Effect of Bridging Atom and Hydroxyl Position on the Antioxidant Capacity of Six Phenolic Schiff Bases

Abdelhakim Kheniche[1,2*], Imededdine Kadi[3], Abderrahim Benslama[4], Samiya Rizoug[2], Sarra Bekri[2], and Abdenassar Harrar[1]

[1]*Laboratory of Inorganic Materials, Faculty of Sciences, University of M'sila, 28000 M'sila, Algeria*

[2]*Department of Chemistry, Faculty of Sciences, University of M'sila, 28000 M'sila, Algeria*

[3]*Research Unit in Medicinal Plants (URPM. 3000, Laghouat) Attached to the Research Center of Biotechnology (CRBT. 25000, Constantine)*

[4]*Department of Biochemistry, Faculty of Sciences, University of M'sila, 28000 M'sila, Algeria*

* ***Corresponding author:***

email:
abdelhakim.kheniche@univ-msila.dz

Received: January 19, 2022
Accepted: July 3, 2022

DOI: 10.22146/ijc.72379

Abstract: *A series of new phenolic Schiff bases* N,N-*bis(2,3-dihydroxybenzyl-idene)-4,4'-diphenylmethane (3-DPM), and* N,N-*bis(2,5-dihydroxybenzylidene)-4,4'-diphenylmethane (5-DPM), for sulfide bridge* N,N-*bis(2,3-dihydroxybenzyl-idene)-4,4'-diphenyl sulfide (3-DPS),* N,N-*bis(2,5-dihydroxybenzylidene)-4,4'-diphenyl sulfide (5-DPS),* N,N-*bis(2,3-dihydroxybenzyl-idene)-4,4'-diphenyl disulfide (3-DPSS), and* N,N-*bis(2,5-dihydroxybenzylidene)-4,4'-diphenyl disulfide (5-DPSS) were synthesized by condensation of substituted 4,4'-diamino-bis-(4-aminophenyl) methane/sulfide with various substituted aldehydes. The synthesized molecules were characterized by physical data, elemental, IR and* ^{1}*H-NMR analyses. The antioxidant ability of compounds was determined through the use* in vitro *assays such as DPPH• scavenging, ABTS, total antioxidant capacity (TAC), hydroxyl radical OH• scavenging, and reducing power capability. The antioxidant activity of the compounds increased slightly after changing the atom bridge and hydroxyl group position. The results showed that the compound 5-DPSS exhibited superior scavenging strength against DPPH (EC_{50} = 7.10 ± 0.16 µg/mL), whereas 3-DPSS showed the highest activity (EC_{50} = 1.36 ± 0.08 µg/mL) when inspected by ABTS in relation to butylated hydroxyanisole (BHA) (EC_{50} = 7.54 ± 0.67). The higher OH• activity was marked by the compound 5-DPS (EC_{50} = 44.9 ± 3.3 µg/mL) related to BHT at (EC_{50} = 98.73 ± 0.3 µg/mL). The compounds 5-DPM demonstrated remarkable activity both reducing power (EC_{50} = 53.2 ± 0.3 µg/mL), and TAC assay (EC_{50} = 620.0 ± 2.4 µg/mL). These results prove that the modification in hydroxyl group position affect the antioxidant ability of Schiff bases.*

Keywords: *Schiff bases; free radicals; antioxidant activity; phenolics*

■ INTRODUCTION

The free radicals are the standard sources in charge of numerous illnesses, for example, age-related maladies, rheumatoid joint inflammation, Infectious ulcer, and malignancy inception [1-5]. These medical issues are conveyed by the response of receptive oxygen species (ROS), usually known as responsive species (RSs) [6-7]. Cancer prevention agents are particles that assume a basic job in securing against oxidant-incited harm and can be displayed as hydrogen donors or electron reducers to the receptive sites in killing free radical [8-9]. It is accounted for those numerous natural atoms executed as generally amazing cell reinforcements. In this manner, it is critical to comprehend the method of activity and effectiveness of these cancer prevention agents [10]. Antioxidants are molecules that play a critical role in protecting against oxidant-induced damage and can be presented as

hydrogen donors or electron reducers to the reactive site in scavenging free radical [11]. It is reported that many organic compounds behave as very powerful antioxidants. It is therefore essential to recognize the mechanism of action and the tendency to act as antioxidants [12-13].

Schiff bases are classes of compounds that contain azomethines bond (C=N) and owe the general formulation RHC=N-R^1 (where R and R^1 may be alkyl, cycloalkyl, aryl or heterocyclic). They are attained by a condensation process of primary aromatic amines and aldehydes or ketones [14]. Schiff bases play a significant role in the biological process with various applications [15-16]. Additionally, phenolic Schiff bases are proved as efficient antioxidants and excellent free radical scavengers [17]. This radical scavenging propriety is mainly due to the transfer of hydrogen atoms from the OH, NH, and SH groups (attached to the aromatic nuclei) to the free radicals [18].

In the light of these data, we are interested in synthesizing new Schiff bases and investigating their antioxidant properties. The modification of the bridge and the position of hydroxyl groups is a useful strategy to ameliorate the antioxidant activity of Schiff bases. For that, eight compounds are synthesized by the insertion of carbon, sulfur, two sulfur atoms between two aromatic rings. The hydroxyl groups are introduced in *ortho* or para position to 3,3'-OH and 5,5′-OH. The aims of this work are to synthesize three series of Schiff bases and to study the effect of the atom bridging on the antioxidant capacity. In addition, the introduction of two hydroxyl groups in different positions in the Schiff bases significantly affects the scavenging efficiency of free radicals in the system, where one is fixed in position 2, and another is at different position in the aromatic ring. The effects of both the bridge and the introduction of the hydroxyl groups were studied. The contributions of the hydroxyl position structures on the antioxidant activity were also investigated.

■ EXPERIMENTAL SECTION

Materials

The chemicals utilized in this work, 4,4′-diaminodiphenylmethane (97%), bis(4-aminophenyl) sulfide (98%), 2,5-dihydroxybenzaldehyde (97%) and bis(4-aminophenyl) disulfide (98%) 2,3-dihydroxybenzaldehyde (98%), and the solvents were purchased from Sigma-Aldrich. 2,2-diphenyl-1-picrylhydrazyl (DPPH; 97%), NaOCl solution (6–14% active chlorine), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; 98%), butylated hydroxy anisole (BHA; 99%), butylated hydroxytoluene (BHT; 99%), 1,10-phenanthroline monohydrate (99%), ascorbic acid (vitamin C; 99%), potassium ferricyanide (99%), potassium persulfate (98%) and trichloroacetic acid (99%), were obtained from Sigma Aldrich and Merck.

Instrumentation

The ^{1}H-NMR spectra were measured through Bruker AC400 spectrometer (400 MHz) using DMSO-d_6. The analysis of the elemental was taken by a Perkin Elmer 2400 (automatic elemental analyzer). The spectra of FTIR were recorded on a Bruker Vector 22 Spectrophotometer (KBr discs).

Procedure

Synthetic procedure of Schiff bases

The ligand was produced by the reported method [19]. Substituted aldehydes (2 mmol), appropriated diamine (1.0 mmol) in 20 mL of pure ethanol, two microliters of acetic acid were added into a (100 mL) round bottom flask, and the product reaction mixture was refluxed for at 78 °C for 3 h. The formed product was filtered and washed with hot ethanol and then dried. The synthesis of monosubstituted products is shown in Fig. 1.

N,N'-bis(2,3-dihydroxybenzylidene)-4,4'-diaminodiphenyl methane: (3-DPM)

2,3-Dihydroxybenzaldehyde (0.276 g, 2 mmol) 4,4′-diaminodiphenylmethane (0.198 g, 1 mmol). Yield 74%; m.p 200 °C; ^{1}H-NMR (100 MHz, $CDCl_3$) δ (ppm) = 13.28 (s, 2H, C_2OH, and C'$_2$OH of 2Ar-OH); 9.17 (s, 2H, C_3OH, and C'$_3$OH of 2Ar-OH); 8.90 (s, 2H, HC=N); 6.78–7.10 (m, 8H, ArH); 5.20 (2H, s); 4.02 (s, 2H, CH_2); FTIR ν (cm^{-1}) 3417 (OH, broad centered at), 1615 (C=N); Microanalysis for $C_{27}H_{22}N_2O_4$ (438.47), Calcd.: C, 73.96; H, 5.06; N, 6.39. Found: C, 73.85; H, 5.06; N, 6.39.

