



## Synthesis, Spectroscopic and Thermal Characterization and Antioxidant Activities of Three Schiff Bases Derived from Aminophenol

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### ABSTRACT

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The main objective of this work is synthesized three Schiff bases: (E)-2-(((2-hydroxyphenyl)imino)methyl)-6-methoxyphenol (I), (E)-2-(((3-hydroxyphenyl)imino)-Methyl)-6-Methoxyphenol(II) and (e)-2-(((4-hydroxyphenyl)imino)methyl)-6-amethoxyphenol(III) derivative of 2-hydroxy-3-methoxybenzaldehyde and ortho/meta / para-aminophenol, their structures were confirmed by spectroscopic methods: UV-Vis, FTIR, (<sup>1</sup>H, <sup>13</sup>C NMR). Firstly, a thermogravimetric analysis (TGA/DTG) study of the composite Schiff bases proved them to be thermally stable. Secondly, the study of the biological properties of the synthesized compounds allowed the evaluation of the antioxidant activity by conventional methods (scavenging of the DPPH radical, and bleaching of β-carotene). The results demonstrated a variable degree of inhibitory effects on the growth of synthesized Schiff bases.

## 1. INTRODUCTION

Schiff bases discovered by Hugo Schiff in 1864 [1], have demonstrated that their structure contains the azomethine group -C=N-. Schiff bases, generally belongs to a very important class of organic compounds with wide range of a potential application, especially in hydrogenation and addition reactions as oxidation catalysts [2-6] and in anti-COVID-19 drug, anticancer drugs and electrochemistry as antibacterial and antiviral agents [7-12]. Schiff bases are comprising of few important ligands that coordinate with metal ions through azomethine nitrogen to form the complexes [13, 14]. The corresponding chelates of Schiff bases have gained significant research interest owing to their ability to stabilize many different metals in various oxidation states [15-19]. They can also be used as an effective corrosion inhibitor since they are capable to form a monolayer spontaneously on the surface of metals [20-22]. The effectiveness of these inhibitors towards corrosion of steel has already been confirmed in hydrochloric and sulfuric acid environments [23-26]. The presence of methoxy (-OCH<sub>3</sub>), hydroxyl (-OH) or methyl (-CH<sub>3</sub>) groups on the aromatic ring improves the inhibitor efficiency of Schiff bases in comparison to the existence of nitro (-NO<sub>2</sub>) and halide groups [27-31]. The inhibitor efficiency primarily resulted

from the adsorption phenomenon at the metal surface due to existence of imine group, electronic cloud, electronegative nitrogen, oxygen and sulfur atoms in the Schiff bases. Mainly, the electron transfer process between metal surface, electrophile and inhibitor, Lewis's base carried out by nucleophilic centers, oxygen and nitrogen atoms. Furthermore, the availability of the benzene rings enhances the creation of adsorption sites for the inhibitor and ultimately enables the formation of monolayer on the metal surface [32, 33] Synthetic phenolic compounds like Schiff bases also exhibit real antioxidant activity and can have potential applications in drugs for the treatment of diseases caused by free radical damage [34, 35].

Despite the works carried out on the Schiff bases mentioned above, these still require in-depth studies, particularly in the field of biology. In the present work, synthesis of three Schiff bases derived from condensation of 2-hydroxy-3-methoxybenzaldehyde and ortho/meta/para-aminophenol with 2-hydroxy-3-methoxybenzaldehyde has been reported. The characterization of synthesized Schiff bases was carried out by UV-Vis, FTIR, <sup>1</sup>H NMR and <sup>13</sup>C NMR techniques. Moreover, antioxidant properties of Schiff bases have been studied using DPPH free radical scavenging assay and β-carotene bleaching test.

