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Synthesis of New Azomethine-N-oxide compounds and Study of Their Antifungal andAntioxidant Activities

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Abstract

In the present research two nitrones, azomethine-N-oxides, were synthesized. The structures of the synthesized compounds have been confirmed on the basis of elemental analysis, FT-IR, ^{1}H-NMR spectral studies. The nitrones were screened for their antifungal activity against *Asperigillus niger* and *Aspergillus flavus* and the zone of inhibition was determined by disc diffusion technique. Both nitrones exhibited promising antifungal activity against fungi as compared with standard drug. The antioxidant properties for the synthesized nitrones were studied by using two in vitro models, such as conjugated diene and reducing power systems. From the results, found that both nitrones have good antioxidant activity compared with using the control, α-tochopherol.

Keywords: Nitrone, antifungal activity, reducing power, conjugated diene and azomethine-N-oxide

1. Introduction

The chemical structure of nitrone, azomethine-N-oxide, in its simplest form can be represented as R'-CH=N(O)-R". Nitrones began to be used in analytical chemistry applications[1]. The characteristic feature of nitrones consists a high reactivity with respect to active radicals. Free radicals play a significant role in the development of a wide range of pathological states, such as atherosclerosis, Alzheimer.s disease, cancer, ischemia, diabetes and ageing[2, 3]. The reaction of the free radical species ($R^{\bullet}$) with a nitrone yields a product termed the spin-adduct R'-CHR-N($O^{\bullet}$)- R"[4]. The nitroxyl free radical spin-adduct is usually much more stable than the free radical therefore making it possible in principle to characterize the original free radical trapped. The nitrone group (-C=N(O)-) acts as a radical scavenger and reduces oxidative stress. The present study is concerned with antioxidant properties of nitrones were assayed by the conjugated diene and reducing power

methods as well evaluation of their antifungal activity[5, 6].

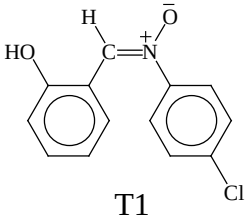
2. Experimental

2.1 Materials and methods.

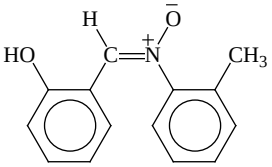
All reagents used were chemically pure. Melting points are uncorrected and were taken in open capillaries on a Gallenkamp apparatus. Elemental analyses (C.H.N.) were recorded using EuroVector model EA3000A (Italy) in the central laboratory of AL-ALBAYET University, Jordan. IR spectra of the nitrones were recorded on FT-IR 8400S SHIMADZU(Japan) as KBr disk in Petrochemical industry company Iraq/Basrah. ^{1}H-NMR spectra of the synthesized compounds were recorded on Bruker model ultra shield 300MHz (Switzerland) using DMSO as solvent and TMS as the internal standard in central laboratories in Al-Albayet University/ Jordan.

2.2 Synthesis of nitrones of salicylaldehyde, T1 and T2[7, 8]

In to 100 ml round bottom flask, N-arylhydroxylamine[9] (0.1 mole), N-(*p*-chlorophenyl)hydroxylamine or N-(*o*-methylphenyl)hydroxylamine, was dissolved in CH_2Cl_2 (30 ml). Salicylaldehyde (0.1 mole) in 10 ml CH_2Cl_2 was added and the mixture was stirred overnight. The reaction mixture was transferred to a separator funnel, and H_2O (40 ml) was added. The organic phase was removed; the aqueous solution was extracted with CH_2Cl_2 (2 x 40 mL), and the organic phases were combined and dried with over Na_2SO_4 then filtered and concentrated in vacuum. The product was purified by column chromatography. Eluent solvent was (hexane: EtOAc 7:3). The information of nitrones are in Table1.



T1



T2

Table 1: The characteristics of synthesized compounds

Compound	Molecular formula	Name of compound	Melting point ^{o}C
T1	$C_{13}H_{10}ClNO$	C-(2-hydroxyphenyl)-N-(4-chlorophenyl) Nitrone	120-122
T2	$C_{14}H_{13}NO$	C-(2-hydroxyphenyl)-N-(2 -methylphenyl) Nitrone	160-162

2.3 Antioxidant activity assay.

1) Conjugated diene method[10, 11].

