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Investigation of the Biological Activity of Modified Branched Poly (P-Hydroxystyrene)

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ABSTRACT

The development of novel antimicrobial polymers is one of the most promising methods for preventing the growth of germs and limiting the spread of infections in a range of applications. Modified polymers have attracted an interest in biological activities. A series of modified poly (p-hydroxystyrene) was screened for in vitro antimicrobial activities against Gram-positive bacteria (Staphylococcus aureus), Gram-negative bacteria (Escherichia coli) and fungi (Candida albicans). Through the utilization of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, the antioxidant activities were examined. To evaluate each compound's affinity and binding pattern towards the *E. coli* protein active site, molecular docking was used. The modification was performed by chloroacetylation, then amination with 1,2-ethylenediamine and finally, the Schiff base compound was produced by condensation of 3-hydroxy-4-methoxybenzaldehyde with the polymer terminated with an amino group. The resulting compounds' structure was confirmed using FT-IR, elemental analysis, TGA and XRD. All synthesized compounds were identified and characterized. The prepared S-BPHS compound showed potential antioxidant and antimicrobial activities.

KEYWORDS: Poly(hydroxystyrene), Schiff base, docking, antibacterial, antioxidant.

1. INTRODUCTION

In numerous fields, infections by pathogenic microbes are an urgent issue, especially in the areas of medical devices, pharmaceuticals, clinic surfaces, dental care, surgical instruments, health care items, sanitary applications, water purification, fibers, food storage and packaging. Microbes (bacteria, fungi, viruses, and protozoa) are responsible for these infections, and they can be found in soil, water and the air. Most of the time, they spread through contact, ingesting or inhalation [1]. These infections can result in septicemia, a dangerous disease that kills a large number of people each year all over the world [2]. The increasing strength of antibiotic-immune people and multidrug-resistant germs, as well as the need for advanced antibiotic formulations to effectively combat them, make treating microbial infections increasingly challenging [3, 4]. A potential strategy to reduce the extent of this issue is prevention, which involves inhibiting the growth and development of bacteria or simply blocking their attachment to various substrate surfaces to prevent their proliferation. Research and development of new antibacterial materials is thus turning out to be a very effective strategy. In addition to conventional disinfectants, low-molecular-weight antibacterial agents can also destroy microorganisms [5]. The short-term antibacterial properties, toxicity to people,

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and environmental contamination caused by residue showed several drawbacks [1, 6, 7]. Polymer molecules can incorporate antimicrobial functional groups to solve concerns associated with low-molecular-weight antimicrobial agents. As various polymers are biocompatible, they are frequently utilized as biomaterials [8, 9]. Antimicrobial polymers are made of compounds that either eliminate or inhibit the growth and proliferation of germs. They offer a preventive treatment that may be utilized to avoid the development of an infection. These polymers, in contrast to antibiotics, reduce the chance that bacteria will become resistant. Chemicals that work against both bacteria and viruses might be added to these polymers. These antibacterial polymers are easily adaptable to broader uses once they are created [1, 10].

Therefore, scientists focused on developing new polymeric materials with outstanding chemical and thermal resilience [11]. Other functional groups can be added to these polymers to give them additional advantageous features [12]. Echeverría *et al*. prepared cellulose modified by 1-methylimidazolium chloride that showed antimicrobial activity against both -ve and +ve bacteria [13]. The production of Schiff base polymers with a broad variety of biological features is formed by the condensation of an aminated polymer with compounds containing carbonyl groups to form compounds with azomethine groups and conjugated bonds, which become more and more relevant [14]. The coupling of azomethine (C=N) in the backbone is accountable for the fundamental characteristics of polymeric Schiff bases [15]. Schiff bases have gained increased importance in the medical and pharmaceutical industries due to a wide range of biological effects it can make, being antibacterial, anti-inflammatory, anticancer, analgesic, antioxidant, antitubercular, anticonvulsant, anthelmintic, and so on [16-18]. The nitrogen atom in azomethine could aid in establishing a hydrogen connection with the active centers of many cell components, which would disrupt normal cell functions [19]. Chelate production improves a drug's lipophilicity and effective permeability at the site of action, which in turn enhances the drug's efficacy. The extent to which Schiff bases exhibit biological activities efficient against a variety of bacterial and fungal species as well as tumors has been shown in several published studies. Kenawy *et al*. synthesized methyl acrylate chitosan condensed with p-nitrobenzaldehyde, which showed significant antimicrobial, anti-biofilm, and antioxidant activities compared with the original chitosan [20].