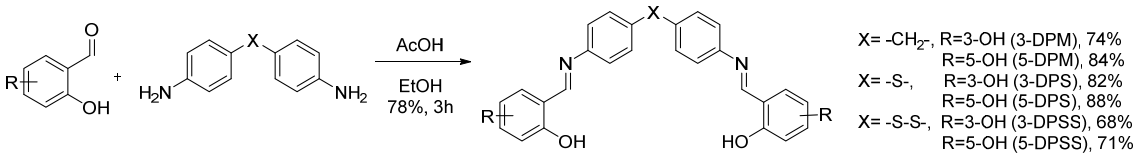

Fig 1. Synthesis of Schiff bases

N,N′-*bis(2,5-dihydroxybenzylidene)-4,4′-diaminodiphenyl methane: (5-DPM)*

2,5-Dihydroxybenzaldehyde (0.276 g, 2 mmol) 4,4′-diaminodiphenylmethane (0.198 g, 1 mmol). Yield 84%; m.p 210 °C; ^{1}H-NMR (25 MHz, $CDCl_3$) δ (ppm) 12.33 (s, 2H, C_2OH and C'$_2$OH of 2Ar-OH), 9.10 (s, 2H, C_5OH and C'$_5$OH of 2Ar-OH), 8.82 (s, 2H, HC=N), 6.80–7.32 (m, 8H, ArH), 5.20 (2H, s), 4.00 (s, 2H, CH_2); FTIR ν (cm^{-1}) 3302 (OH), 1602 (C=N); Microanalysis for $C_{27}H_{22}N_2O_4$ (438.47), Calcd.: C, 73.96; H, 5.06; N, 6.39. Found: C, 73.81; H, 5.04; N, 6.41.

N,N′-*bis(2,3-dihydroxybenzylidene)-4,4′-diaminodiphenyl sulfide: (3-DPS)*

2,3-Dihydroxybenzaldehyde (0.276 g, 2 mmol) bis(4-aminophenyl) sulfide (0.216 g, 1 mmol). Yield 82%; m.p 210 °C; ^{1}H-NMR (25 MHz, $CDCl_3$) δ (ppm) 13.78 (s, 2H, C_2OH and C'$_2$OH of 2Ar-OH), 9.48 (s, 2H, C_5OH and C'$_5$OH of 2Ar-OH), 8.88 (s, 2H, HC=N), 6.80–7.75 (m, 8H, ArH); FTIR ν (cm^{-1}) 3400 (OH), 1620 (C=N), 880 (C-S); Microanalysis for $C_{26}H_{20}N_2O_4S$ (456.11), Calcd.: C, 68.41; H, 4.42; N, 6.14; S, 7.02. Found: C, 68.39; H, 4.40; N, 6.13; O, 14.04; S, 7.03.

N,N′-*bis(2,5-dihydroxybenzylidene)-4,4′-diaminodiphenyl sulfide: (5-DPS)*

2,5-Dihydroxybenzaldehyde (0.276 g, 2 mmol) bis(4-aminophenyl) sulfide (0.216 g, 1 mmol). Yield 88%; m.p > 210 °C; ^{1}H-NMR (25 MHz, $CDCl_3$) δ (ppm) 12.01 (s, 2H, C_2OH and C'$_2$OH of 2Ar-OH), 9.10 (s, 2H, C_5OH and C'$_5$OH of 2Ar-OH), 8.83 (s, 2H, HC=N), 6.82–7.60 (m, 8H, ArH), 5.20 (2H, s); FTIR ν (cm^{-1}) 3417 (OH), 1612 (C=N), 879 (C-S); Microanalysis for $C_{26}H_{20}N_2O_4S$ (456.11), Calcd.: C, 68.41; H, 4.42; N, 6.14; S, 7.02. Found: C, 68.39; H, 4.40; N, 6.13; O, 14.04; S, 7.03.

N,N′-*bis(2,3-dihydroxybenzylidene)-4,4′-diaminodiphenyl disulfide: (3-DPSS)*

2,5-Dihydroxybenzaldehyde (0.276 g, 2 mmol) bis(4-aminophenyl) disulfide (0.248 g, 1 mmol). Yield 68%; m.p > 210 °C; ^{1}H-NMR (25 MHz, $CDCl_3$) δ (ppm) 12.33 (s, 2H, C_2OH and C'$_2$OH of 2Ar-OH), 9.10 (s, 2H, C_5OH and C'$_5$OH of 2Ar-OH), 8.82 (s, 2H, HC=N), 6.80–7.32 (m, 8H, ArH), 5.20 (2H, s), 4.00 (s, 2H, CH_2); FTIR ν (cm^{-1}) 3425 (OH), 1620 (C=N), 881 (C-S); Microanalysis for $C_{26}H_{20}N_2O_4S_2$ (488.09), Calcd.: C, 63.92; H, 4.13; N, 5.73; O, 13.10; S, 13.12. Found: C, 63.90; H, 4.13; N, 5.72; O, 13.12; S, 13.11.

N,N′-*bis(2,5-dihydroxybenzylidene)-4,4′-diaminodiphenyl disulfide: (5-DPSS)*

2,5-Dihydroxybenzaldehyde (0.276 g, 2 mmol) bis(4-aminophenyl) disulfide (0.248 g, 1 mmol). Yield 71%; m.p > 210 °C; ^{1}H-NMR (25 MHz, $CDCl_3$) δ (ppm) 12.01 (s, 2H, C_2OH and C'$_2$OH of 2Ar-OH), 9.10 (s, 2H, C_5OH and C'$_5$OH of 2Ar-OH), 8.82 (s, 2H, HC=N), 6.80–7.60 (m, 8H, ArH); FTIR ν (cm^{-1}) 3340 (OH), 1604 (C=N), 879 (C-S); Microanalysis for $C_{26}H_{20}N_2O_4S_2$ (488.09), Calcd.: C, 63.92; H, 4.13; N, 5.73; O, 13.10; S, 13.12. Found: C, 63.91; H, 4.12; N, 5.73; O, 13.13; S, 13.12.

Radical scavenging capacity

Free radical scavenging capacity. The antioxidant potential of the Schiff base compounds to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was assessed spectrophotometrically following the method reported by Benslama et al. [20]. Briefly, a 320 µL of DPPH methanolic solution (0.6 mg/100 mL) was added to the 80 µL of the compound prepared at various concentrations (solubilized in *N*,*N*-dimethylformamide). The resulting mixture was maintained in the dark at room temperature. After incubation for 30 min, the absorbance of the mixture was recorded at 517 nm, and the antiradical power was expressed as half-maximal effective concentration (EC_{50}) as compared to the control. The percent of antiradical capacity was then calculated according to Eq. (1):

$$\text{DPPH scavenging } (\%) = \frac{(A_c - A_s) \times 100}{A_c} \quad (1)$$

where A_c is the absorbance of control and A_s is absorbance of sample.

