistry 2009; 115(4): 1524-8.
37. Rosa Martha Perez Gutierrez. Inhibition of Advanced Glycation End-Product Formation by *Origanum majorana* L. *in vitro* and in Streptozotocin-Induced Diabetic Rats. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine. Volume 2012, Article ID 598638, 8 pages
38. Ahmad MS, Ahmed N. Antiglycation properties of aged garlic extract: possible role in prevention of diabetic complications. Journal Nutrition 2006; 136 (Suppl. 3): 796S-9S.
39. Monnier VM. Intervention against the Maillard reaction *in vivo*. Archives of Biochemistry and Biophysics 2003; 419(1): 1–15
40. Yokozawa T, Nakagawa T. Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. Food Chem Toxicol 2004; 42(6): 975-81.
41. Lunceford N, Gugliucci A. Ilex paraguariensis extracts inhibit AGE formation more efficiently than green tea. Fitoterapia 2005; 76(5): 419-27.
42. Chaudhary AK, Reddy GR, Blair IA, Marnett LJ. Characterization of an N6-oxopropenyl-2'-deoxyadenosine adduct in malondialdehyde-modified DNA using liquid chromatography/electrospray ionization tandem mass spectrometry. Carcinogenesis 1996; 17(4): 1167-70.
43. Chen HJ, Chung FL. Epoxidation of trans-4-hydroxy-2-nonenal by fatty acid hydroperoxides and hydrogen peroxide. Chem Res Toxicol. 1996; 9(1): 306-12.
44. Ramakrishnan N, McClain DE, Catravas GN. Membranes as sensitive targets in thymocyte apoptosis. *Int J Radiat Biol.* 1993; 63(6): 693-701.