ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF A SERIES OF AROMATIC NITRONES

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Abstract.- *Microbiological tests of a series of nitrones were performed by measuring the minimum inhibitory concentration (MIC) on sixteen targeted microorganisms. The results obtained showed that these compounds exhibited good antimicrobial activity and that this activity was dependent on the substituent on the aryl group of the nitrone. The addition of a surfactant was found to enhance this activity. In addition, the antioxidant activity tests of these nitrones was assessed using three chemical tests: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical, β-carotene bleaching, and "ferric reducing antioxidant power" (FRAP) method. The tests showed that these compounds exhibited an antioxidant capacity similar or better than α-phenyl-N-tert-butylnitrone (PBN) which was used as a reference.*

Keywords: *antioxidant activity, β-carotene test, microbiological test, aromatic nitrone.*

ACTIVITÉ ANTIMICROBIENNE ET ANTIOXYDANTE D'UNE SÉRIE DE NITRONES AROMATIQUE

Résumé.- *Des tests microbiologiques d'une série de nitrones sont effectués. Les résultats obtenus montrent que les composés présentent une bonne activité antimicrobienne et que cette activité est sensible à la substitution. Aussi, l'addition de surfactant a amélioré cette activité. Les résultats sont affinés par la mesure de la CMI sur seize microorganismes cibles. De même, des tests d'activité antioxydant des nitrones en utilisant trois tests chimiques: la méthode de DPPH «1,1-dipheny1-2 picrylhydrazule», la méthode de blanchissement de β carotène et la méthode FRAP «ferric réducing antioxydant» sont réalisés. Ces tests montrent que les composés présentent un pouvoir antioxydant similaire ou meilleur à celui de la nitrone de référence l'α-phényl-N-tertbutylnitrone (PBN).*

Mots clés: *activité antioxydante, test β-carotène, teste microbiologique, nitrone aromatique.*

Introduction

Nitrones are dipolar compounds that contain the azomethine group *N*-oxide ($C=N^{+}-O^{-}$). They are intermediate compounds of many products of biological interest [1,2]. Two common nitrones are known: 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) and *α*-phenyl-*N*-*tert*-butyl nitrone (PBN) (fig.1). The therapeutic effectiveness of nitrones has been recognized for many years [3]. Several nitrones have been synthetized and applied in medicine [4-6] since the therapeutic success of the first nitrone used, *α*-phenyl-*N*-*tert*-butylnitrone (PBN), which showed a protective activity in the case of cerebral ischemia [7]. However, there have been a few reports on their antimicrobial activity [8-10]. A study of their propensity of trapping free radicals *in vitro* or *in vivo* was reported [11].

Figure 1.- The two common nitrones

In a continuation of our work on the biological activity of nitrones [12], we herein present results on the antimicrobial and antioxidant properties of some aromatic ones (fig. 2). A novel facet of this work is the application of a surfactant to enhance these activities by mimicking the biological environment.

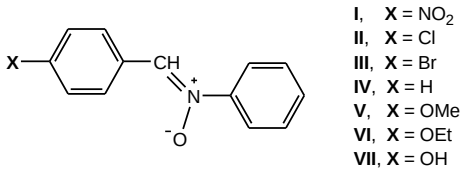

Figure 2.- Aromatic nitrones I-VII

1.- Experimental section

1.1- Chemicals and equipment

Solvents and chemicals were purchased either from Fluka or Prolabo. The chemicals were reagent grades with a purity exceeding 95%. Prior to their use, the microorganisms used were obtained from LBSM Lab (Ecole Normale Supérieure de Kouba, Algiers, Algeria). The experimental aromatic nitrones were synthesized as reported earlier [13]. The antimicrobial activity was determined by the antibiogram method, and the minimum inhibitory concentration (MIC) was realized by the method of agar dilution. The antioxidant activity was evaluated by means of three chemical tests: Ferric reducing antioxidant power (FRAP) [14], *β*-carotene method [15-17], and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [15,18,19]. The latter chemical tests involved either 'simple electron transfer' (SET) or 'hydrogen atom transfer' (HAT) mechanisms or both [20].