ABTS radical-scavenging activity. To set the antioxidant ability of the synthesized phenolic Schiff bases, we adopted the $ABTS^+$ method from [21], with a little alteration. A solution of $ABTS^+$ (7 mM) was mixed with solution of potassium persulfate (2.4 mM) to get a fresh stock solution. Thereafter, the solution used for ABTS screening was produced by combining the stock solutions of potassium persulfate and $ABTS^+$ in equal amounts for 12–14 h in the dark at room temperature. A specific absorbance of $ABTS^+$ at 0.705 ± 0.01 units at 734 nm desired for the analysis was attributed to diluting 1 mL of $ABTS^+$ solution in 60 mL of methanol. A fresh $ABTS^+$ solution was made up for each assay. The sample (50 µL) was mixed with 1 mL of the $ABTS^+$ solution, and then the absorbance at 734 nm was recorded after 6 min for each measurement. The percentage inhibitions of the synthesized SBs were calculated using Eq. (2):

$$\text{ABS scavenging } (\%) = \frac{(A_c - A_s) \times 100}{A_c} \quad (2)$$

where A_c is the absorbance of the ABTS free radical solution recorded without a sample and A_S is the absorbance of ABTS free radical solution with the sample. All tests and analyses were realized in triplicate and the outcomes attained were averaged. The inhibition percentage was plotted against concentration, and a straight line was generated, and the EC_{50} values of the Schiff bases were calculated from this graph, explicitly, the amount of antioxidant Schiff bases necessary to diminish the 50% of the initially ABTS radical concentration.

Hydroxyl radical (OH•) -scavenging activity. The hydroxyl radical scavenging effect of compounds was evaluated by [21]. However, 60 µL of $FeSO_4 \cdot 7H_2O$ solution (1 mM) was added to 90 mL of a 1,10-phenanthroline (1 mM) aqueous solution, and 2.4 mL of phosphate buffer (0.2 M, pH 7.8) were added to the resulting mixture following by the addition of 1.5 mL of different concentrations of the sample, then 150 µL of hydrogen peroxide (0.17 mM) in sequence. The mixture was then incubated in the water bath at 37 °C for 30 min. The decrease in the concentration of OH• was estimated spectrophotometrically by measurement of absorbance at 560 nm, and the antiradical capacity was expressed as EC_{50} the values, i.e., the concentration of the studied compound, which induces a decrease of 50% in the absorbance of 560 nm compared to the control. All readings were taken in triplicate, and Butylhydroxy toluene (BHT) was used as a positive control.

The percent inhibition was calculated according to Eq. (3).

$$\text{Hydroxyl} - \text{radical scavenging } (\%) = \frac{(A_c - A_s) \times 100}{A_c} \quad (3)$$

where A_c is the absorbance of the control solution without the sample and A_S is the absorbance of the sample solution with the sample.

Reducing capacity

The reducing effect of the compounds was measured following the methods [22]. A volume of 20 µL of the compound at various concentrations was combined with 100 µL of potassium ferricyanide $[K_3Fe(CN)_6]$ solution (1%) and 80 µL of phosphate buffer solution (0.2 M, pH 6.6). The resulting mixture was incubated for 20 min at 50 °C. Then, 100 µL of trichloroacetic acid (10%) was also added to terminate the reaction, and the entire was centrifuged for 10 min at 2800 r/min. Finally, the supernatant solution (100 µL) of was combined with 20 µL of $FeCl_3$ solution (0.1%) and distilled water (100 µL). Then, the mixture was incubated for 10 min, and the absorbance was recorded at 700 nm. The antioxidant strength of samples was estimated using a ferrous iron standard curve, absorbance as a function of concentration, and results are expressed as Fe^{2+} concentration (µg/mL) corresponding to the half absorbance value in the standard curve $A_{0.5}$.

Total antioxidant capacity (TAC)

This assay was based on the capacity of an antioxidant agent to reduce the molybdenum (VI) to molybdenum (V) and generate a green phosphomolybdate complex (V) which to be estimated spectrophotometrically at 695 nm. Equal volumes of sodium phosphate (28 mM), sulfuric acid (0.6 M), and ammonium molybdate (4 mM) were taken in a beaker to get the phosphomolybdate reagent. To take the test, 300 µL of sample in several concentrations of Schiff bases (50–500 µg/mL in DMSO)

was mixed with phosphomolybdate reagent and incubated in a water bath for 90 min at 95 °C. The mixture was allowed to settle at room temperature, and absorbance was determined at 765 nm. Instead of the sample, DMSO was used in the blank. According to the equation obtained from the calibration curve, the total antioxidant capacity was estimated as µg/mL ascorbic acid equivalent (AAE) [23].

Statistical analyses

The experiments were performed in triplicates, and data were presented as mean ± SD. The EC_{50} (50% inhibition concentration) values were estimated using the linear regression method. The Graphpad Prism7 was determined to analyze the data. However, the statistical differences between the experimental groups and standard were analyzed using the Student *t*-test, and the difference were considered statistically significant where $p < 0.05$.

■ RESULTS AND DISCUSSION

Synthesis

The present work involved the preparation of the phenolic Schiff bases by condensation reaction. All the synthesized molecules were powder solids and found to be insoluble in dichloromethane and soluble in methanol and acetonitrile. The analytical data agree well with the formula of the products and were recognized through elemental analysis and spectroscopic data (UV-Vis, IR, and ^{1}H-NMR). The relevant FTIR data of the synthesized compounds presented standard features in appointed regions and characteristic bands in the other zones. In all the compounds under study, the aromatic (C–H) bond manifested in the range (3024–3082 cm^{-1}) [24], whereas the aliphatic (C–H) of 3-DPM and 5-DPM bond appeared in the range of (2924 cm^{-1}). The clear band in the range of (1604–1628 cm^{-1}) was attributed to the azomethine bond (C=N) [25]. The aromatic (C=C) bond appeared in the range of (1520–1566 cm^{-1}) [26].

The ^{1}H-NMR spectra were used in structure elucidation and identification of the synthesized phenolic Schiff bases. The ^{1}H-NMR spectra of synthesized compounds (3-DPM; 5-DPM, 3-DPS, 5-DPS, 3-DPSS, 5-DPSS) displayed the azomethine proton (-HC=N) at and 8.93, 8.77, 8.87, 8.79, 8.87, and 8.83 ppm respectively as singlet [27-28] and aromatic protons at 6.78–7.38 ppm range as multiplets [29-30]. Furthermore, the aliphatic protons ($-CH_2$) shows a singlet at 4.02 ppm for the compound 3-DPM and 5-DPM [31]. The identified signals of all the protons of the phenolic Schiff bases were in agreement in their expected region.

Radical Scavenging Capacity

DPPH• scavenging activity

From Fig. 2, the curves of the antioxidant effect of all the Schiff base compounds appear almost the same evolutionary trend. The sharp rise in the DPPH• inhibition curve from 10 up to 180 µg/mL, taking a constant trend (stationary phase) from 200 µg/mL. The compound 5-DPSS has the best anti-free radical power with a percentage inhibition of 94.43% with a concentration threshold of 200 µg/mL. The oxidation power of the samples is related to the presence of compounds that exert actions by splitting the chain of free radicals via hydrogen atom donation [32]. The best anti-free radical activity is attributed to the compound 5-DPSS with an EC_{50} value of 7.10 ± 3.2 µg/mL, while the weak activity is recorded for the product 5-DPM (20.97 ± 0.89 µg/mL). The percentage inhibition of the DPPH• increases with increasing concentration in a dependent manner, either for the Schiff base compounds or for standards [33-35]. The inhibition efficiency of synthesized compounds was found to be in ascending order of 5-DPM < 3-DPSS < 3-DPS < 3-DPM < 5-DPS < 5-DPSS (Fig. 2). Based on the EC_{50} values (Table 1) among the six products tested, the 5-DPSS compound was noted as the most active, with an antioxidant activity equal to (7.10 ± 0.16) µg/mL. The DPPH scavenger potential increased in that order as follow: 5-DPSS (7.10 ± 0.16 µg/mL) > 5-DPS (7.20 ± 0.20 µg/mL) > 3-DPM (8.37 ± 0.34 µg/mL) > 3-DPS (8.84 ± 0.34 µg/mL) > 3-DPSS (11.34 ± 0.17 > 5-DPM (20.97 ± 0.89 µg/mL). At the same time, the positive control, BHA and BHT showed a close potential 5.73 ± 0.41 and 22.32 ± 1.19 µg/mL, respectively, than the six products. The lowest antioxidant potential was assigned for 5-DPM with 20.97 ± 0.89 µg/mL. It was clear that the compounds having sulfur bridging atom and second hydroxyl group in position 5 showed the best free radical scavenging activities in the DPPH test.

