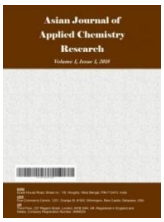

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Synthesis, Antibacterial and Antioxidant Activities of Some Tridentate Substituted Salicylaldimines

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Authors' contributions

This work was carried out in collaboration between all authors. Author AIOA designed the study, performed all the analyses, wrote the protocol, and wrote the first draft of the manuscript. Author JOB managed the literature searches. Author SOS managed the statistical analysis and the interpretation of the statistical results of the study. All authors read and approved the final manuscript.

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ABSTRACT

Five substituted tridentate salicylaldimines, (E)-2-((2-hydroxybenzylidene)amino)phenol, (E)-2-(((2-hydroxyphenyl)imino)methyl)-4-nitrophenol, (E)-4-chloro-2-(((2-hydroxyphenyl)imino)methyl) phenol, (E)-2-(((2-hydroxyphenyl)imino)methyl)-4-methoxyphenol, (E)-4-bromo-2-(((2-hydroxy-phenyl)imino)methyl)-6-methoxyphenol were synthesised and characterised by elemental analysis, IR, UV and NMR (^{1}H and ^{13}C). They were screened against some multi-drug resistance Gram-positive (*Streptococcus agalactiae* and *Staphylococcus aureus*), and Gram-negative (*Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Salmonella typhimurium*) organisms by the agar-well diffusion method. The total antioxidant capacities of the salicylaldimines were determined by the phosphomolybdenum assay. Their antibacterial and antioxidant activities were screened to understand the substituents effects. The result showed that the methoxy-substituted compound exhibited the highest antibacterial and antioxidant activities

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while the nitro-substituted compound exhibited the least activities. This implies that the electron-donating group of the compound increases its antibacterial and antioxidant activities. The one-way analysis of variance was performed with MINITAB 17 at 95% confidence level.

Keywords: Schiff base; substituents; antioxidants; characterised; antibacterial.

1. INTRODUCTION

Salicylaldimines are 2-hydroxyl Schiff bases formed by the reaction between salicylaldehyde and a primary amine [1-3]. Schiff bases are aldehyde or ketone like compounds in which the carbonyl (C=O) group is replaced by an imine or azomethine (−HC=N−) group. Salicylaldimines have considerable biological importance because of the presence of many active donor atoms (N and O) in molecules of these compounds and of being analogous to biological systems to some extent. They may contain a variety of substituents with different electron-donating or electron-withdrawing groups and therefore may have interesting chemical properties. They have attracted much attention due to their biological activities [4]. They have a wide range of applications in medicinal and pharmaceutical chemistry [5-9,10]. For instance, they have been used as anti-inflammatory [11], analgesic [12], antimicrobial [1,7,13-19], anticonvulsant [20], antitubercular [21-24], anticancer [25-27], antioxidant [7,17-19,28], anthelmintic and antimalarial [29,30], which make them gain importance in medicinal and pharmaceutical fields.

Salicylaldimines commonly act as chelating ligands and the chemistry of a metal complex is greatly influenced by the properties of the ligand. Since the presence of functional groups and substituents on the ligands affect the nature of metal complex obtained, knowledge of ligand properties can afford synthesis of metal complexes with tunable properties [4]. As an additional contribution to understand the substituent effects on the antibacterial and antioxidant activities of Schiff bases, the present study was conducted to report the synthesis, antibacterial and antioxidant activities of some tridentate substituted salicylaldimines.

2. MATERIALS AND METHODS

2.1 Reagents

Salicylaldehyde, 5-methoxysalicylaldehyde, 5-bromo-3-methoxysalicylaldehyde, 5-nitrosalicylaldehyde, 5-chlorosalicylaldehyde, and *o*-aminophenol were purchased from Merck (Germany) and used as supplied. The solvent DMSO (dimethyl sulfoxide) and absolute ethanol were of analytical grade and were used without further purification. Elemental analysis was carried out on Finnigan Flash EA 1112 series. The electronic spectra were recorded on Shimadzu UV-2600 series (Japan), in DMSO. The infrared spectra were recorded on a Perkin-Elmer 400 FT-IR/FT-FIR while the NMR spectra were recorded on Bruker Avance III 600 in deuterated DMSO solution with tetramethylsilane (TMS) as an internal reference.