The UV-visible analyses were conducted on a SCHIMADZU brand 160 double beam UV-visible spectrophotometer, using ethanol as solvent for DPPH test, water for FRAP test, and emulsion for *β*-carotene.

1.2.- Antimicrobial activity of nitrones

1.2.1.- Antibiotic susceptibility method or Kirby-Bauer disk susceptibility test

Paper disk (Whatman # 3) of 6 mm diameter was aseptically soaked in 20, 40 or 60 µL of a solution of 2 mg of nitrone in 1 mL of a chloroform/1% Tween mixture, and then sterilized with UV radiation. The disk was then placed aseptically in a Petri dish containing the ISP2 (International *Sterptomyces* Project) medium (10 g/l agar) that was seeded previously by the tested microorganism. To ensure better diffusion and distribution of the nitrone within the medium and a momentary inhibition of the growth of the targeted organism, the dish was allowed to stand at 4°C for 3 h, and then incubated at 30°C for 24 h for bacteria or 48 h for fungi. The result was estimated as the inhibition zone or halo diameter.

The ISP2 medium was composed of 4 g/l of yeast extract, 10 g/l of malt extract, 4 g/l of glucose, and 20 g/l of agar. The pH of the solution was adjusted to 7.2 before its sterilization in autoclave for 20 min at 120°C.

1.2.2.- Determination of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentration (MIC) of the assayed nitrone towards the targeted microorganism was determined by the conventional method using the agar dilution in a Petri dish. The DMSO solution of a nitrone was added to the agar system to produce the following final concentrations: 20, 40, 60, 80, 100, 150, 200, 300, and 600 µg/l. A blank test was done in the absence of nitrone, with DMSO alone, and under the same conditions. The agar medium was seeded on surface with the targeted microorganism by spotting a drop of 1µl of the microbial suspension; the density of inoculum was 10^5 colony forming units (CFU) per spot (on the agar medium). Two runs were carried out for each bacterial and fungal strain. The reading of the results started after 24-36 h of incubation at 30°C for bacteria and yeast, and after 36-72 h of incubation for the filamentous fungi. This reading is related to evaluate the microorganism growth and to determine the lowest concentration of antibiotic that prevents the microorganism totally from growing.

1.3.- Antioxidant capacity of nitrones

1.3.1.- Free radical trapping with DPPH

In this method, a color change of the DPPH alcoholic solution occurs upon its reduction with the antioxidant, from deep purple (dark violet) to faint yellow; the former color is attributed to the DPPH radical form which absorbs at λ_{max} = 517 nm, and the latter color for the reduced form of DPPH. The decline in the intensity of the absorbance band λ_{max} = 517 nm of the DPPH radical form is function of the antioxidant activity.

A solution of DPPH radical was prepared by dissolving 4 mg in 100 ml of DMSO. Solutions (5×10^{-3} M) in DMSO of nitrone and the control antioxidants (3,5-di-*tert*-4-butylhydroxytoluene, BHT, and commercial mixture of 2-*tert*-butyl-4-hydroxyanisole, 2-BHA, and 3-*tert*-butyl-4-hydroxyanisole, 3-BHA) were freshly prepared. 200 µL of each of these solutions were introduced into a dry and sterilized test tube. To this were added 800 µl of DMSO and 1 ml of DPPH solution. The whole system was then stirred, and then the

tube was kept under darkness at 25°C for 30 min. Afterwards, the UV absorbance at λ_{max} = 517 nm was measured. Three runs were performed for every nitrone solution. The antioxidant activity (***AA***) was calculated from Eq. (1)

$$\boldsymbol{AA}\ (\%) = (Abs_{blank} - Abs_{test})/Abs_{blank} \times 100 \quad (1)$$

Where Abs_{blank} and Abs_{test} stand for the absorbances of the blank solution (DPPH in methanol) and the test solution, respectively.