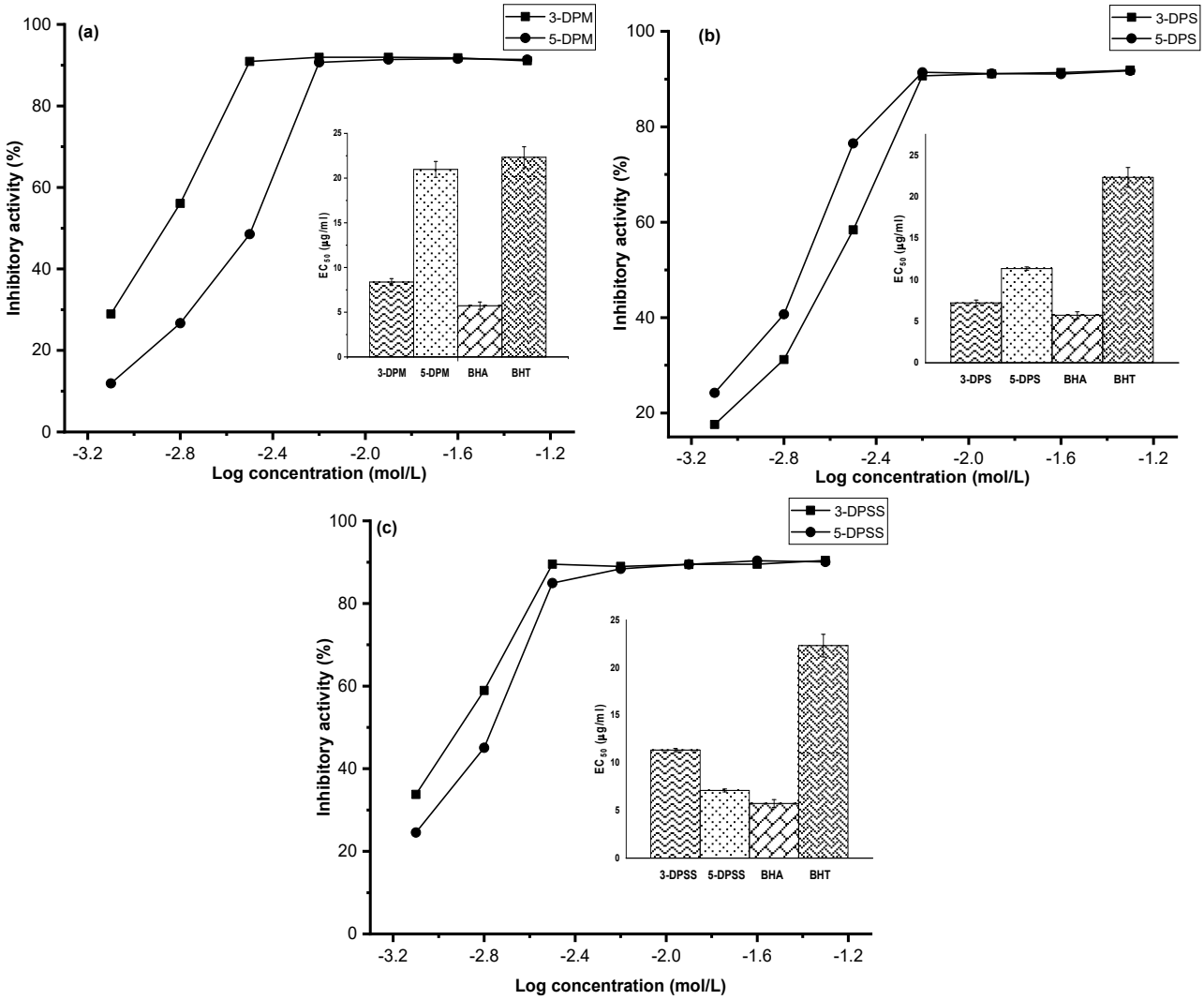

Fig 2. Free radical scavenging activity DPPH• (%) of: (a) DPM, (b) DPS, (c) DPSS. Error bar indicates SD standard deviation (n = 3)

Table 1. The antioxidant activity of the products synthesized and standards

Product	EC_{50} values (µg/mL)			$A_{0.5}$	EAA
	DPPH	ABTS	OH•	Reducing power	TAC
3-DPM	8.37 ± 0.38	1.49 ± 0.05	227.7 ± 5.1	71.1 ± 0.1	1680.0 ± 0.01
5-DPM	20.97 ± 0.89	3.23 ± 0.01	143.2 ± 16.0	53.2 ± 0.3	620.0 ± 2.4
3-DPS	8.84 ± 0.34	1.77 ± 0.08	209.2 ± 7.3	139 ± 1.3	1153.3 ± 5.7
5-DPS	7.20 ± 0.20	1.95 ± 0.01	44.9 ± 3.3	138.9 ± 1.0	949.16 ± 1.44
3-DPSS	11.34 ± 0.17	1.36 ± 0.08	244.1 ± 16.0	75.5 ± 0.5	1193.3 ± 5.7
5-DPSS	7.10 ± 0.16	2.64 ± 0.05	232.5 ± 3 .93	68.2 ± 0.2	730.0 ± 4.6
BHA	5.73 ± 0.41	7.54 ± 0.67			
BHT	22.32 ± 1.19	1.55 ± 0.26	98.73 ± 0.3	99.2 ±0.41	100.0 ±0.21

ABTS radical scavenging activity

A significant attribute of the antioxidants is the free radical proton scavenging. A recognized protonated radical ABTS has distinctive absorbance maxima at 734 nm, which declines with the quenching of the proton free radicals [36]. The Schiff base series, with

phenyl, diphenylmethane, diphenyl sulfide, and diphenyl disulfide bridges, were powerful and impressive scavenger effect of the ABTS free radical (Fig. 3), and this capacity was consistent with those to those of BHA and BHT (Table 1) that are employed as standard drugs. The lower concentrations of the tested samples were more potent in quenching $ABTS^+$ free radicals in the system [37]. The three series of Schiff bases exhibit powerful scavenging of the $ABTS^+$ radical relative to those of the standards (BHA and BHT). 3-DPSS revealed the highest activity with an EC_{50} of about (1.36 ± 0.08 µg/mL) amongst the synthesized diphenolic Schiff bases series. The ABTS radical scavenging capacity of the tested compounds can be ranked in the order: 3-DPSS > 3-DPM > 3-DPS > 5-DPS > 5-DPSS > 5-DPM.

From the calculated values of EC_{50}, we can conclude that the compounds that have the hydroxyl group in position three present more potent scavenging effects than position 5 in the other Schiff bases series. Furthermore, the sulfur atom bridge performed well compared to that of carbon. The results of the ABTS radical test by the Schiff bases were shown to be much better than those of DPPH•. Many factors like solubility of the compounds' stereoselectivity of the radicals or the indifferent testing systems have been conveyed to affect the aptitude of compounds to react and inhibit different radicals [37-38]. Hong et al. [39] observed that some compounds that present ABTS scavenging response did not display DPPH scavenging ability. In this study, the compounds showed strong scavenging effect against