For each synthesized nitrone, amounts (0.5–5 mg/ml) in ethanol (100 µl) were mixed with 2 ml of 10 mM linoleic acid emulsion in 200 mM sodium phosphate buffer (pH 6.5) in test tubes and placed in darkness at 37 ^{o}C to accelerate oxidation. After incubation for 15 h, 6 ml of 60% methanol in distilled water were added, and the absorbance of the mixture was measured at 234 nm against a blank UV-visible spectrophotometer. The antioxidant activity was calculated as follows: antioxidant activity (%) = [(DA_{234} of control – DA_{234} of sample)/DA_{234} of control]-100. A control consisted of ethanol and the reagent solution without synthesized compounds added and the procedure was carried out as described above. α-Tochopherol was used for comparison.

2) Reducing power antioxidant[12]

The reducing power was determined according to the following procedure. Each nitrone (0.5–5 mg/ ml) in ethanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.5) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 ^{o}C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 200g for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank in UV-visible spectrophotometer. A higher absorbance indicates a higher reducing power. α-Tocopherol was used as controls.

2.4 Antifungal activity.

The test was performed according to the disk diffusion method. The prepared nitrones were tested against pathogenic fungi of *Asperigillus niger* and *Aspergillus flavus* were obtained from Department of Biology/ University of Basrah. Nitrones were dissolved (1000 µg/ml) in dimethylsulfoxide (DMSO). The growth medium is Sabarod's agar media was used and was poured into the petri-dishes and allowed to solidify. Whatman No. 1 filter paper disk of 6 mm diameter were sterilized by autoclaving. The sterile disks were impregnated with different compounds (1000 µg/ml). The impregnated disks were placed on the media suitably spaced apart and the plates were incubated for 72 hr. At the end of the period, the inhibition zones formed on media were measured with a zone in millimeters[13, 14].

3. Results and discussion

3.1 Elemental analysis

The results of the elemental analysis of prepared compounds (Table 2) are in a good agreement with the calculated values.

Table 2: Elemental analysis data.

Compound	Elemental analysis %	Found	Calculated
T1	C	62.70	63.04
	H	4.10	4.07
	N	5.73	5.66
T2	C	74.39	73.99
	H	5.71	5.77
	N	6.46	6.16

3.2 IR spectra

In the IR spectra as shown in Figures 1 and 2, the nitrones exhibited characteristic bands in the region 1652-1654 cm^{-1} which is characteristic of the azomethine nitrone group (–HC=N(O)-)[15]. Both nitrones showed strong absorption bands due to the out of plane (o. o. p.) C–H bending of the aromatic ring in the range 682–756 cm^{-1}. The peak observed in the range 3056–3096 cm^{-1} in IR spectra was assigned to aromatic stretching vibration of C-H bond[16]. Also both compounds show very strong bands at 1452-1595 cm^{-1} attributed to the C=C group of the aromatic ring. The absence of the band at 3300-3400 cm^{-1} in the IR spectra of nitrones showed a strong evidence of strong intramolecular H-bonding. This observation was found to be in accordance with the literature report on similar systems[17]. Compound T2 showed the absorption bands at 2862 cm^{-1} and 2992 cm^{-1} which assignable to the stretching vibration of the

aliphatic C-H bond subjected to the methyl group. The other characteristic absorption bands[15, 16, 18] for the two compounds are listed in Table 3.