2.2 Synthesis of Schiff bases

A 0.015 mole of *o*-aminophenol in 15 ml of absolute ethanol was added to a stirring solution containing 0.015 mole of the appropriate salicylaldehyde in 10 ml absolute ethanol. The resulting mixture was stirred for 2 hrs. The precipitates were filtered and washed with cold ethanol, recrystallised from ethanol and dried in a desiccator over silica gel for two days.

2.3 Antibacterial Activity

The antibacterial potentials of the samples were evaluated by the agar-well diffusion method as described by Ghosh et al. [19] against multi-drug resistance Gram-positive (*Streptococcus agalactiae and Staphylococcus aureus),* and Gram-negative *(Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa and Salmonella typhimurium*) organisms. The bacteria isolates were sub-cultured in Nutrient agar and incubated at 37°C for 24 hours. All the bacteria cultures were adjusted to 0.5 McFarland standards. An amount of 20 ml sterilised nutrient agar medium was dispensed into each petridish aseptically and allowed to gel. The plates were swabbed with inocula of the test organisms and kept for 15 minutes for adsorption onto the gel. Using a sterile cork borer of 6 mm diameter, wells were bored into the seeded agar plates, and these were loaded with different concentrations of the samples. The plates were allowed to stand in the refrigerator for 1 hour to allow proper diffusion of the sample into the medium and incubated at 37°C for 24 hours before visual assessment of the inhibition zones. Antimicrobial activities were

expressed as inhibition diameter zones in millimetre (mm). The determinations were made over the concentration levels of 5 mg/l, 10 mg/l and 15 mg/l. The mean from each of these levels represented a single reading and the final zone of inhibition was the mean over the three levels for each compound. Gentamycin was used as the control.

2.4 Phosphomolybdate Total Antioxidant Capacity (PTAC) Assay

The total antioxidant capacities (TAC) of the samples were determined by the phosphomolybdenum assay using ascorbic acid as the standard. An aliquot of 1.0 ml of extract (1000 µg) solution was combined with 1.0 ml of reagent (0.6 M sulphuric acid, 28 µM sodium phosphate and 4 µM ammonium molybdate). The tubes were capped and incubated in a hot water bath at 95°C for 90 min and cooled to room temperature. The absorbance of the aqueous solution of each mixture was measured at 695 nm in UV spectrophotometer. The blank solution having only reagent solutions was treated and analysed in a similar manner as described above. The total antioxidant capacity was expressed as equivalents of ascorbic acid.

3. RESULTS AND DISCUSSION

3.1 Synthesis

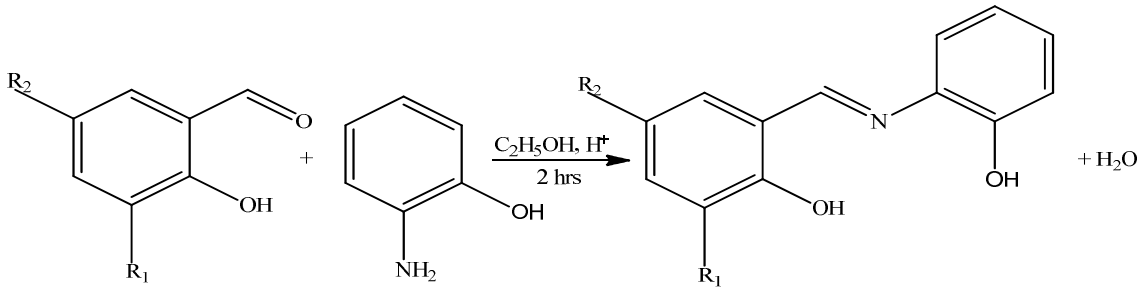
The condensation (Scheme 1), of *o*-aminophenol and corresponding substituted salicylaldehyde yielded the following Schiff bases: **I** (*E*)-2-((2-hydroxybenzylidene)amino)phenol, **II** (*E*)-2-(((2-hydroxyphenyl)imino)methyl)-4-nitrophenol, **III** (*E*)-4-chloro-2-(((2-hydroxyphenyl)imino)methyl) phenol, **IV** (*E*)-2-(((2-hydroxyphenyl)imino) methyl)-4-methoxyphenol and **V** (*E*)-4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-6-methoxyphenol.