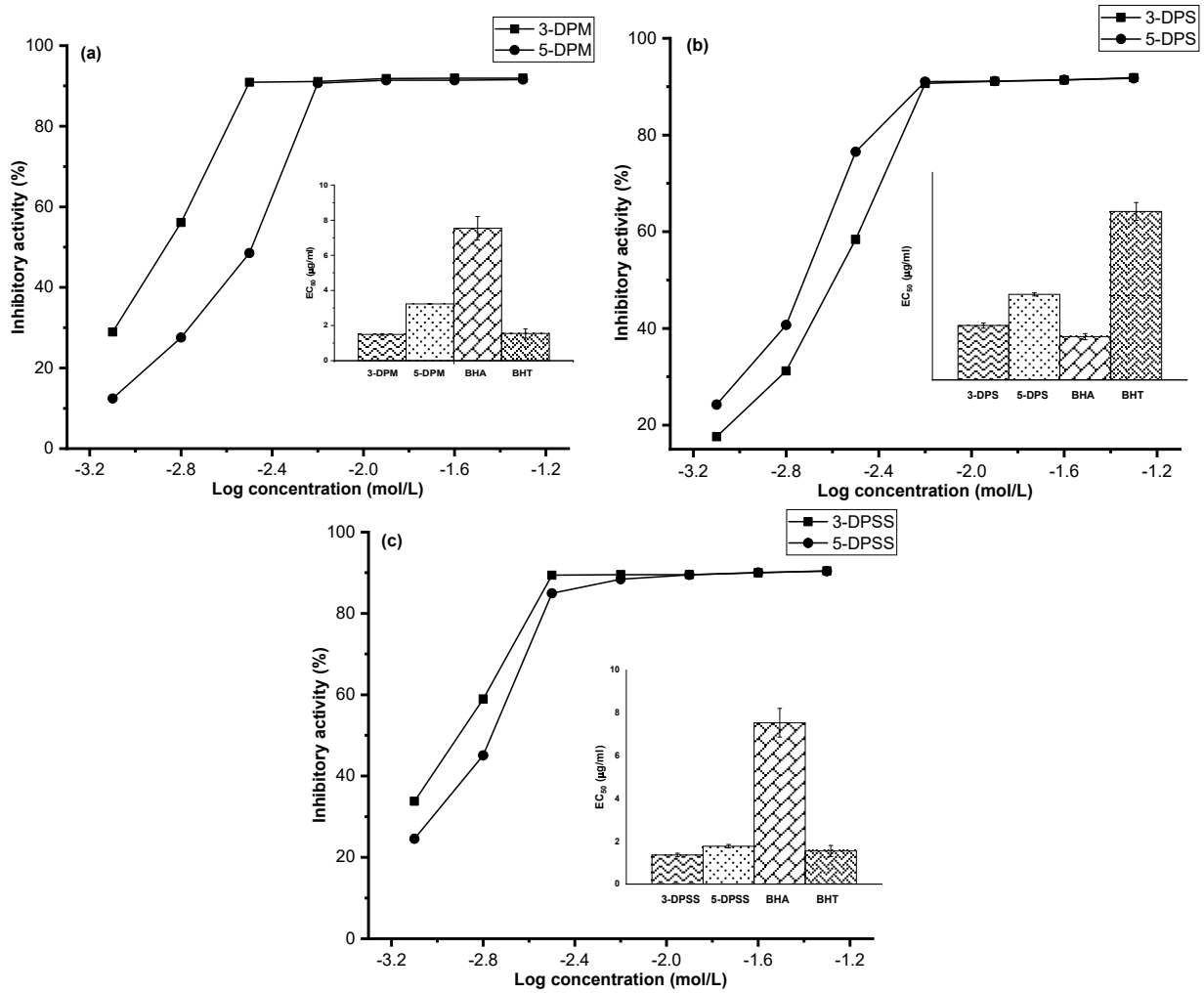

Fig 3. Free radical scavenging activity ABTS• (%). (a) DPM, (b) DPS, (c) DPSS. Error bar indicates SD standard deviation (n = 3)

DPPH and ABTS•. This supplementary showed the capacity of the extracts to quench different free radicals in different systems, signifying that they may be beneficial therapeutic agents for remedying radical-related pathological injury.

Hydroxyl radical OH• scavenging capacity

The six synthesized compounds showed different scavenging power for the OH• (Fig. 4). It is important to note that the compound which has the best inhibitory activity was 5-DPS (44.9 ± 3.3 μg/mL) among the others, which appears weak than BHT (98.73 ± 0.3 μg/mL), while the lowest was found in the compound 3-DPSS (244.1 ± 16.0 μg/mL). In addition, it is clearly seen that the compounds having two hydroxy groups at position 2-5 are more effective than the catecholic compounds (at position 2-3) in the same and the different series [40]. The statistical results obtained show that the activity of scavenging OH• by the compound 5-DPS has a significant difference of $p < 0.05$ compared to the standard, which clearly explains the insignificant action of this compound in terms of activity and efficiency as the standard (BHT).

The results indicated that the substituents on the phenyl ring have a great influence on the antioxidant activity expressed in the activity of scavenging DPPH•. Schiff's bases carrying the 2,3-dihydroxysalicylidene fragment on the benzene ring with sulfur bridge were found to be the best scavengers of DPPH•. This result was expected since it was well known that the catechol moiety, two adjacent hydroxyl groups influence antioxidant activity [41]. Thus, the hydrogen bonds between the two adjacent hydroxyl groups involve

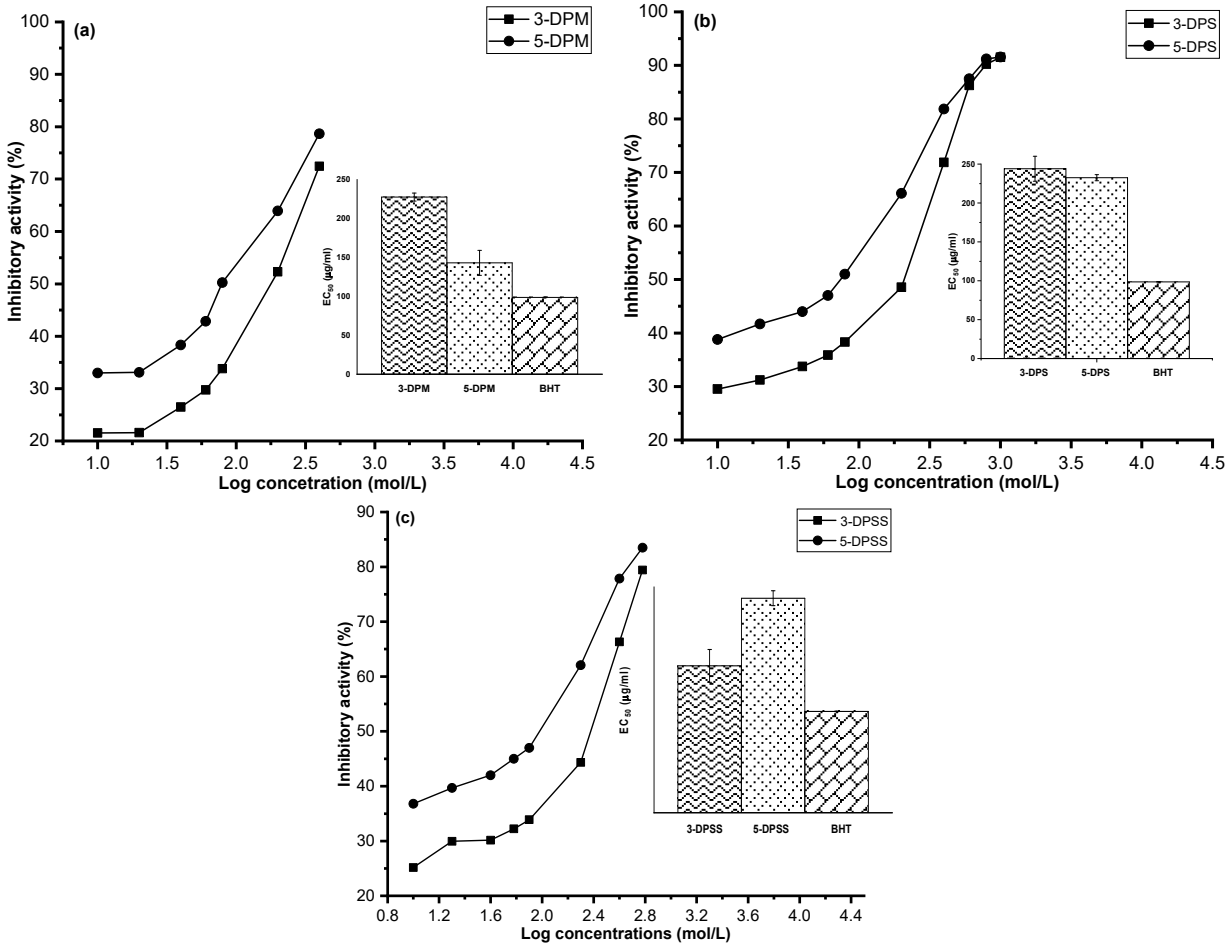

Fig 4. The OH• scavenging activity (%) of 3-DPSS and 5-DPSS. (a) DPM, (b) DPS, (c) DPSS. Error bar indicates SD standard deviation (n = 3)

electrostatical interactions between the proton of the hydroxyl in position three and the oxygen of the hydroxyl number 2. This weakens the OH bond, which consequently makes hydrogen more labile, therefore more reactive, and more ready to react with the free radical [42]. In addition, the oxidation potential of the hydroxyl group plays an important role in determining the entity responsible for the activity.