1.3.2.- *β-Carotene bleaching*

This method consists in the follow-up of the discoloration of *β*-carotene at λ_{max} = 470 nm, which stemmed from its oxidation by the products of the decomposition of linoleic acid. The addition of an antioxidant would induce a delay in the kinetics of this discoloration.

An emulsion of *β*-carotene/linoleic acid was produced by dissolving 2 mg of *β*-carotene in 1 ml of chloroform, followed by addition of 25 µl of linoleic acid and 200 mg of Tween. After evaporating chloroform completely by means of a rotary evaporator, 100 ml of oxygen-saturated water were added to afford an emulsion which was vigorously agitated. A test tube was charged with 2.5 ml of the thus-made emulsion to which 350 µl of nitrone solution (5×10^{-3} M in DMSO) was added. The mixture was incubated under darkness at room temperature. This experiment was triplicated for each nitrone solution. The same procedure was applied to the control solutions (BHT and BHA solutions) and to a blank one (ethanol only) under identical conditions. The discoloration kinetics of the emulsion in the presence and in the absence of nitrone was tracked at regular time intervals during 48 h. The relative antioxidant activity (*RAA*) was drawn from Eq. (2).

$$RAA\ (\%) = (Abs_{test}/Abs_{BHT})\ x100 \quad (2)$$

Where Abs_{test} and Abs_{BHT} are the absorbance values after 48 h of the tested sample and BHT, respectively.

1.3.3.- Ferric reducing antioxidant power (FRAP)

This method relies on the ability of an experimented species of reducing ferric ion (Fe^{3+}) of the complex $K_3Fe(CN)_6$ to ferrous one (Fe^{2+}). An increase in the absorbance of the reaction medium at λ_{max} = 700 nm is usually assigned to an increase in the reducing power of the experimented species.

To 1 ml of the nitrone solution (5×10^{-3} M in DMSO) 2.5 ml of phosphate buffer solution (0.2 M, pH = 6.6) and 2.5 ml of potassium ferricyanide solution (1%) were added. The mixture was incubated at 50 °C for 30 min after which 2.5 ml of trichloroacetic acid solution (10%) were added. The whole system was then centrifuged for 10 min at 3000 rpm. 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ solution (0.1%) were added to a 2.5 ml portion of the supernatant layer, and the UV-visible absorbance of the generated mixture was measured at λ_{max} = 700 nm. Ascorbic acid, BHT and BHA were employed as positive controls under identical conditions.

3.- Results and discussion

3.1. - Microbial assays

Preliminary microbial assays of the synthesized aromatic nitrones (fig. 2) in form of chloroform solutions and in the presence of Tween, a non-ionic surfactant, were performed. The latter surfactant, devoid of antimicrobial activity, would tremendously improve the diffusion of the antibiotic (nitrone) into the aqueous culture medium.

The targeted microorganisms were: two fungal strains (*Aspergillus niger* and *Fusarium oxysporum* f. sp. *lini*), one yeast (*Candida albicans*), and three bacterial strains (2 Gram-positive: *Bacillus subtilis* and *Staphylococcus aureus*, one Gram-negative: *Escherichia coli* Gram). The different results are summarized in table I.

Table I.- Diameters of inhibition zones of the different microorganisms with synthesized aromatic nitrones (- : No activity; D: delay in growth; N: net inhibition)