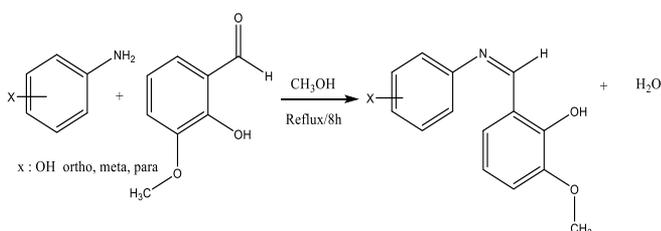
## 2. EXPERIMENTAL

### 2.1 Materials and instruments

All the required analytical grade chemicals were purchased from Sigma-Aldrich and used without further purification. The reactions were observed through thin layer chromatography (TLC) on 0.25 mm thick E-Merck plates (60F-254) of silica gel. The eluent was mixture of methanol and dichloromethane with stoichiometric ratio as 4.5:0.5 and the spots were visualized under UV light. Melting point of the compounds was measured by a digital apparatus (Koeffler Banc). The UV-Vis and FTIR spectroscopic measurements were performed at room temperature using JASCO V- 650 and Perkin Elmer spectrophotometer, respectively. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of Schiff bases were recorded by Bruker Advance III HD 400 MHz NMR spectrometer in DMSO- $d_6$  with tetramethylsilane as an internal standard. Perkin Elmer TGA 4000 apparatus was used to perform thermal analyses (TGA/DTG) in nitrogen atmosphere for temperature range of 50 to 600°C.

### 2.2 Synthesis of the Schiff bases

The Schiff bases (E)-2-(((2-hydroxyphenyl)imino)methyl)-6-méthoxyphenol (I), ((E)-2-(((3-hydroxyphenyl)imino)methyl)-6-méthoxyphenol (II) and (E)-2-(((4-hydroxyphenyl)imino)methyl)-6-méthoxyphenol (III) have been synthesized by condensation of a equimolar (1:1) methanolic solution of 2-aminophenol (4 mmol, 0.43652 g), 3-aminophenol, (4 mmol, 0.43652 g), 4-aminophenol(4 mmol, 0.43652 g) and 2-hydroxy-3-methoxybenzaldehyde (4 mmol, 0.6086 g) under reflux conditions for 8 hours (Figure 1).



**Figure 1.** Schematic of reaction for the synthesis of Schiff bases I, II and III

The color of the reaction mixtures turns to orange and light orange. The solutions were then allowed to cool under glace. The orange and light oranges precipitates were formed after few days and were collected by filtration followed by washing with methanol and diethyl ether. The monitoring of reactions was carried out by thin layer chromatography (TLC), the reaction yield and melting point for all three synthesized compounds is given in Table 1. The structure synthesized Schiff bases have been studied through FTIR, ( $^1\text{H}$ ,  $^{13}\text{C}$ ) NMR and UV-Vis spectral data.

**Table 1.** Physical data of Schiff bases (I-III)

Schiff bases	Molecular formula	Color	Yield (%)	M.P. (°C)
I	C <sub>14</sub> H <sub>13</sub> NO <sub>3</sub>	Orange	40.74	195
II	C <sub>14</sub> H <sub>13</sub> NO <sub>3</sub>	Light orange	55.29	162
III	C <sub>14</sub> H <sub>13</sub> NO <sub>3</sub>	Light orange	57.88	150

### 2.3 Antioxidant activities

#### 2.3.1 Radical scavenging activity DPPH

The scavenging activity of free radical was evaluated by adopting the procedure as described in literature [36]. In this test, the antioxidant reduces the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) with violet color and demonstrated absorption at 517 nm. When DPPH is mixed with a hydrogen atom donor, a stable non-radical form of DPPH-H obtained with the change in color from violet to pale yellow [37, 38]. Methanolic solution (1250  $\mu\text{l}$ ) of DPPH (0.1 mmol/l) was added to 50  $\mu\text{l}$  of the tested sample (I-III) dissolved in methanol and butylatedhydroxytoluene (BHT) solutions with different concentrations. The reaction mixture was stirred vigorously and kept at room temperature in dark for 30 min. The percentage of free radical scavenging activity for various compounds was studied with the help of following equation [39]:

$$RSA_{DPPH} \% = \left[ \frac{A_{control} - A_{sample}}{A_{control}} \right] \times 100 \quad (1)$$

where, A control and A sample are absorbance for DPPH solution and the samples, respectively. The variation in percentage of scavenging activity was plotted as a function of sample concentration and IC<sub>50</sub> was calculated. The IC<sub>50</sub> represents the inhibition concentration of test samples that reduces up to 50% of the initial free radical. All tests were repeated thrice and the IC<sub>50</sub> values were reported as mean  $\pm$  SD.