Čačić et al. [43] proved that the catecholic part 2.3-dihydroxybenzylidene (phenolic ring carrying two hydroxyl groups in *ortho* position; adjacent) is crucial for the anti-radical activity, as well as other authors [44-45], who investigated coumarin derivatives with *o*-dihydroxy phenolic groups. When two hydroxyl groups are at position 2-5 of the phenyl ring, a stable phenoxyl radical is formed, which allows an oxygen atom to share a positive charge, which causes stabilization by delocalization. When two hydroxyl groups are in position 2-4 of one of the phenyl rings, oxygen cannot share a positive charge, and this influences the scavenging DPPH activity [46].

Reducing Power

The reduction capacity is an important property of potential antioxidant activity. The antioxidant compounds can give electrons or hydrogen atoms to the reactive radicals, reducing them into more stable and unreactive species [47]. Generally, antioxidant compounds provoke the reduction of Fe^{3+}/ferricyanide complex to the ferrous (Fe^{2+}) form owing to their reductive potency [48]. According to this method, the reduction is expressed as an increasing of absorbance at 700 nm, in which higher absorbance suggests a higher ferric reducing antioxidant power. In the FRAP assay, higher $FeSO_4{\cdot}7H_2O$ equivalent signifying powerful antioxidant activity. Hence, the compound 5-DPM has relatively high ferric reducing power ability (53.2 ± 0.3 mg/mL), while compound 3-DPS (139 ± 1.3 mg/mL) exhibits inefficient ferric reducing activity (Fig. 5). As shown in Fig. 5, the reducing power of Schiff bases was augmented comparable to standard antioxidants by increasing concentration. The reducing power of samples and standard antioxidants decreased in the order of 5-DPM > 5-DPSS > 3-DPM > 3-DPSS > 5-DPS > 3-DPS. The above-cited results indicate that compounds that have a hydroxyl group in position 5 possess powerful reductive capacity than the catechol form. The FRAP assay, overall, as the nonradical method, has been disputed to have a slight relationship with the radical scavenging method (HAT mechanism) happening in lipid systems, and it has a deprived correlation with other antioxidant activity measurements. It is, hence, advised that this assay may well be used in combination with further methods in discriminating dominant mechanisms for diverse antioxidants [49].

Total Antioxidant Capacity (TAC)

The results showed that all of the compounds tested have remarkable reducing power with values between 620 to 1680 μg EAA.mg of the product (Fig. 7). The most active compound is 3-DPM and the weakest 5-DPM. The decreasing classification of the tested is as follows: 3-DPM > 3-DPSS > 3-DPS > 5-DPS > 5-DPSS > 5-DPM.

All compounds bearing a hydroxyl group in the *para* and *ortho* position did not show significant antioxidant activity compared with the standards BHA and BHT. Compounds having two hydroxyl groups in the *para* position exhibit moderate antioxidant activity relative to the *ortho* position [50]. This indicates that the change in the position of the hydroxyl groups on the phenyl ring has a great influence on the antioxidant activity expressed in the activity of scavenging DPPH•,

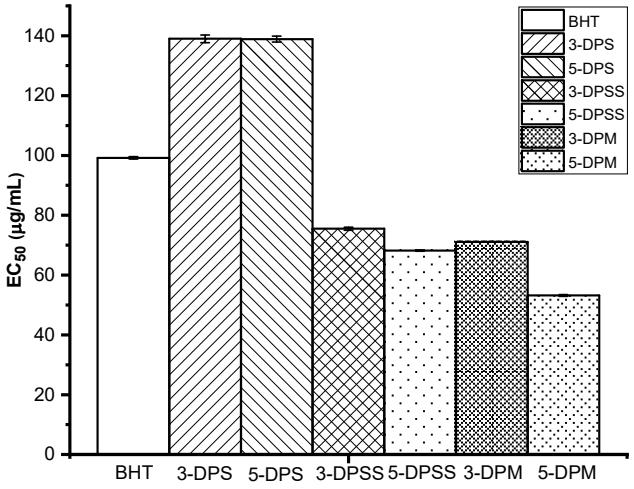

Fig 5. Reducing power of the synthesized Schiff bases

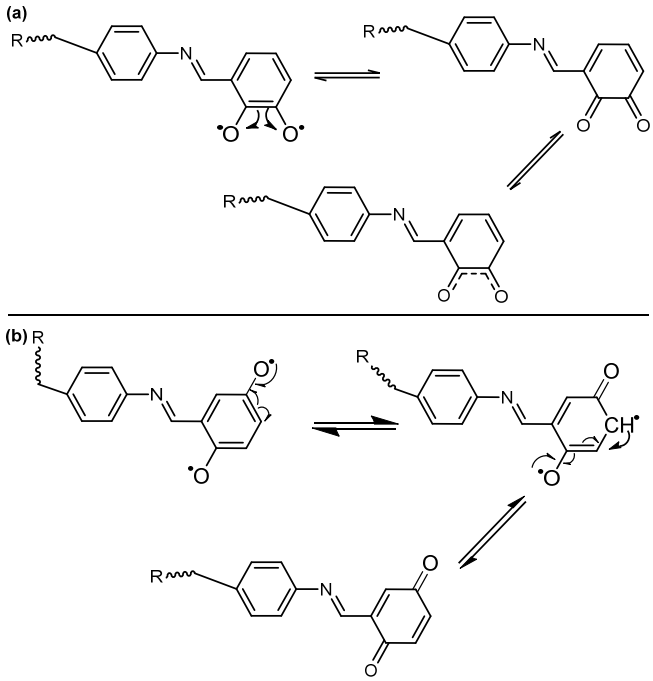

Fig 6. (a) Structure of the stable quinoid form of the compound 3-DPM stabilized by electron delocalization (b) Stable basic Schiff structure of position 2.5 with post electron/hydrogen contribution, with possibility of stabilization by delocalization

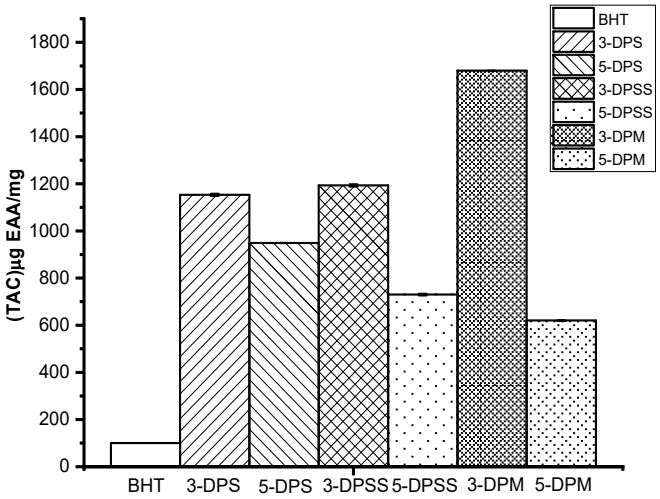

Fig 7. TAC of Schiff bases

that it carries in its structure the catecholic form. Regarding to the reducing power, the compound 5-DPM (53.2 ± 0.3) with the methylene bridge has considerable reducing power performance compared to the standards BHA (99.2 ± 0.41) (Table 1).