So, this study involves the modification of poly(p-hydroxystyrene) to form a polymer-based Schiff base. The novel polymer was evaluated for antimicrobial and antioxidant activities.

2. MATERIAL AND METHODS

2.1. Materials

Branched poly (p-hyroxystyrene) [BPHS] was purchased from TriQuest, LP, A ChemFirst Company. Chloroacetyl chloride and pyridine were provided by Acros Organics (New Jersey, USA). Ethylenediamine [EDA], 3-hydroxy-4-methoxy benzaldhyde and 2,2-diphenyl-1-picrylhydrazyl were obtained from Sigma-Aldrich, USA. Dimethyl sulfoxide (DMSO), Dimethyl Formamide (DMF), diethyl ether and diluted HCl were purchased from El-Gomhouria Chemicals Company, Egypt**.**

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2.2. Instrumentation

FT-IR (fourier-transform infrared) spectra were recorded on a Massachusetts, US Perkin Elmer 1430 ratio. The compounds were scanned against a blank pellet backdrop of KBr in the 400–4000 cm-1 wavelength range at the Tanta University Faculty of Science in Egypt. XRD spectra were acquired using the CuKα target (1.5406 Å) at 40 kV and 30 mA on an APD 2000 PRO diffractometer (GNR analytical instruments group, Italy). Elemental Analyzer Model 1106 from Carlo Erba Strumentazione in Milan, Italy, was used at the Microanalytical Center-Cairo University in Cairo, Egypt, prior to the use of elements in microanalysis. Thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTG) were performed at Tanta University's Faculty of Science using a Perkin Elmer TGA 4000 Thermogravimetric analyzer. Alumina crucibles containing 5–10 mg of sample were filled with N2 at a flow rate of 20 ml/min, and the samples were scanned at a rate of $30°C$/min between 50 and $800°C$.

2.3. Chloroacetylation of Branched Poly(p-hyroxystyrene) [Cl-BPHS]

In a round-bottomed flask, 1.93 mmol of poly(p-hyroxystyrene) was dissolved in 60 mL of DMF before the addition of 56.96 mmol of pyridine. The flask was transferred to an ice bath to cool the mixture, and then 57.76 mmol of chloroacetyl chloride was added dropwise with stirring for 3 h. The reaction was continued for 3 days at R.T. The resultant product was obtained by precipitation in 1N HCl then filtered off and washed with distilled water [21].

2.4. Synthesis of Aminated Branched Poly(p-hyroxystyrene) with EDA [AM-BPHS]

To a solution of chloroacetylated branched poly(p-hyroxystyrene) [3.2 g, 1.17 mmol] dissolved in 20 mL DMF at $50\ °C$, ethylene diamine [1.9 g, 33.2 mmol] was added dropwise and refluxed for 4 days at $80°C$. The aminated polymer was precipitated by pouring in hot water, filtered and washed several times with diethyl ether [22].

2.5. Synthesis of Branched Poly(p-hyroxystyrene) Schiff base [S-BPHS]

Aminated polymer (AM-BPHS) [3.154 g, 1 mmol] was mixed with 2.4 g 3-hydroxy-4-methoxy benzaldehyde in 20 mL DMF and refluxed for four days at $90\ °C$. The product was obtained by adding it to hot water, then separated by filtration [22].

2.6. Molecular Docking

For the docking investigation on Escherichia coli (PDB code: 7AB3) obtained from the protein database bank (PDB), the molecular operation environment (MOE, 2015.10) application was utilized. All of the minimizations were done using the Merck molecular force field (MMFF94) until the gradient of the root-mean-square deviation (RMSD) was at 0.01 kcal/mol/A. The output data for the final score function (S) was recorded for the ligand [23-25].