3.2 Characterisation of the Schiff Bases

The air stable compounds were obtained as solids in good yields, their colours ranged from orange-wine-yellow. Their analytical data have been summarised in Table 1.

The Important IR, ^{1}H NMR and UV of the Schiff Bases are shown in Table 2. The IR spectral data of each of the Schiff Bases confirmed the formation of the azomethine bond ν(−HC=N). Their IR spectral data showed the azomethine ν(HC=N) bands in the range of 1627-1614 cm^{-1}. All the compounds displayed a band at 1306-1247 cm^{-1} which was assigned to the phenolic stretching ν(C−O) vibration while the hydroxyl (O−H) band appeared in the range of 3747-3067 cm^{-1} [15,23,31-36].

The ^{1}H NMR spectra of the Schiff bases (Figs. 1-5) showed two singlet signals at δ 13.78-13.07 ppm and δ 9.86-9.68 ppm, which were assigned to two phenolic –OH protons [9,15,23,35,37]. All the Schiff bases showed a singlet signal at δ 9.26-8.91 ppm attributed to the azomethine (−HC=N) protons [9,17,22,31]. The aromatic protons appeared as multiplets at δ 7.68-6.82 ppm [9,34,36,38,39]. One sharp singlet signal assigned to the protons of methoxy (–OCH_3) groups appeared at δ 3.71 and 3.80 ppm in the spectra of compounds **IV** and **V,** respectively [9,23]. The carbon-13 NMR spectra of the compounds exhibited singlet signals assigned to the azomethine carbon in the range 162.21-160.12 ppm. This further confirms the formation of the Schiff bases.



(**I**) R_1= R_2= H
(**II**) R_1= H, R_2= NO_2
(**III**) R_1= H, R_2= Cl
(**IV**) R_1= H, R_2= OCH_3
(**V**) R_1= OCH_3, R_2= Br

Scheme 1. Synthesis of Schiff bases (I-V)

Table 1. Analytical data of the Schiff bases

Compounds	Empirical formula	Molecular weight (g/mol)	Yield (%)	Elemental analysis (%)		
				C	H	N
I	$C_{13}H_{11}NO_2$	213.23	86%	73.22 (73.3)	5.21 (5.77)	6.57 (6.16)
II	$C_{13}H_{10}N_2O_4$	258.23	80%	60.50 (60.47)	3.89 (3.90)	10.82 (10.85)
III	$C_{13}H_{10}ClNO_2$	247.68	82%	63.03 (63.04)	4.07 (4.07)	5. 68 (5.66)
IV	$C_{14}H_{13}NO_3$	243.26	82%	69.10 (69.12)	5.40 (5.39)	5.80 (5.76)
V	$C_{14}H_{13}NO_3$	322.15	84%	52.18 (52.20)	3.80 (3.75)	4.46 (4.35)