■ CONCLUSION

In this work, we have synthesized six phenolic Schiff bases compounds. The synthesized compounds were characterized by using various spectroscopic methods, as well as the evaluation of their antioxidant power by different methods, scavenging of the DPPH•, scavenging of the OH•, ABTS•, TAC and reducing power. Examination of the various results obtained by scavenging the DPPH• and ABTS, allowed us to conclude that Schiff bases have hydroxyl group in position 5 exhibit better antioxidant activity compared to the ones having hydroxyl group in position 3. In contrast, the ABTS assay showed that the catechol Schiff bases compounds having a hydroxyl group in position 3 reveal good antioxidant activity in comparison with their analogues having a hydroxyl in position 5. On the one hand, the antioxidant profile of the various compounds synthesized exhibits a dose-dependent power. The observation of the EC_{50} values of the ABTS method shows that the 3-DPSS compound reveals the best value by mean of EC_{50} (1.77 ± 0.08 µg/mL), the compounds: 3-DPS, 3-DPSS, 3-DPS, 5-DPSS, 3-DPM and 5-DPM, presented an inhibitory power superior to that of BHA (7.54 ± 0.67 µg/mL). Noting that all compounds have uneven results, whether OH•, reducing power, or TAC.

■ REFERENCES

[1] Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C.J., and Valko, M., 2017, Targeting free radicals in oxidative stress-related human diseases, *Trends Pharmacol. Sci.*, 38 (7), 592–607.

[2] Archibong, A.E., Rideout, M.L., Harris, K.J., and Ramesh, A., 2018, Oxidative stress in reproductive toxicology, *Curr. Opin Toxicol.*, 7, 95–101.

[3] Hoffmann, M.H., and Griffiths, H.R., 2018, The dual role of Reactive Oxygen Species in autoimmune and inflammatory diseases: Evidence from preclinical models, *Free Radical Biol. Med.*, 125, 62–71.

[4] Phaniendra, A., Jestadi, D.B., and Periyasamy, L., 2015, Free radicals: Properties, sources, targets, and

their implication in various diseases, *Indian J. Clin. Biochem.*, 30 (1), 11–26.

[5] Gebicki, J.M., 2016, Oxidative stress, free radicals and protein peroxides, *Arch. Biochem. Biophys.*, 595, 33–39.

[6] McInnis, S.M., Desikan, R., Hancock, J.T., and Hiscock, S.J., 2006, Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: Potential signalling crosstalk?, *New Phytol.*, 172 (2), 221–228.

[7] Hu, S., Yao, G., Wang, Y., Xu, H., Ji, X., He, Y., Zhu, Q., Chen, Z., and Sun, Y., 2014, Transcriptomic changes during the pre-receptive to receptive transition in human endometrium detected by RNA-Seq, *J. Clin. Endocrinol. Metab.*, 99 (12), E2744–E2753.

[8] Goodman, M., Bostick, R.M., Kucuk, O., and Jones, D.P., 2011, Clinical trials of antioxidants as cancer prevention agents: past, present, and future, *Free Radicals Biol. Med.*, 51 (5), 1068–1084.

[9] Khan, N., and Mukhtar, H., 2015, Dietary agents for prevention and treatment of lung cancer, *Cancer Lett.*, 359 (2), 155–164.

[10] Ghasemi, S., and Lorigooini, Z., 2016, A review of significant molecular mechanisms of flavonoids in prevention of prostate cancer, *J. Chem. Pharm. Sci.*, 9 (4), 3388–3394.

[11] Tan, H.W., Mo, H.Y., Lau, A.T., and Xu, Y.M., 2018, Selenium species: Current status and potentials in cancer prevention and therapy, *Int. J. Mol. Sci.*, 20 (1), 75.

[12] Postigo, C., and Barceló, D., 2015, Synthetic organic compounds and their transformation products in groundwater: Occurrence, fate and mitigation, *Sci. Total Environ.*, 503-504, 32–47.

[13] Gobec, M., Tomašič, T., Markovič, T., Mlinarič-Raščan, I., Dolenc, M.S., and Jakopin, Ž., 2015, Antioxidant and anti-inflammatory properties of 1,2,4-oxadiazole analogs of resveratrol, *Chem.-Biol. Interact.*, 240, 200–207.

[14] Alam, M.S., Choi, J.H., and Lee, D.U., 2012, Synthesis of novel Schiff base analogues of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one and their evaluation for antioxidant and anti-inflammatory activity, *Bioorg. Med. Chem.*, 20 (13), 4103–4108.

[15] Wu, H., Pan, G., Bai, Y., Wang, H., Kong, J., Shi, F., Zhang, Y., and Wang, X., 2014, Preparation, structure, DNA-binding properties, and antioxidant activities of a homodinuclear erbium(III) complex with a pentadentate Schiff base ligand, *J. Chem. Res.*, 38 (4), 211–217.

[16] Poladian, Q., Şahin, O., Karakurt, T., İlhan-Ceylan, B., and Kurt, Y., 2021, A new zinc(II) complex with N_2O_2-tetradentate Schiff-base derived from pyridoxal-S-methylthiosemicarbazone: Synthesis, characterization, crystal structure, DFT, molecular docking and antioxidant activity studies, *Polyhedron*, 201, 115164.

[17] Adeleke, A.A., Zamisa, S.J., Islam, M., Olofinsan, K., Salau, V.F., Mocktar, C., and Omondi, B., 2021, Quinoline functionalized Schiff base silver (I) complexes: Interactions with biomolecules and *in vitro* cytotoxicity, antioxidant and antimicrobial activities, *Molecules*, 26 (5), 1205.

[18] Shahraki, S., Delarami, H.S., Mansouri-Torshizi, H., and Nouri, H., 2021, Investigation of kinetics and thermodynamics in the interaction process between two pyridine derived Schiff base complexes and catalase, *J. Mol. Liq.*, 334, 116527.

[19] Kheniche, A., Ourari, A., Dakhouche, A., Ghanem, A., Min, W., and Meguellati, K., 2018, Electrochemical and theoretical studies influencing the effect of hydroxyl position of tetraphenolic Schiff bases towards corrosion inhibition of mild steel in 1 M HCl, *J. Fundam. Appl. Sci.*, 10 (3), 209–238.

[20] Benslama, A., Harrar, A., Gul, F., and Demirtas, I., 2017, Phenolic compounds, antioxidant and antibacterial activities of *Zizyphus lotus* L. leaves extracts, *Nat. Prod. J.*, 7 (4), 316–322.

[21] Sahoo, A.K., Narayanan, N., Sahana, S., Rajan, S.S., and Mukherjee, P.K., 2008, *In vitro* antioxidant potential of *Semecarpus anacardium* L., *Pharmacologyonline*, 3 (3), 27–35.

[22] Benslama, A., and Harrar, A., 2016, Free radicals scavenging activity and reducing power of two

Algerian Sahara medicinal plants extracts, *Int. J. Herb. Med.*, 4 (6), 158–161.

[23] Aboseada, H.A., Hassanien, M.M., El-Sayed, I.H., and Saad, E.A., 2021, Schiff base 4-ethyl-1-(pyridin-2-yl) thiosemicarbazide up-regulates the antioxidant status and inhibits the progression of Ehrlich solid tumor in mice, *Biochem. Biophys. Res. Commun.*, 573, 42–47.

[24] Xing, A., Zeng, D., and Chen, Z., 2022, Synthesis, crystal structure and antioxidant activity of butylphenol Schiff bases: Experimental and DFT study, *J. Mol. Struct.*, 1253, 132209.