Table3: FT-IR data (cm^{-1}) of prepared compounds

Compound	C=N	N-O	C-O	C=C	Ar-H		Other vibrations
					bending	stretching	
T1	1654	1278-1311	1033	1467-1595	682 756	3066	C-Cl 1089
T2	1652	1276-1301	1029	1452-1593	756 700	3056 3096	aliphatic C-H 2992, 2862

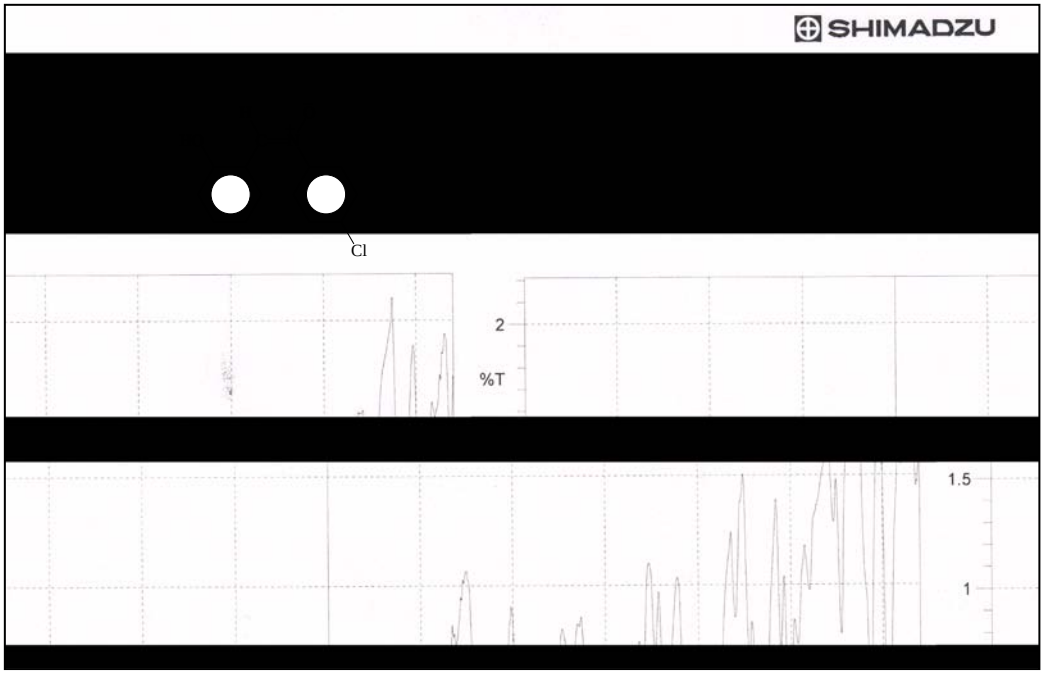


Figure 1: FT-IR spectrum of compound T1

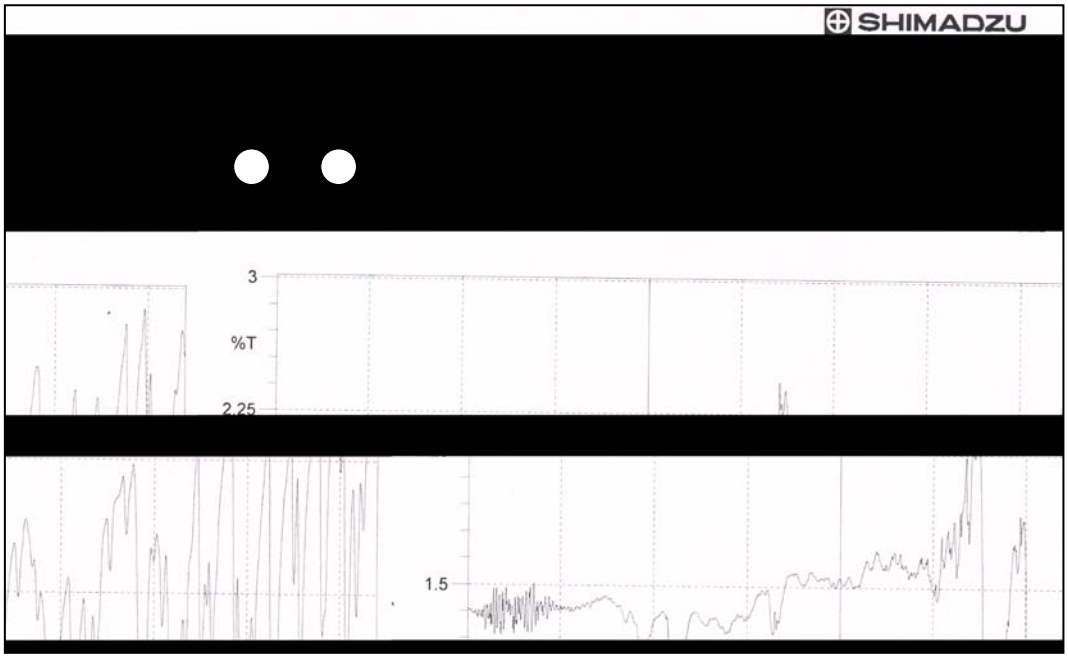


Figure 2: FT-IR spectrum of compound T2

3.3 ^{1}H-NMR spectra

The ^{1}H-NMR spectral data in deturated DMSO solution of the prepared nitrones are listed in Table 4. The spectra of the compounds, as shown in Figures 3 and 4, showed a singlet at 8.855 ppm (T1) and 8.851 ppm (T2) which has been assigned to the proton H-α for the azomethine nitrone (–HC=N(O)-)[15]. It should be noted that the phenolic protons have always given a sharp singlet at high δ values 12.51 ppm for compound T1 and 12.63 ppm for compound T2 which confirms its involvement in an intramolecular hydrogen bonding[17]. The multi signals within the regions 6.995-8.386 ppm and 6,999-8.043 ppm are assigned to the aromatic protons of T1 and T2, respectively. Methyl protons in compound T2 appears as a singlet signal at 2.450 ppm as shown in Figure 4[19, 20].

Table 4: ^{1}H-NMR data [δ (ppm), J (Hz)] of synthesized compounds

Compound	H-α (s)	O-H (s)	Ar-H (m)	Other signals
T1	8.855	12.51	6.995-8.386 (8H)	-------
T2	8.851	12.63	6.999-8.043 (11H)	C-H(CH_3) 2.450