#### 2.3.2 $\beta$ -carotene bleaching test

The inhibition of volatile organic compounds and conjugated double bond hydroperoxides appeared in oxidation of linoleic acid was measured to estimate the antioxidant activity of Schiff bases (I-III) [40]. The emulsion of linoleic acid was prepared by dissolving 0.5 mg of  $\beta$ -carotene in 1ml of chloroform; 25 $\mu\text{l}$  of linoleic acid and 200 mg of Tween 40 were added. Afterwards, rota vapor at 40°C was used to evaporate chloroform followed by addition of 100 ml of distilled water to obtain the emulsion. 35 ml of the tested sample prepared in methanol (2mg/ml) was added to the 2.5 ml of emulsion [41]. Similar method (distilled water, methanol) was used for both positive and negative standard BHT. The absorbance was observed at 490 nm for different time spans like, 0, 1, 2, 3, 4, 6 and 24 hrs. of incubation in dark at room temperature. The inhibition (%) of  $\beta$ -carotene decomposition by tested samples (I-III) was estimated by the following equation [42]:

$$AA(\%) = \frac{A_{sample}}{A_{BHT}} \times 100 \quad (2)$$

where: AA%, A<sub>sample</sub> and A<sub>BHT</sub> are the percentage of the antioxidant activity, absorbance with tested products and absorbance with positive control BHT.

## 3. RESULTS AND DISCUSSION

The prepared Schiff bases (I-III) are found to be non-hygroscopic and colored solids. They are, insoluble in water while soluble in DMF and DMSO. The structure of prepared Schiff bases (I-III) was established through various spectroscopic techniques (UV-Vis, IR,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR).

### 3.1 Spectroscopic analysis

#### 3.1.1 UV-Vis analysis

UV-Vis spectra of prepared Schiff bases (I-III) are illustrated in Figure 2 (a), (b) and (c), respectively. The absorption bands appeared in the region from 200-280 nm are attributed to the  $\pi \rightarrow \pi^*$  transitions of the benzene rings [43-46] and an absorption band appeared in the region from 280-350 nm is assigned to  $n \rightarrow \pi^*$  transition of azomethine group [46, 47]. However, in the visible region, from 400-550 nm, absorption corresponds to the  $n-\pi^*$  intramolecular charge transfer interaction [46, 47].