Key: Calculated values are in parenthesis

Table 2. Important IR, ^{1}H NMR and UV of the Schiff bases

Compounds	IR (cm^{-1})			NMR (^{1}H and ^{13}C)		UV-Vis (nm)	
	O−H	C=N	C−O	δ(ppm)	Assignments	n-π*	π-π*
I	3746	1627	1274	13.78 9.73 8.92 7.56-6.84 162.21	(s, 1H, −OH) (s, 1H, −OH) (s, 1H, −HC=N) (m, 8H, $CH_{Aromatic}$) (s, 1C, −CH=N)	297	353
II	3067	1614	1306	13.50 10.34 9.26 8.56-6.84 160.59	(s, 1H, −OH) (s, 1H, −OH) (s, 1H, −HC=N) (m, 7H, $CH_{Aromatic}$) (s, 1C, −CH=N)	297	353
III	3730	1615	1272	13.77 9.76 8.92 7.68-6.83 160.51	(s, 1H, −OH) (s, 1H, −OH) (s, 1H, −HC=N) (m, 7H, $CH_{Aromatic}$) (s, 1C, −CH=N)	282	360
IV	3747	1626	1247	13.07 9.68 8.89 7.29-6.82 3.71 161.52	(s, 1H, −OH) (s, 1H, −OH) (s, 1H, −HC=N) (m, 7H, $CH_{Aromatic}$) (s, 3H, $CH_{Methoxy}$) (s, 1C, −CH=N)	270	370
V	3740	1615	1253	13.37 9.86 8.91 7.36-6.83 3.79 160.12	(s, 1H, −OH) (s, 1H, −OH) (s, 1H, −HC=N) (m, 6H, $CH_{Aromatic}$) (s, 3H, $CH_{Methoxy}$) (s, 1C, −CH=N)	290	350

**Key: s = singlet, m = multiplet.*

The electronic spectral data of the Schiff bases recorded two absorption peaks at 297-270 nm and 370-350 nm assigned to transitions of $n-\pi^*$ of the azomethine and $\pi-\pi^*$ of the aromatic ring in the Schiff bases, respectively.

3.3 Antimicrobial Activity

The mean of the inhibition zones from the results of the antimicrobial activities of the compounds are displayed in Table 3. The results revealed that all the synthesised compounds were active against all the bacterial strains to a varying extent except compound **II,** which was inactive against *S. typhimurium*. Compound **IV** with the electron-donating methoxy-substituent ($–OCH_3$) performed the highest activity to all the bacterial strains. This is in line with reports that $–OCH_3$ substituent increases antibacterial

activity [4]. The nitro-substituted ($-NO_2$) compound (**II**) exhibited the least activity to the bacterial strains. All the compounds were active against *S. agalactiae* which is resistant to Gentamycin.

The resistance of some of the pathogens towards the tested compounds can be attributed to the existence of cell wall in the bacteria, which reduces the permeability of the tested compounds. The activity against them can be attributed to the higher lipophilicity of the compounds [4].

3.4 Total Antioxidant Capacity

The difference in the total antioxidant capacities of the Schiff bases is presented in Table 4. This could be explained by the presence of different substituents on the compounds. The effect of the substituents on the total antioxidant capacities of the Schiff bases is same as their effects on the antimicrobial activities. Compound **IV** showed the highest total antioxidant capacities while compound **II** revealed the least capacities. Hence, compound **IV** was found to be a better free radical scavenger.