[25] Daravath, S., Rambabu, A., Ganji, N., Ramesh, G., and Lakshmi, P.V.A., 2022, Spectroscopic, quantum chemical calculations, antioxidant, anticancer, antimicrobial, DNA binding and photo physical properties of bioactive Cu(II) complexes obtained from trifluoromethoxy aniline Schiff bases, *J. Mol. Struct.*, 1249, 131601.

[26] Islam, M.M., Pal, T.K., Paul, S., Uddin, M.N., Sheikh, M.C., Alam, M.A., and Hossen, J., 2022, Computational, Hirshfeld surface, and molecular docking analysis of 2-(((4-methoxyphenyl)imino) methyl)-4-nitrophenol: *In-vitro* anticancer, antimicrobial, anti-inflammatory, and antioxidant studies, *Results Chem.*, 4, 100331.

[27] Çalışkan, N., Usta, A., Beriş, F.Ş., Baltaş, N., and Çelik, E., 2020, Synthesis, antibacterial and antioxidant activities of some new N substituted azachalcone, Schiff base and pyrazole derivatives, *Lett. Org. Chem.*, 17 (8), 631–638.

[28] Muğlu, H., Yakan, H., Misbah, A.G.A., Çavuş, M.S., and Bakır, T.K., 2021, Synthesis, structure characterization and quantum chemical study on relationship between structure and antioxidant properties of novel Schiff bases bearing (thio)/carbo hydrazones, *Res. Chem. Intermed.*, 47 (12), 4985–5005.

[29] Affat, S.S., 2021, Experimental and theoretical studies of new Schiff base as a corrosion inhibitor in acidic media and study antioxidant activity, *Iran. J. Chem. Chem. Eng.*, Article in Press.

[30] Prestiani, A.E., and Purwono, B., 2017, Styrene and azo-styrene based colorimetric sensors for highly selective detection of cyanide, *Indones. J. Chem.*, 17 (2), 238–247.

[31] Güngör, Ö., and Gürkan, P., 2014, Synthesis and characterization of higher amino acid Schiff bases, as monosodium salts and neutral forms. Investigation of the intramolecular hydrogen bonding in all Schiff bases, antibacterial and antifungal activities of neutral forms, *J. Mol. Struct.*, 1074, 62–70.

[32] Maduwanthi, S.D.T., and Marapana, R.A.U.J., 2021, Total phenolics, flavonoids and antioxidant activity following simulated gastro-intestinal digestion and dialysis of banana (*Musa acuminata*, AAB) as affected by induced ripening agents, *Food Chem.*, 339, 127909.

[33] Irfan, A., Imran, M., Al-Sehemi, A.G., Shah, A.T., Hussien, M., and Mumtaz, M.W., 2021, Exploration of electronic properties, radical scavenging activity and QSAR of oxadiazole derivatives by molecular docking and first-principles approaches, *Saudi J. Biol. Sci.*, 28 (12), 7416–7421.

[34] Abuelizz, H.A., Taie, H.A.A., Bakheit, A.H., Marzouk, M., Abdellatif, M.M., and Al-Salahi, R., 2021, Biological evaluation of 4-(1*H*-triazol-1-yl) benzoic acid hybrids as antioxidant agents: *In vitro* screening and DFT study, *Appl. Sci.*, 11 (24), 11642.

[35] Karkar, B., Şahin, S., and Güneş, M.E., 2021, Evaluation of antioxidant properties and determination of phenolic and carotenoid profiles of chestnut bee pollen collected from Turkey, *J. Apic. Res.*, 60 (5), 765–774.

[36] Zheng, Y.Z., Deng, G., and Zhang, Y.C., 2022, Multiple free radical scavenging reactions of flavonoids, *Dyes Pigm.*, 198, 109877.

[37] Enbaraj, E., Dhineshkumar, E., Jeyashri, K., Logeshwari, G., Mohana, V., Manikandan, H., Rajathi, V., Chakkaravarthy, J., Govindaraju, R., and Seenivasan, M., 2021, Novel synthesis, spectral characterisation and DFT calculation of (3,4-bis((*E*)-(substituted-dichlorobenzylidene)amino) phenyl)(phenyl) methanone derivatives, *Mater. Today: Proc.*, 42, 982–988.

[38] Mar, J.M., da Silva, L.S., Moreira, W.P., Biondo, M.M., Pontes, F.L.D., Campos, F.R., Kinupp, V.F., Campelo, P.H., Sanches, E.A., and Bezerra, J.A., 2021, Edible flowers from *Theobroma speciosum*: Aqueous extract rich in antioxidant compounds, *Food Chem*., 356, 129723.

[39] Hong, Y., Wang, Z., Barrow, C.J., Dunshea, F.R., and Suleria, H.A.R., 2021, High-throughput screening and characterization of phenolic compounds in stone fruits waste by LC-ESI-QTOF-MS/MS and their potential antioxidant activities, *Antioxidants*, 10 (2), 234.

[40] Bendary, E., Francis, R.R., Ali, H.M.G., Sarwat, M.I., and El Hady, S., 2013, Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds, *Ann. Agric. Sci.*, 58 (2), 173–181.

[41] Hernández, J.A., Jiménez, A., Mullineaux, P., and Sevilia, F., 2000, Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences, *Plant, Cell Environ.*, 23 (8), 853–862.

[42] Marc, G., Stana, A., Oniga, S.D., Pîrnău, A., Vlase, L., and Oniga, O., 2019, New phenolic derivatives of thiazolidine-2,4-dione with antioxidant and antiradical properties: Synthesis, characterization, in vitro evaluation, and quantum studies, *Molecules*, 24 (11), 2060.

[43] Čačić, M., Pavić, V., Molnar, M., Šarkanj, B., and Has-Schön, E., 2014, Design and synthesis of some new 1,3,4-thiadiazines with coumarin moieties and their antioxidative and antifungal activity, *Molecules*, 19 (1), 1163–1177.

[44] Pele, R., Marc, G., Stana, A., Ionuț, I., Nastasă, C., Tiperciuc, B., Oniga, I., Pîrnău, A., Vlase, L., and Oniga, O., 2022, Synthesis of new phenolic derivatives of quinazolin-4(3H)-one as potential antioxidant agents-*In vitro* evaluation and quantum studies, *Molecules*, 27 (8), 2599.

[45] Pedersen, J.Z., Oliveira, C., Incerpi, S., Kumar, V., Fiore, A.M., De Vito, P., Prasad, A.K., Malhotra, S.V., and Saso, L., 2007, Antioxidant activity of 4-methylcoumarins, *J. Pharm. Pharmacol*., 59 (12), 1721–1728.

[46] Teran, R., Guevara, R., Mora, J., Dobronski, L., Barreiro-Costa, O., Beske, T., Pérez-Barrera, J., Araya-Maturana, R., Rojas-Silva, P., Poveda, A., and Heredia-Moya, J., 2019, Characterization of antimicrobial, antioxidant, and leishmanicidal activities of Schiff base derivatives of 4-aminoantipyrine, *Molecules*, 24 (15), 2696.

[47] Dawidowicz, A.L., Wianowska, D., and Olszowy, M., 2012, On practical problems in estimation of antioxidant activity of compounds by DPPH$^{\bullet}$ method (Problems in estimation of antioxidant activity), *Food Chem.*, 131 (3), 1037–1043.

[48] Farhoosh, R., Johnny, S., Asnaashari, M., Molaahmadibahraseman, N., and Sharif, A., 2016, Structure–antioxidant activity relationships of *o*-hydroxyl, *o*-methoxy, and alkyl ester derivatives of *p*-hydroxybenzoic acid, *Food Chem.*, 194, 128–134.

[49] Martins, A.C., Bukman, L., Vargas, A.M.M., Barizão, É.O., Moraes, J.C.G., Visentainer, J.V., and Almeida, V.C., 2013, The antioxidant activity of teas measured by the FRAP method adapted to the FIA system: Optimising the conditions using the response surface methodology, *Food Chem.*, 138 (1), 574–580.

[50] Prior, R.L., Wu, X., and Schaich, K., 2005, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, *J. Agric. Food Chem*., 53 (10), 4290–4302.