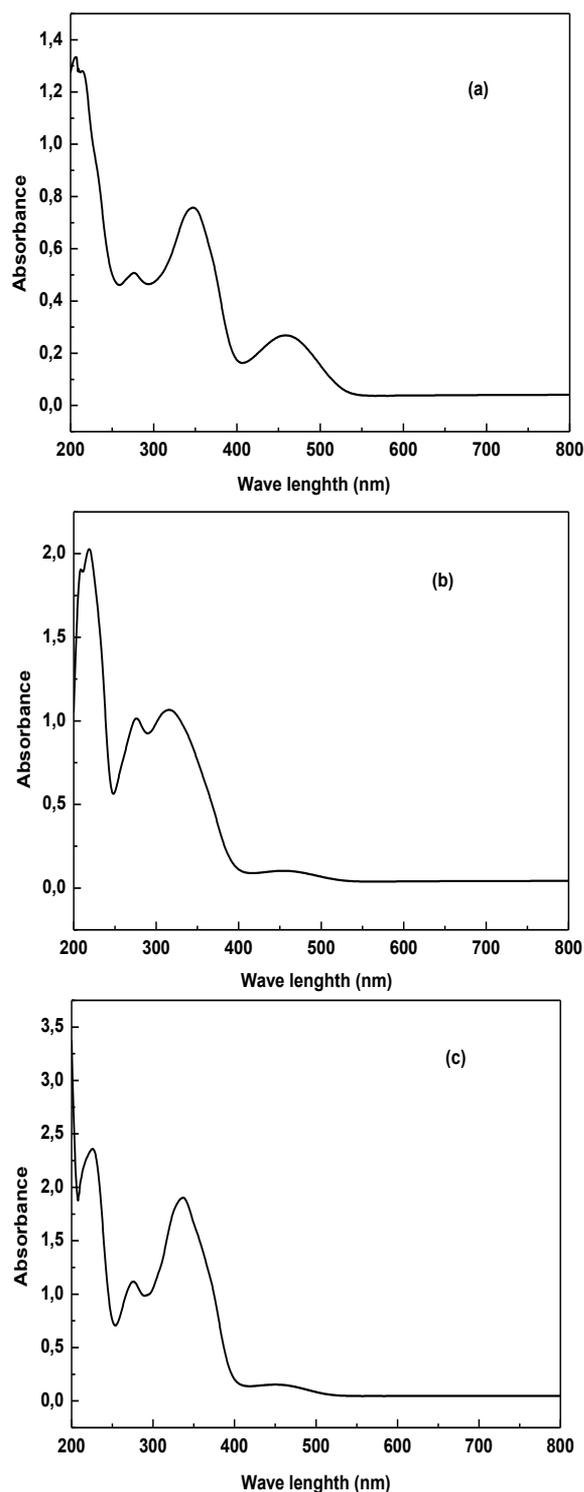


Figure 2. UV-Vis spectrum of: (a) I, (b) II and (c) III

#### 3.1.2 FTIR analysis

The data regarding significant infrared bands for synthesized Schiff bases (I-III) extracted from Figure 3 (a), (b) and (c), are given in Table 2.

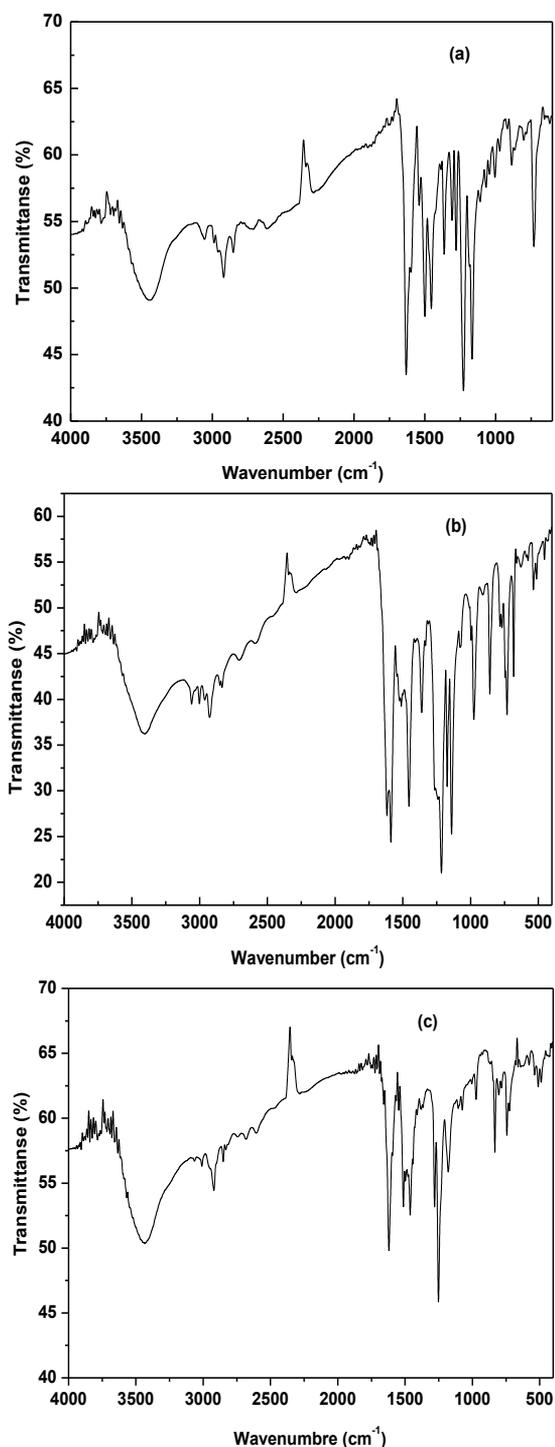


Figure 3. FT-IR spectrum of: (a) I, (b) II and (c) III

Table 2. Experimental IR results ( $\text{cm}^{-1}$ )

Schiff bases	I	II	III
$\nu(\text{O-H})$	3445	3411	3435
$\nu(\text{C-H})_{\text{Ar}}$	3056	3057	3013
$\nu(\text{C-H})_{\text{as,s}}$	2990-2850	2998-2824	2924-2817
$\nu(\text{-CH=N-})$	1627	1631	1618
$\delta(\text{O-H})$	1223	1223	1239
$\nu(\text{C=C})_{\text{Ar}}$	1504	1454	1517
$\delta(\text{C-H})$	1368-1290	1366-1221	1461- 1284
$\nu(\text{C-O})_{\text{as,s}}$	1226-1167	1176-1138	1240-1176