Microorganism	Amount of nitrones (µg)	Zone inhibition diameter (mm)						
		PBN	I	II	III	IV	V	VI
Bacillus subtilis **ATCC 6633**	20	-	-	-	-	9 (D)	8 (D)	-
	40	-	7 (N)	-	-	11 (D)	9 (D)	-
	100	8 (N)	10 (N)	9 (N)	8 (D)	12 (N)	9 (N)	-
Staphylococcus aureus **43300**	20	-	-	8 (N)		-		17 (N)
	40	-	-	10 (N)	-	-	16 (N)	18 (N)
	100	-	8 (N)	13 (N)	-	19 (N)	20 (N)	20 (N)
Escherichia coli **CIP 54.8**	20	-	-	-	-	-	-	-
	40	-	-	-	-	7 (D)	-	-
	100	-	-	-	-	8 (D)	-	-
Aspergillus niger **ATCC 16404**	20	-	-	-	-	-	8 (D)	/
	40	-	-	-	-	-	9 (D)	13 (N)
	100	-	8 (N)	-	-	-	11 (N)	18 (N)
Fusarium oxysporum* f. sp. *lini	20	-	10 (D)	8 (D)	9 (D)	10 (D)	14 (N)	/
	40	-	12 (N)	11 (N)	11(D)	13 (N)	18 (N)	14 (N)
	100	9 (N)	16 (N)	14 (N)	15(N)	14 (N)	16 (N)	16 (N)
Candida albicans **ATCC 16404**	20	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-
	100	-	-	-	-	12 (N)	12 (N)	-

We previously reported that these nitrones had no antibacterial activity and their fungal activity was limited only to non-substituted, methoxylated and ethoxylated *C*, *N*-diphenyl nitrones [12]. The present study demonstrated that the adjunction of Tween allowed better solubility of the nitrones in the aqueous culture medium, and consequently enhancing their biological activity. Indeed, as shown in Table I, their antibacterial capacity was greater than that of PBN. This activity increase with nitrone concentration, and the activity dependent on the strain tested. For instance, the growth of *Bacillus subtilis*, known for its high sensitivity towards antibiotics, could be inhibited by non-substituted and methoxylated *C*, *N*-diphenyl nitrones at low concentrations; however, higher concentrations were required for the remaining nitrones to induce equal inhibition. Furthermore, the biological activity was found to be depended on the nature of the substituent on the aromatic ring of the nitrone. For example, *Echerichia coli* showed an appreciable solidity towards all nitrones with the exception of non-substituted *C*, *N*-diphenyl nitrone. In addition, *Candida albicans* resisted towards all nitrones but failed towards non-substituted

and methoxylated *C*, *N*-diphenyl nitrones.

To endorse these observations, the minimum inhibitory concentrations (MIC) of nitrones on sixteen targeted microorganisms were measured, and were compared with that of PBN. Most of the tested microorganisms are either pathogenic or toxigenic towards humans and are multi-antibiotic resistant [21].

Microorganisms with these properties are: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* S1, *Escherichia coli* E195, *Klebsiella pneumoniae* E40, *Candida albicans*, *Saccharomyces cerevisiae* ATCC 4226, *Fusarium culmorum* FC200, *Fusarium graminearum* FG3, *Fusarium monileforme*, *Fusarium oxysporum* f. sp. *lini*, *Fusarium proliferatum*, *Aspergillus ochraceus*, *Aspergillus flavus* AF3, *Aspergillus carbonarius* M333, *Penicillium glabrum* PG1, and *Mucor ramannianus*. Table II showed the MICs of the aromatic nitrones of the pathogenic microorganisms. A significant effect of substitution in the aryl group of the nitrone on their antimicrobial activity is observed. In fact, nitrones **I** and **II** with nitro and chlorine substituents, respectively, exhibited MICs equal or greater than 600 µg/ml against all tested microorganisms. The MICs of brominated aromatic nitrone **III** towards all the tested microorganisms were equal or higher than 300 µg/ml. Those of non-substituted, methoxylated, and ethoxylated aromatic nitrones **V**, **VI**, and **VII**, ranged between 75 and 150 µg/ml against yeast strains, between 30 and 150 µg/ml against filamentous fungi, between 40 and 150 µg/ml against the Gram-positive bacteria, and finally between 50 et 300 µg/ml against Gram-negative bacteria.

These results would indicate that methoxylated and ethoxylated aromatic nitrones **VI** and **VII** proved to have higher antimicrobial activity than PBN; in most cases, their MICs were lower than those of PBN towards all microorganisms tested.