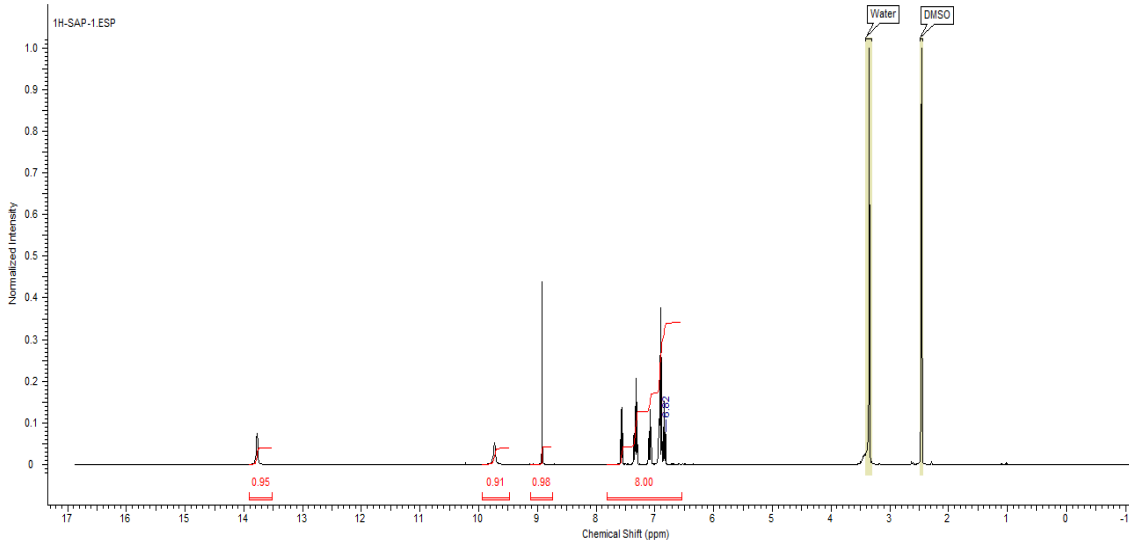


Fig. 1. The proton (^{1}H), NMR spectrum of compound (I)

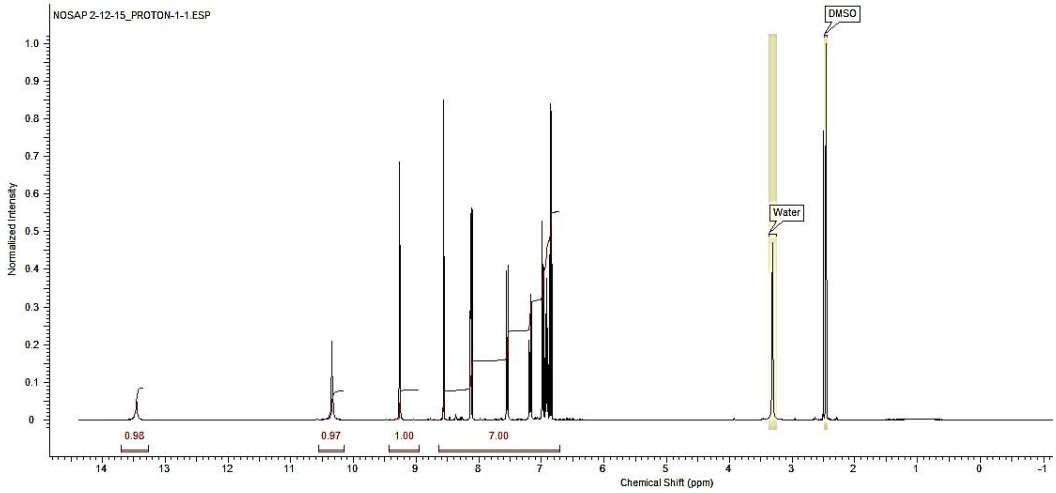


Fig. 2. The proton (^{1}H), NMR spectrum of compound (II)

Table 3. Mean of the zones of inhibition showing the antimicrobial potentials of the compounds (1-V) over the range of concentrations used

	Mean (n=3), zones of inhibition (mm)						
Compound	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***S. agalactiae***	***S. aureus***	***S. typhimurium***	***P. mirabilis***
I	16.33±0.58[a]	15.67±0.58[b]	17.67±1.16[c]	14.00±0.00[d]	16.33±0.58[f]	15.67±0.58[g]	14.00+0.00
II	13.67±0.58	15.00±0.00[b]	12.00±0.00	15.33±2.31[d]	8.00±0.00	0.00±0.00	11.00+0.00
III	15.33±0.58[a]	13.33±4.16	16.00±0.00[c]	20.00±0.00[e]	15.33±3.51[f]	16.00±1.73[g]	20.00+0.00
IV	22.00±0.00	20.00±0.00	30.00±0.00	22.00±0.00[e]	30.00±0.00	18.00±0.00	25.00+0.00
V	11.33±0.58	13.00±0.00	13.67±2.89	13.33±2.89	15.00±1.73[f]	11.00±0.00	12.33+0.58
DMSO	–	–	–	–	–	–	–
Gentamycin	20	18	20	–	20	11	20

Key: a, b, c, d, e, f, and g: Not significant (p>0.05) difference in zones of inhibition of the compounds in a given organism.