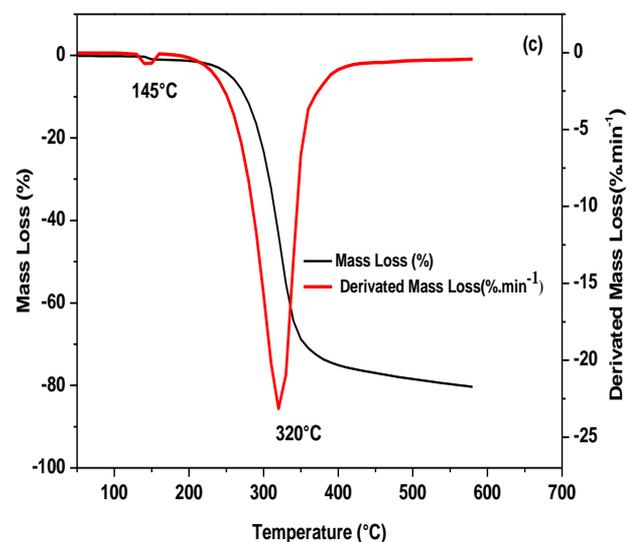


### 3.1.4 $^{13}\text{C}$ NMR analysis

The  $^{13}\text{C}$  NMR spectra of Schiff bases (I-III) are shown in Figure 5 (a), (b) and (c) respectively. The signals of characteristic carbon like methoxy carbons are observed at 56.336, 56.376 and 56.349 ppm [49, 58], aromatic ring carbon atoms are observed in the range from 109 to 159 [58]. The signals of azomethine carbons appeared at 162.005, 163.741 and 163.741 ppm, respectively [47, 49]. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra confirm the formation of Schiff bases.

### 3.1.5 Thermal behavior

The decomposition temperature of the Schiff bases is an important parameter to study for the synthesis of these ligands in order to understand the thermal stability of these products. In this section we will focus on the TGA/DTG curves of the Schiff bases (I-III) are illustrated in Figure 6 (a), (b) and (c) revealed that the decomposition of Schiff bases begins at about 285°C however; these are thermally stable at 190, 200 and 230°C, is no decomposition or chemical transformation below these temperatures respectively. Some studies have shown that the thermal stability of the prepared Schiff bases may be due to the presence of inter- or intra-molecular H-bonding in Schiff bases [59-61].



**Figure 6.** TGA curves (black) and DTG curves (red) of: (a) I, (b) II and (c) III

## 3.2 Antioxidant activities

### 3.2.1 Radical scavenging activity DPPH

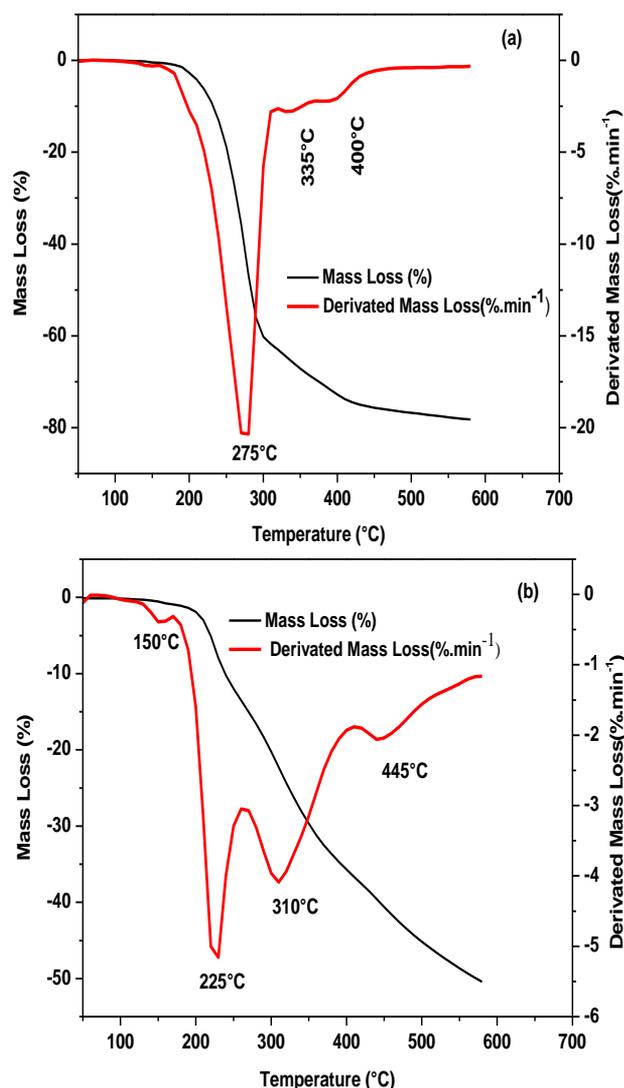
N,N-diphenyl-N'-picrylhydrazyl (DPPH) assay was used to access the antioxidant activity of synthesized Schiff bases (I-III). (Figure 7). There is a proportional increase in the percentages of inhibition of the Schiff base as a function of concentration; the basic Ligand of Schiff presents an activity to trap the radical DPPH. This activity is linked to the mobility of the Basic Ligand hydrogen atom of Schiff in the presence of a free radical DPPH, the atom H is transferred to it to transform it into a stable DPPH molecule, this causes a decrease in the Concentration of free radicals and also the decrease in absorbance over the reaction time to the exhaustion of the antioxidant hydrogen donor.