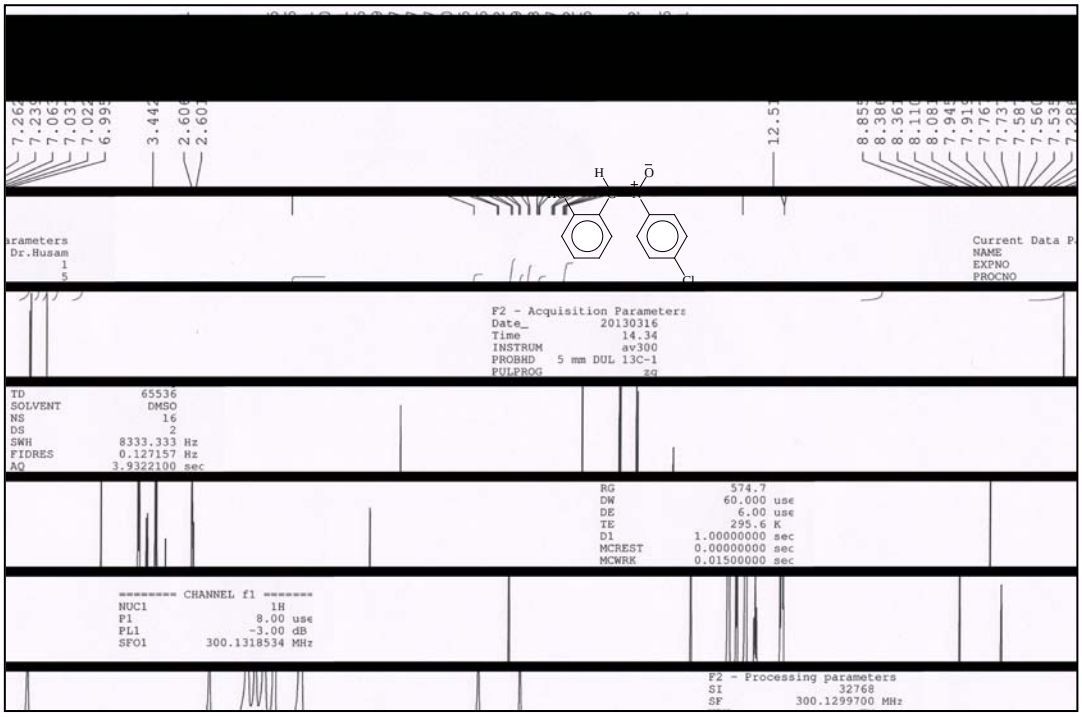


Figure 3: ^{1}H-NMR spectrum of compound T1

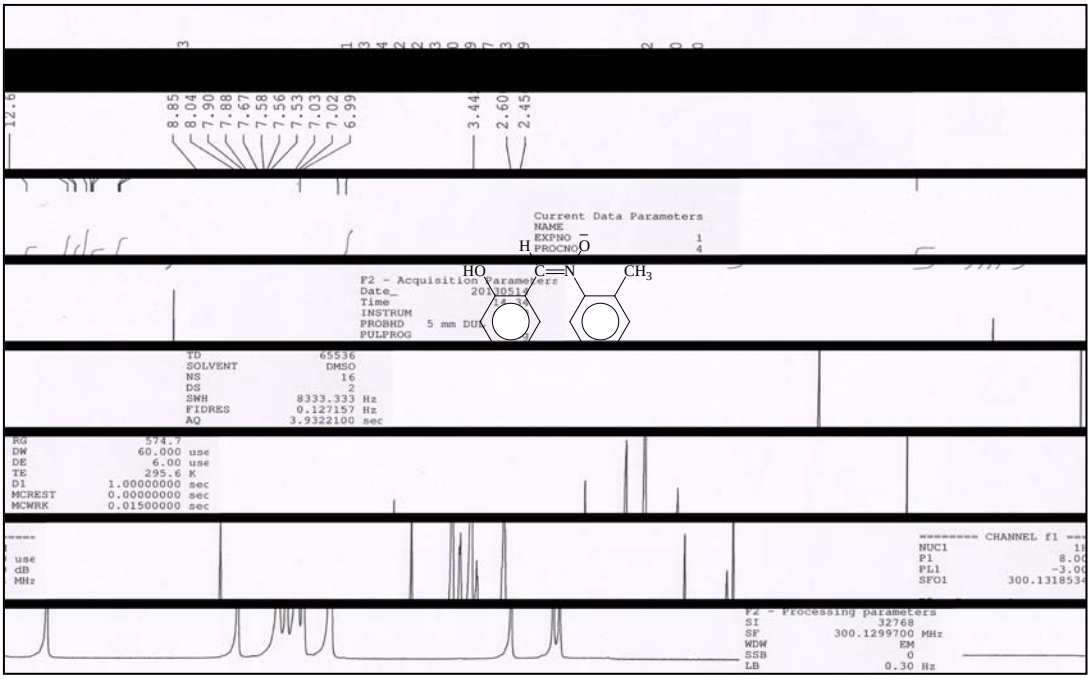


Figure 4: ^{1}H-NMR spectrum of compound T2

3.4 Antioxidant activity

a) Conjugated diene method

The conjugated diene method is concerned with the ability of compound of scavenging the free radicals of linoleic acid which was absorbed at wave length 234 nm [21]. By using this method, both nitrones (T1 and T2) showed an increasing in antioxidant activities as concentration increased from 0.5 mg/ml to 5 mg/ml. As shown in Figure 5, at concentration 0.5 mg/ml the antioxidant activity of nitrone T1 is above 50% as compared to nitrone T2 which indicates that T2 have good antioxidant activity. The antioxidant activity of T2 at high concentration (5 mg/ml) is higher than T1. Generally, the antioxidant activity for T1 at 0.5 mg/ml to 2 mg/ml is higher than that to T2, while the activity of T2 is good at concentrations 3 mg/ml to 5 mg/ml as compared with T1. The antioxidant activity of α-tochopherol is approximated to both nitrones. However, the antioxidant of α-tochopherol is equal to the activity of nitrone T2.