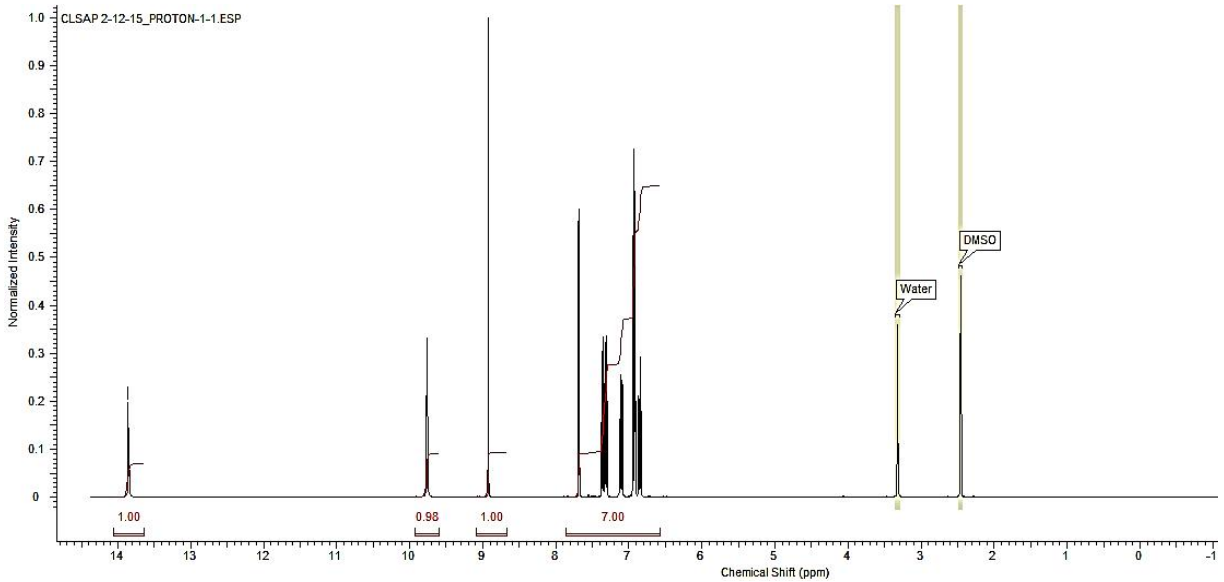


Fig. 3. The proton (^{1}H), NMR spectrum of compound (III)