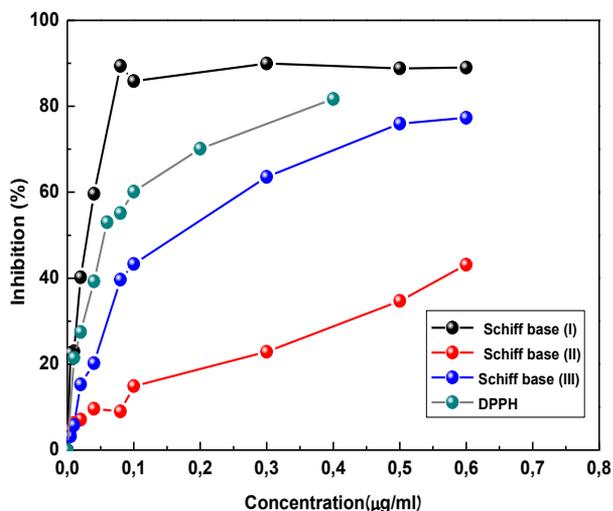
**Table 3.** Antioxidant activity of Schiff bases (I-III)

Compounds	DPPHIC50 $\mu\text{g/ml}$
<b>I</b>	$0.0117 \pm 0.0000915062$
<b>II</b>	$0.1647 \pm 0.00087575$
<b>III</b>	$0.0609 \pm 0.00299386$
<b>BHT</b>	$0.0231 \pm 0.0017$

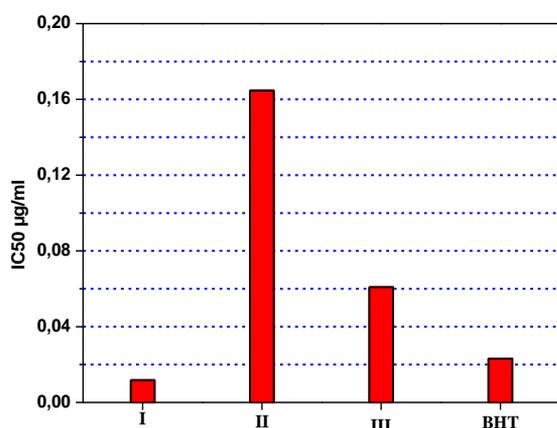
The overall DPPH radical scavenging activity of Schiff bases I, II and III is given in Table 3. The small value of IC50 indicated high free radical scavenging activity of samples a minute amount is enough to reduce the concentration of DPPH by 50%. Although, all the compounds showed radical scavenging activities in DPPH assay but Schiff base I with hydroxyl group at ortho position is appeared as strong antioxidant than Schiff base III with a para-OH group and Schiff base II with meta-OH group (Table 3).

It has also been observed that the Schiff base I demonstrated a higher radical scavenging activity as compared to the standard BHT (Figure 8).