Table II.- Minimum inhibitory concentrations, MICs of aromatic nitrones against the tested microorganisms (a: Gram-positive bacteria; b: Gram-negative bacteria; c: yeast; d: fungi, D: delay in growth)

Microorganisms		MIC (µg/ml) of tested nitrones						
		I	**II**	**III**	**IV**	**V**	**VI**	**PBN**
a	***Bacillus subtilis* (ATCC 6633)**	> 600	> 600	> 300	150	40	40	150
	***Staphylococcus aureus* (S1)**	> 600	> 600	> 300	150	75	50	> 300
b	***Klebsiella pneumoniae* (E40)**	> 600	> 600	> 300	300	300 (D)	300 (D)	> 300
	***Escherichia coli* (E195)**	> 600	> 600	> 300	300	300 (D)	300 (D)	> 300
c	***Candida albicans***	> 600	> 600	300	150 (D)	75	75	> 300
	***Saccharomyces cerevisia* (ATCC 4226)**	> 600	> 600	300	150 (D)	75	75	50
d	***Fusarium culmorum* (FC200)**	> 600	> 600	300	150 (D)	75	75	> 300
	***Fusarium graminearum* (FG3)**	600 (D)	> 600	300	150 (D)	50	20	> 300
	Fusarium monileforme	> 600	600	300	150	75	75	150 (D)
	Fusarium oxysporum* f. sp. *lini	> 600	> 600	300	150	50	50	> 300

	Fusarium proliferatum	> 600	> 600	300	150	75	75	300 (D)
	Aspergillus ochraceus	600 (D)	> 600	300	150	50	50	300
	***Aspergillus flavus* (AF3)**	> 600	600	300	150	75	75	> 300
	***Aspergillus carbonarius* (M333)**	600	> 600	300	150	50	30	300
	***Penicillium glabrum* (PG1)**	600	> 600	300	100	75	75	> 300
	Mucor ramannianus	600	600	300	150	75	75	> 300

3.2.- Antioxidant assays

Results of the tests of antioxidant activity of nitrones **I-VII** and of the controls are summarized in table III.

Table III.- Results of antioxidant assays of nitrones (ND = non determined)

Nitrone and standard antioxidant	DPPH	FRAP	*β*- Carotene
I	4.36	0.6240	ND
II	5.95	0.6142	ND
III	3.73	0.6030	ND
IV	8.65	0.5251	20.88
V	5.57	0.5853	24.99
VI	4.61	0.5246	23.67
VII	15.66	0.7292	31.19
BHA	89.91	0.5599	99.99
BHT	84.93	0.6162	99.62
PBN	9.07	0.7600	19.56
Negative Control		0.0661	07.56

The antioxidant activity of nitrones is clearly related to the substituent on the aromatic ring as revealed by DPPH free radical scavenging results. It is interesting to note the better activity of the hydroxylated nitrone **VII**, with a DPPH trapping of 15.66%; indeed, its DPPH trapping exceeded those of the remaining ones by almost three to five fold, resulting from its double scavenging action. The first action is the trapping of the free radical by the nitrone group via its *α*-carbon atom of azomethine group *N*-oxide ($C=N^{+}-O^{-}$), giving rise to a spin nitroxide adduct as shown in figure 3.

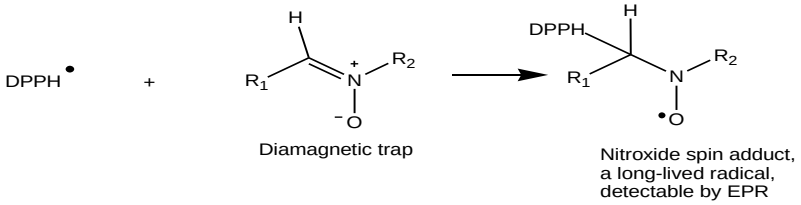

Figure 3.- Mechanism of the DPPH radical trapping by nitrone group.