2.7. Antimicrobial Activities of Selected Derivatives of BPHS

The antibacterial action of modified polymers was evaluated against a selection of Gram-positive (Staphylococcus aureus), Gram-negative (Escherichia coli) bacteria and their

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antifungal actions (Candida albicans) by using the disc diffusion method [26]. In our study, nutrient agar for bacteria and Sabouraud Dextrose agar for fungi were used. On each plate, 100L of standardized microbial suspensions (0.5 McFarland turbidity standards) were applied to the entire surface. Separate paper discs of standard-sized Whatman filter paper with a diameter of 5 mm were sterilized. Tested materials were immersed in DMSO at a concentration of 1 mg/mL then sterilized paper discs were put in them. The paper discs were aseptically placed onto the petri plates. The culture plates were incubated at $36^{o}C$ and after 24 hours, the inhibition zones were measured by a ruler in mm. Each test was repeated three times. Using the same procedure, the antifungal Colitrimazole and the antibiotic Ampicillin were also tested as standards for antibacterial activity using the same dose and solvent [27, 28]. The % activity index was derived from applying this equation:

$$\%\ \text{Activity Index} = \frac{\text{Zone of inhibition by test compound (diametre)}}{\text{Zone of inhibition by standard (diametre)}} \times 100 \quad (1)$$

2.8. Antioxidant Activity of Some Polymer Derivatives

In this work, the DPPH assay (a stable 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay) was used to evaluate the antioxidant action of new modified compounds. The synthesized samples were introduced at doses ranging from 10 to 100 µM in DPPH solution (0.01 mmol). The compounds' scavenging capability was recorded by a spectrophotometer. The absorbance of the DPPH· solution was measured at 517 nm at time = 0 min (A_0) and after 30 min of incorporating the sample into the DPPH· solution (A_{30}) at R.T. The scavenging activity was determined by calculating the rate of inhibition ($I\%$) according to this equation:

$$I\% = 100(A_0 - A_{30})/A_0\%. \quad (1)$$

The inhibitory concentration (IC_{50}) of each sample was determined after 30 minutes of reaction and compared to that of L-ascorbic acid (Vitamin C) as a standard [29, 30].

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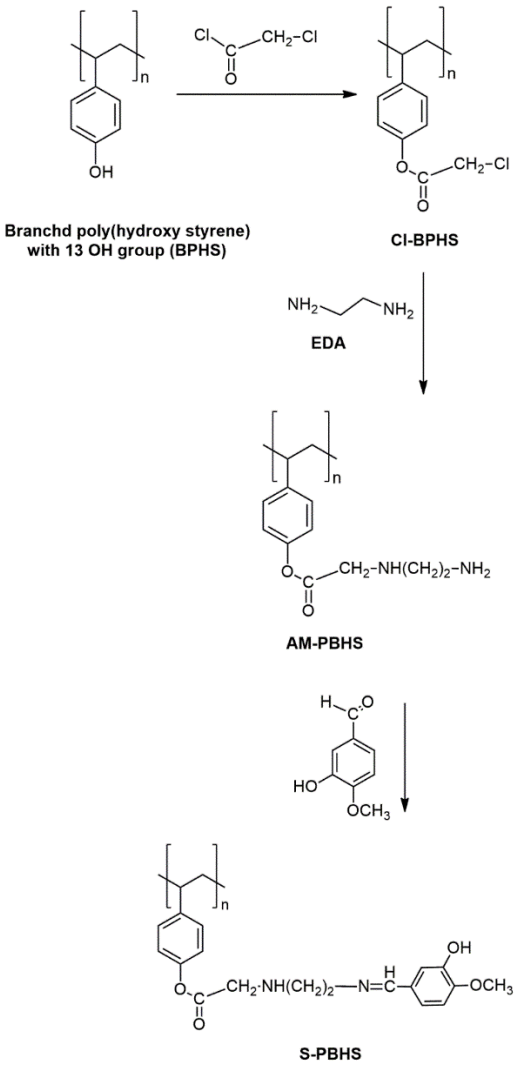

Fig. 1: Synthesis of aminated polymer [AM-BPHS] and schiff base derivative [S-BPHS].