**Figure 7.** Curves of the Radical Scavenging Activity of Schiff bases (I, II and III) and BHT



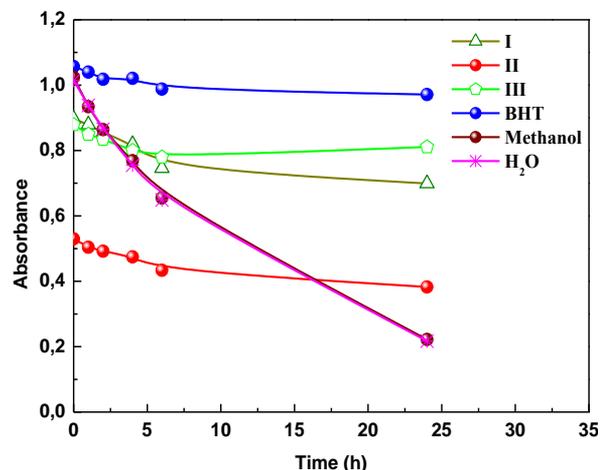
**Figure 8.** Histograms of IC<sub>50</sub> of different compounds and BHT standard

### 3.2.2 β-carotene bleaching test

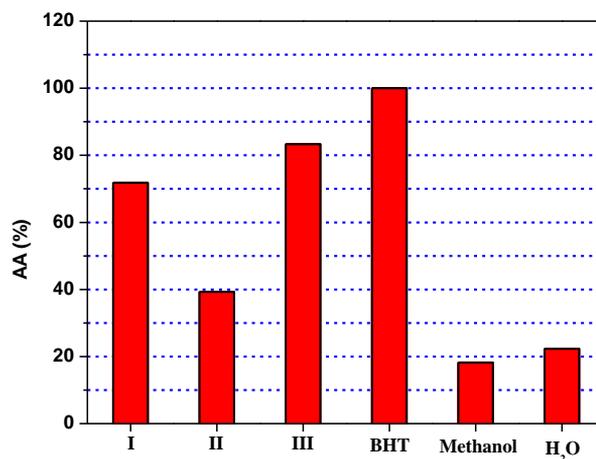
The mechanism of β-carotene bleaching resulted from the presence of hydroperoxides arises from linoleic acid. The hydro peroxide oxidizes the β-carotene and reduces the intensity of orange color of β-carotene. The antioxidants are used to minimize this oxidation process of the β-carotene. In the incubation period, the absorbance of β-carotene is stable in case of BHT, while in case of methanol and water; β-carotene oxidizes very quickly. The bleaching test revealed that, β-carotene oxidizes slowly in the presence of Schiff bases I, II and III (Figure 9).

In the presence of the antioxidant BHT, the absorbances of the β-carotene solution remain stable throughout the incubation period. The synthesized schiff bases showed remarkable antioxidant activity. The negative control sample oxidizes the fastest. However, after 24 hours, Schiff base III demonstrated highest antioxidant activity as 83.32% as compared to Schiff bases I and II as 71.81 and 39.3%, respectively. The antioxidant activity of the synthesized products can be summarized as: eau < methanol < II < I < III < BHT.

Figure 10 shows that the antioxidant activity of Schiff bases (I-III) is far better than BHT and the negative control samples. The greater effectiveness of BHT in preventing the bleaching of β-carotene can be attributed to their greater solubility in the linoleic acid system.



**Figure 9.** β-carotene–linoleic acid assay of Schiff bases (I-III)



**Figure 10.** Percent inhibition histogram AA% after 24 h, compared to BHT as positive control and to negative controls

## 4. CONCLUSIONS

In summary, three Schiff bases (E)-2-(((2-hydroxyphenyl)imino)methyl)-6-méthoxyphenol (I), (E)-2-(((3-hydroxyphenyl)imino)-methyl)-6-méthoxyphenol (II) and (E)-2-(((4-hydroxyphenyl)imino)methyl)-6-méthoxyphenol (III) have been synthesized by a simple condensation of 2-hydroxy-3-methoxybenzaldehyde with ortho/meta/para-aminophenol, respectively in methanol. The synthesized compounds were characterized through UV-Vis, FTIR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral data. The FTIR spectra depicted the presence of functional groups of Schiff bases (I-III) and validates the formation of imines bond (-C=N-). <sup>1</sup>H-NMR, <sup>13</sup>C-NMR spectral data confirm the molecular structures of these compounds. Furthermore, synthesized Schiff bases are solid with sharp melting points indicating the purity of compounds. Moreover, the thermal analysis illustrates a good thermal stability of these Schiff bases up to 210°C. DPPH free radical scavenging method was used to determine the antioxidant activity. The results revealed that the radical scavenging activity of the compound I was much higher than BHT. The values of IC<sub>50</sub> demonstrated that antioxidant activities of synthesized compounds are in order as; II < III < I. Schiff bases (I-III) may have potential applications in context of their excellent antioxidant activity.

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