b) Reducing power method

The determination of ferric reducing antioxidant power is a simple direct test for measuring antioxidant capacity. This assay method is often used to evaluate the ability of material to donate of electron. The presence of antioxidant in the tested samples would result in Fe^{+3} reduction complex to the ferrous ion (Fe^{+2}). The resultant Fe^{+2} can be monitored by UV-visible spectrophotometer of Prussian blue, which absorbs at 700 nm [22]. The reducing power of nitrones increased readily along with the increasing of concentration. The results of the reducing power at different concentrations (0.5 to 5 mg/ml) of T1 and T2 are shown in Figure 6. At concentrations 0.5 mg/ml and 1 mg/ml the reducing power were in the order T2 > α-tochopherol > T1. The reducing powers of nitrone T1 at concentrations above 1 mg/ml were higher than those from T2 and α-tochopherol. At all concentrations the reducing power of T2 is high as compared with α-tochopherol and are

parallel. Overall, the reducing power at concentrations 2-5 mg/ml are in the order T1 > T2 > α-tochopherol. From the results, the activities of nitrones are good as compared with α-tochopherol which is used as a control.

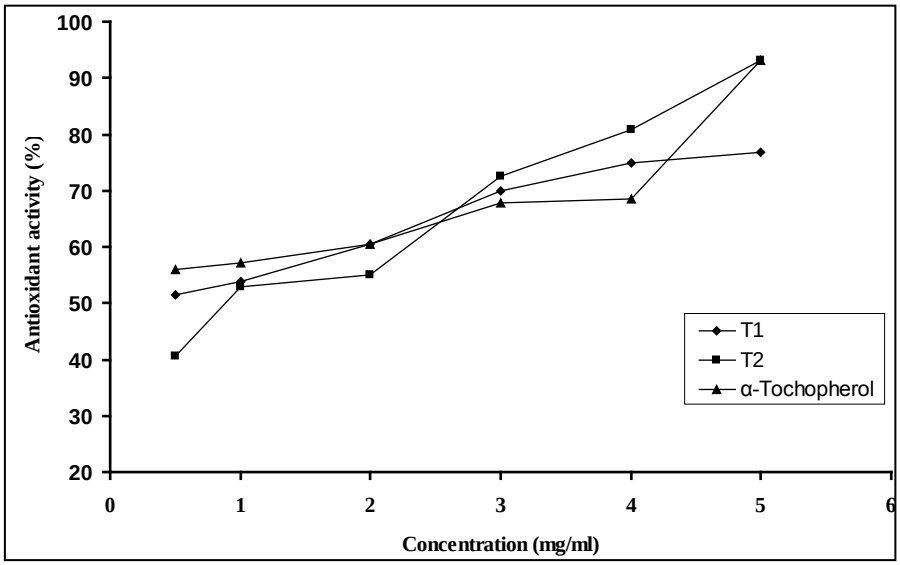


Figure 5: Antioxidant activities of T1, T2 and α-tochopherol

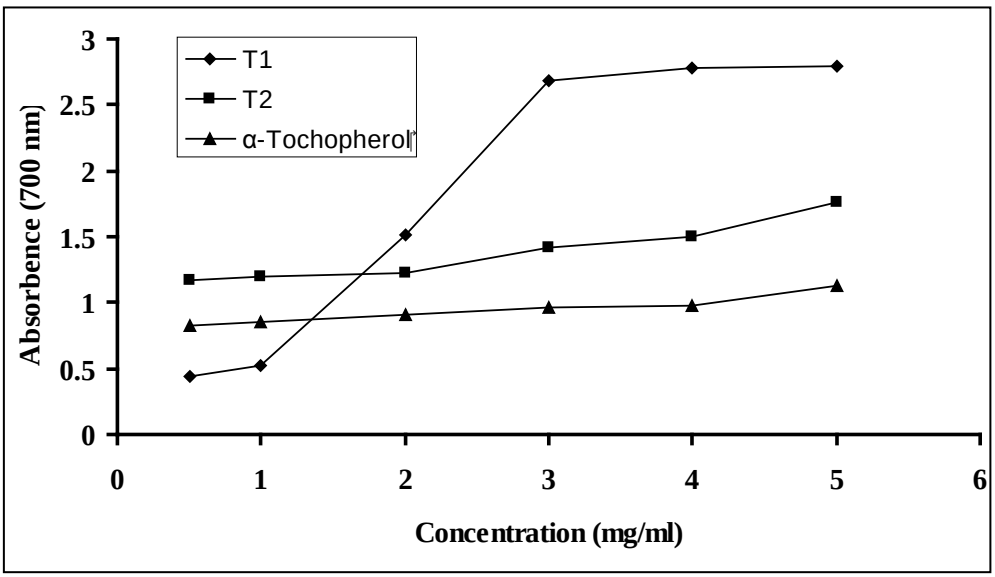


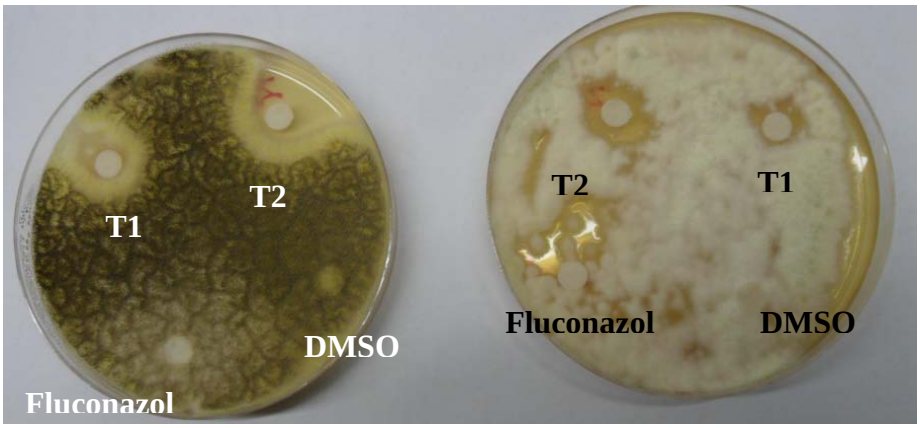
Figure 6: Reducing power activities of T1, T2 and α-tochopherol

3.5 Antifungal activity