The second trapping action is linked to the hydroxyl group of the phenol moiety. Phenols are commonly known as powerful free radical scavenging, according to the mechanism traced in figure 4. Phenols are known for their inhibition of the radical polymerization of vinyl monomers. Furthermore, this action of phenols was observed in the study of the antiradical activity of a set of *C*-aryl, *N*-alkyl nitrones by DPPH trapping

method [22]. Moreover, the radical trapping of hydroxyl group in *para* position of benzene ring was more evident than in *meta* position.

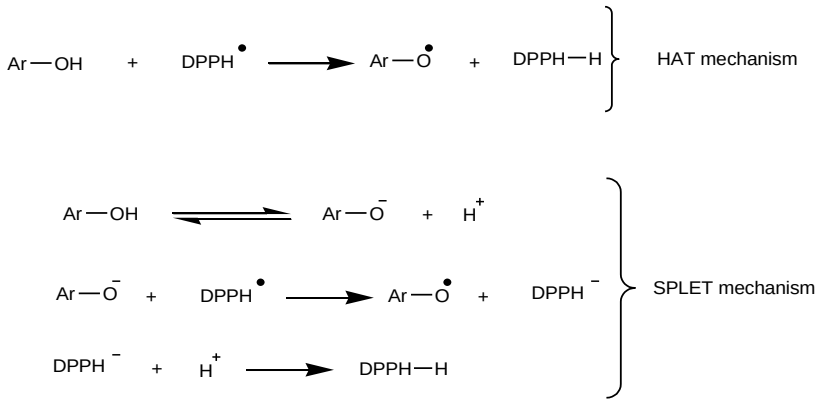

Figure 4.- Mechanisms of DPPH radical trapping by phenols Ar-OH: Hydrogen atom transfer, HAT; Sequential proton-loss electron-transfer, SPLET.

Even though the antioxidant activity of the nitrones is far lower than that of standard phenolic oxidants: BHT and BHA, the present results are promising because their DPPH trapping nears (in the case of **I**-**VI**) or higher (in the case of **VII**) that of PBN. Moreover, the *β*-carotene method revealed an antioxidant property for nitrones **IV**-**VII** greater than that of PBN. However, with the exception of nitrones **IV** and **VI**, the results of FRAP method suggest that the remaining nitrones have about equal or higher antioxidant activity than those of standards.

Conclusion

The *C*-aryl-*N*-phenylnitrones present an antimicrobial activity that can be improved by addition of a surfactant. The extent of such activity depends on the substituent on the aryl group of the nitrone. Because they display MICs lower than that of PBN, nitrones with methoxy and ethoxy substituents may be conceived for meningitis treatment as proved for PNB.

Through all the three methods (DPPH, FRAP, *β*-carotene), these nitrones were shown to have antioxidant activity lower than that of BHT and BHA, but greater than that of PBN. Best of all, the antioxidant activity of the phenolic nitrone approaches that of the standards.

References

[1].- Merino P., Tejero T., 1999.- Polyalkoxy nitrones as chiral building blocks in asymmetric synthesis, Molecules, 4: 169–179.

[2].- Dagoneau C., Tomassini A., Denis J.-N., Vallée Y., 2001.- A short synthesis of γ-amino acids from nitrones: synthesis of vigabatrin, Synthesis, 1: 150–154.

[3].- Floyd R. A., Hensley K., Forester M. J., Kelleher-Andersson J. A., Wood P. L., 2002.- Nitrones, their value as therapeutics and probes to understand aging, Mechanisms of Ageing and Development, 123: 1021–1031.

[4].- Floyd R. A., Hensley K., Forester M. J., Kelleher-Andersson J. A., Wood P. L., 2002.- Nitrones as neuroprotectants and antiaging drugs, Annals of the New York Academy of Science, 959: 321–329.