3. RESULTS AND DISCUSSION

The polymer was functionalized by the addition of chloroacetyl chloride to pyridine to obtain chloroacetylated polymer that can be used to form aminated polymer (AM-BPHS) by the addition of ethylenediamine to chloroacetylaed polymer, as shown in Fig. 1. In order to obtain the Schiff base compound, the polymer with the terminal amino group was condensed with 3-hydroxy-4-methoxybenzaldhyde. The compounds' structures were verified by FT-IR, elemental analysis, XRD and TGA analysis.

3.1. Characterization of the Modified Polymer

The FT-IR spectrum for chloroacetylated polymer [Cl-BPHS] (Fig. 2 (B) showed all the characteristic peaks belong to poly (p-hydroxy styrene), with a decrease in the peak intensity and a slight shift of the OH peak from 3439 to 3420 cm-1 as well as the appearance of peaks at 1734 cm-1 corresponding to the C=O, 1164 and 689 cm-1 corresponding to CH2Cl presence in the chloroacetyl group [21].

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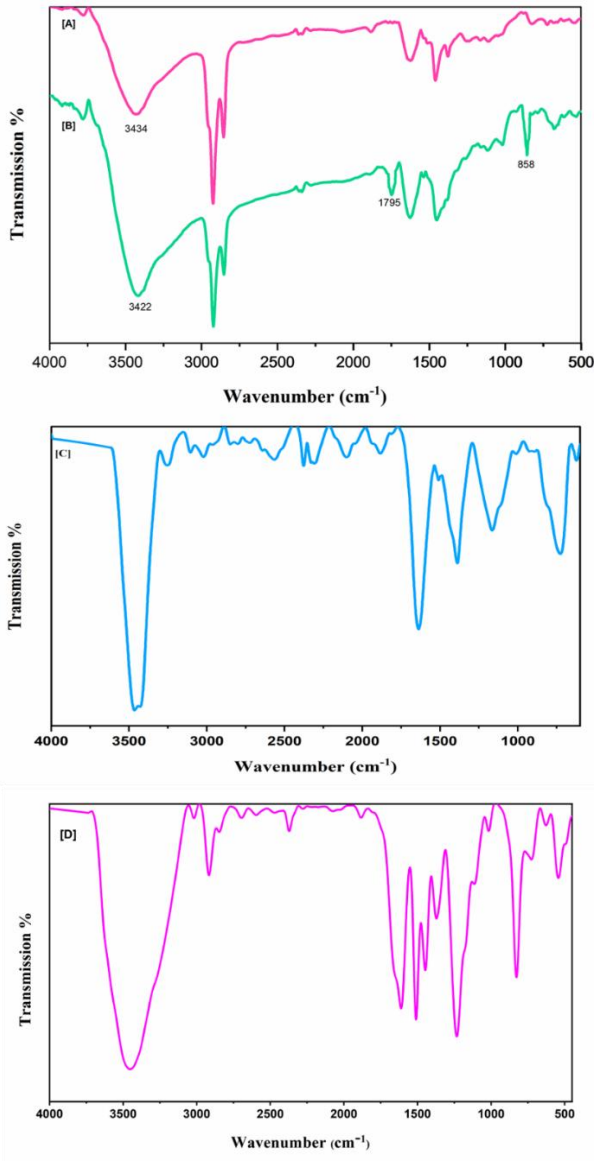

Fig. 2: FT-IR spectra of [A] BPHS, [B] chloroacetylated polymer Cl-BPHS, [C] aminated poly(p-Hydroxystyrene) [AM-BPHS] and [D] schiff base polymer (S-BPHS).

The spectrum of AM-BPHS (Fig.2 (C)) showed strong bands at 3465-3426 cm-1 that exist due to the stretching vibrations of NH or OH. A strong absorption band at 1638 cm-1 was for (C=O) that shifted due to the near amino group (the amide carbonyl stretch) [25], and the band at 1164 cm-1 was for C-N stretch in aliphatic amine. The Schiff base spectrum (Fig.2 (D) showed peaks at 3436 cm-1 for the OH group and 1597 cm-1 which can be assigned to the N=CH group. The peaks were at 1233 cm-1 for –C–N stretch [31, 32].