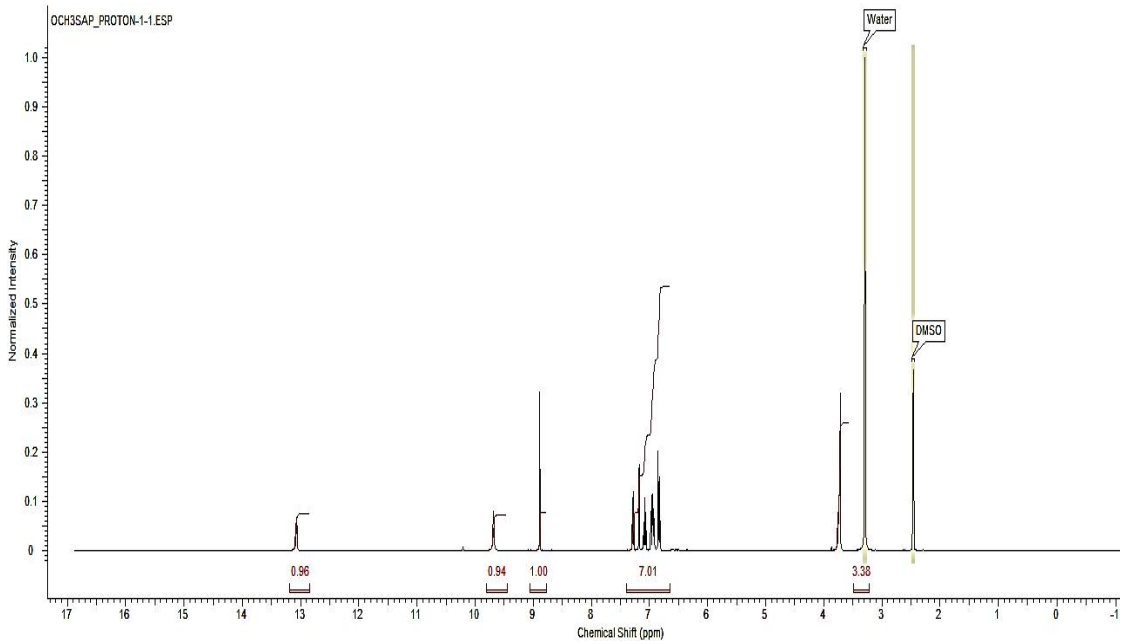


Fig. 4. The proton (^{1}H), NMR spectrum of compound (IV*)*

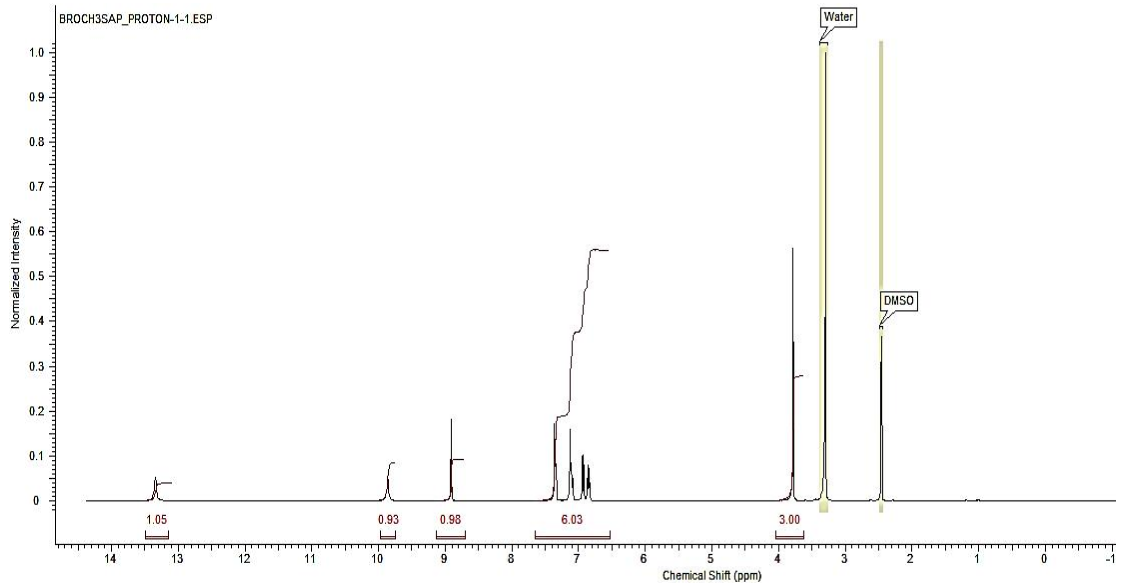


Fig. 5. The proton (^{1}H), NMR spectrum of compound (V)

Table 4. Total antioxidant capacity (TAC)

Samples	I	II	III	IV	V
TAC µg per mg AA	0.68	0.52	0.73	0.78	0.56

Key: *AA = ascorbic acid*

4. CONCLUSION

The present study revealed that the methoxy-substituted Schiff base exhibited the highest antibacterial and antioxidant activities. The antibacterial and total antioxidant activities results revealed the order of activity of the compounds as **IV** > **III** > **I** > **V** > **II**

indicating a correlation between the antimicrobial activity and the TAC. Thus, it can be concluded that the electron-donating methoxy group enhances the antibacterial and antioxidant activities of the studied compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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