From the results (Table 5) obtained by the *in vitro* antifungal activity it is found that the nitrone T2 is more active against all tested fungi. Generally, both nitrones have good antifungal activity (Figure 7) as compared with fluconazole as standard drug, which active against *A. flavus* while inactive against *A. flavus.*

Table 5: Antifungal activity of N-oxides and fluconazole against *A. niger* and *A. flavus*

Compound(1000 µg/ml)	Inhibition zone diameter (mm)	
	A. niger	*A flavus.*
T1	13	6
T2	14	8
Fluconazole	no inhibition	10



A. niger *A. flavus*

Figure 7: Antifungal ctivity of nitrones and fluconazole against *A. niger* and *A. flavus*

4. Conclusions

In the present study two azomethine-N-oxide were prepared which identified with C.H.N., FT-IR and ^{1}H-NMR. The results of antifungal activity were showed that compounds have good and very good antioxidant activities as compared with controls.

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تحضير مركبات ازوميثاين-N-اوكسايد جديدة ودراسة فعاليتها المضادة للأكسدة والفطريات

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المستخلص

تضمنت الدراسة تحضير مركبين نوع ازوميثاين-N-اوكسايد (نايترون). شخصت المركبات المحضرة باستخدام التحليل العنصري الدقيق وكذلك باستخدام الطرق الطيفية منها مطيافية الاشعة تحت الحمراء ومطيافية الرنين النووي المغناطيسي. درست الفعالية المضادة للفطريات ضد نوعين من الفطريات وكانت النتائج جيدة مقارنة بالدواء المستخدم كمرجع. كذلك درست الفعالية المضادة للاكسدة ووجد ايضا ان المركبين يمتلكان فعالية جيدة جدا مقارنة بالمرجع المستخدم وهو فيتامين E.