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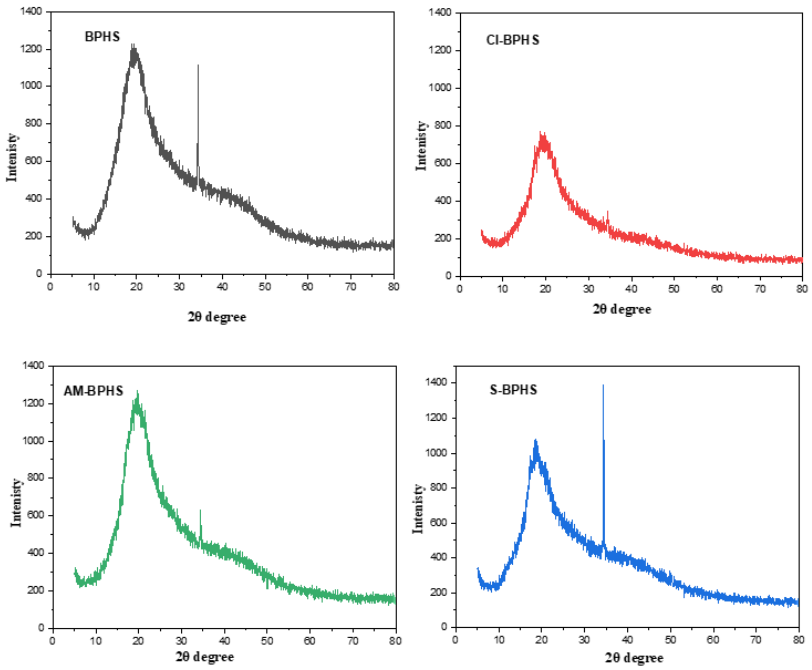

Fig. 3: XRD spectra of BPHS, Cl-BPHS, AM-BPHS and S-BPHS.

The composition of the modified polymers was also determined using C, H, N elemental analysis for the compounds. The results scheduled in Table 1 revealed that the nitrogen percent in AM-BPHS predicted polymer amination, while the results for S-BPHS suggested Schiff base formation.

Table 1: Elemental analysis of Branched poly (p-hydroxystyrene) [BPHS] and its modified derivatives

Sample	C %		H %		N %		Cl %	
	Calculated	Found	Calculated	Found	Calculated	Found	Calculated	Found
BPHS	79.75	78.09	6.97	6.325	-	-	-	-
Cl-BPHS	62.12	60.71	6.16	5.358	-	-	16.67	15.42
AM-BPHS	65.12	69.71	7.32	6.98	12.72	11.49	-	-
S-BPHS	68.46	68.86	6.05	6.684	7.6	7.4	-	-

To illustrate the physical characteristics and crystallinity of the modified polymer, XRD analysis was performed. The results are shown in Fig. 3, and the peaks' data in Table 2. The broad peak at roughly 2θ= 19.34o and the sharp peak at 2θ= 34.34o for BPHS, which were induced by stronger inter- and intra-molecular hydrogen bonding of the hydroxyl groups in BPHS, indicated the semicrystallinity of the sample. For Cl-BPHS, H-bonding deformation occurred. So, the peak at 34° almost vanished and the particular peak at 19.34° became less strong, causing the amorphous structure. After modification using ethylene diamine (AM-BPHS), the diagram revealed the same crystallography of BPHS. However, the intensity of the peak at 2θ=34.34^{o} was slightly decreased, i.e., a reduction in the modified polymer's crystallinity brought about by the introduction of amino groups to the polymer surface, which altered the polymer's intra- and intermolecular hydrogen interactions. However, the Schiff base diagram revealed a weakening and small displacement of the peak at 2θ=34^{o}, indicating a

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change in the compounds' crystallinity and the production of less crystalline or amorphous samples that were influenced by hydrophobic force, π-π stacking, and special hindrance. This may be the result of hydrogen bond distortion and a decrease in the amount of free amino groups on the aminated polymer following the creation of the Schiff base [33].