[5].- Seo Y. M., Nam K. H., Kang P. S., Ko S. B., Oh E., Sung M. T., Choi B. W., Lee B. H., Park J. H., 2007.- New cholinesterase inhibitor, lipoic acid-nitrone derivatives, Bulletin of the Korean Chemical Society, 27: 225–228.

[6].- Green A. R., Ashwood T., Odergren T., Jackson D. M., 2003.- Nitrones as neuroprotective agents in cerebral ischemia, with particular reference to NXY-059. Pharmacology and Therapeutics,100: 195–214.

[7].- Coutts R. T., Hindmarsh K. W., Myers G. E., 1970.- Quinoline *N*-oxides and hydroxamic acids with antibacterial properties, Canadian Journal of Chemistry, 48: 2393–2396.

[8].- Thirumalaikumar M., Sivasubramanian S., Ponnuswamy A., Mohan P., 1996.- Synthesis, reactions and antimicrobial studies of α-Aryl-*N*-(2-nitrocyclohexyl) nitrones. European Journal of Medicinal Chemistry, 31: 905–908.

[9].- Danish I. A., Prasad K.J.R., 2003.- A one pot synthesis and evaluation of 13-oxo-quino[3,4-*b*]carbazol-*N*-oxides as antimicrobial agents. Acta Pharmaceutica, 53: 287-294.

[10].- Molnar M., Lennmyr, F., 2010.- Neuroprotection by S-PBN in hyperglycemic ischemic brain injury in rats. Upsala journal of medical sciences,115: 163–168.

[11].- Hamza K., Touati A., Ait Yahia A., Meklat A., 2008.- Mise en évidence de l'activité antimicrobienne de deux séries de nitrones et d'isoxazolidines synthétisées. Sciences et Technologie C, 28: 65–72.

[12].- Hamza K., Touati A., Ait Yahia A., Baltas M., Moulay S., 2010.- Study of the reactivity of aromatic nitrones towards acrylonitrile in microemulsion: a green chemistry. Asian Journal of Chemistry, 22: 1231–1244.

[13].- Oyaizu M., 1986.- Studies on products of browning reaction. antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics, 44: 307–315.

[14].- Bourkhiss M., Hnach M., Paolini J., Costa J., Farah A., Satrani B., 2010.- Propriétés antioxydantes et anti-inflammatoires des huiles essentielles des différentes parties de *Tetraclinis Articulata* (Vahl) Masters du Maroc. Bulletin de la Société royale des sciences de Liège, 79: 141–154.

[15].- Marco G. J., 1968.- A Rapid method for evaluation of antioxidants. Journal of the American Oil Chemists Society, 45: 594–598.

[16].- Miller H. E., 1971.- Simplified method for the evaluation of antioxidants. Journal of the American Oil Chemists Society, 48: 91–91.

[17].- Bois M. S., 1958.- Antioxidant determinations by the use of stable free radical. Nature, 181: 1199–1200.

[18].- Brand-Williams W., Cuvelier M.E., Berset C., 1995.- Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28: 25–30.

[19].- Prior R. L., Wu X., Schaich K., 2005.- Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53: 4290–4302.

[20].- Aouiche A., Sabaou N., Meklat A., Zitouni A., Mathieu F., Lebrihi A., 2012.- Activité antimicrobienne de *Streptomyces* sp. pal111 d'origine saharienne contre divers microorganismes cliniques et toxinogènes résistants aux antibiotiques, Journal de Mycologie Médicale, 22: 42–51.

[21].- Samadi A., Soriano E., Revuelta J., Valderas C., Chioua M., Garrido I., Bartolomé B., Tomassolli I., Ismaili L., Gonzáles-Lafuente L., Villarroya M., Garcia A.G., Oset-Gasque M.J., Marco-Contelles J., 2011.- Structure, theoretical and experimental *in vitro* antioxidant/pharmacological properties of *α*-aryl, *N*-alkyl nitrones, as potential agents for the treatment of cerebral ischemia. Bioorganic and Medicinal Chemistry, 19: 951–960.