Table 2. Data analysis for polymer and their derivatives

Compounds	2Θ (°)	d spacing (A°)	Area under peak	Peak height	FWHM
BPHS	19.37	4.57	2943.6	859.06	0.587
	34.31	2.6	158.27	671.19	0.296
Cl-BPHS	19.76	4.39	1590.28	541.64	0.506
	34.43	1.97	56.19	110.98	0.169
AM-BPHS	19.55	4.5	2387.04	882.58	0.68
	34.4	2.67	56.94	107.48	0.62
S-BPHS	18.62	4.73	1680.19	798.69	0.4287
	34.4	2.6	333.9	650.4	0.24

TGA and DTG analysis were done to confirm the thermal stability of the modified polymer and the resulted data is illustrated in Table 3 and Fig, 4.

Table 3. Thermal analysis data of BPHS and its modification

Sample code	$T_{50\%}$ (°C)	DTG $T_{MAX.}$ (°C)
BPHS	426	428
Cl-BPHS	419	425
AM-BPHS	430	431
S-BPHS	420	426

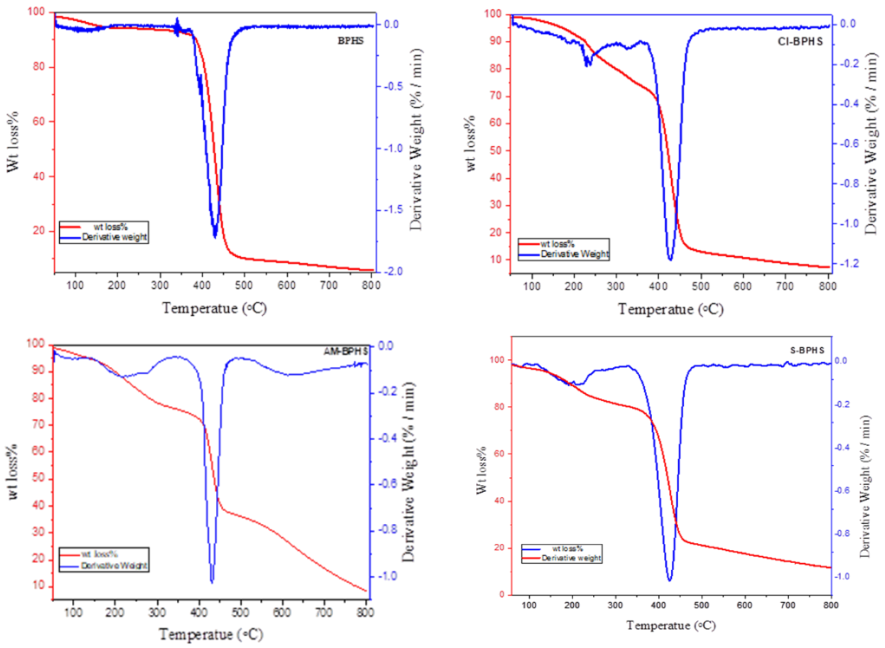

Fig. 4: TGA-DTG thermographs of AM-BPHS and S-BPHS schiff base derivatives.

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For the parent polymer thermograph, the first step of decomposition, confirmed the removal of water molecules within the 50–250°C temperature range at the highest temperature of 121°C in the DTG curve. The next stage occurred with a notable difference between 250 and 800°C with a sharp curve at 428°C in the DTG graph. while the first stage of Cl-BPHS was related to the isolation of the chloroacetyl group, the second was related to the breakdown of the main chain with two maximum temperatures at 328 and 425°C. AM-BPHS thermograph showed three steps: the first step was from 50- 332 ºC with a weight loss of 23.3 %, the second was to 476 ºC with 62.6 % wt loss, and the third was to 800 ºC with 92 % a weight loss. Because the inclusion of amino groups increased the thermal stability of the aminated polymer, AM-BPHS had a residual mass of 8.3%, indicating that it was more thermally stable than the other. While the Schiff base S-BPHS thermogram showed two steps. the first of which was from around 90-360 oC with weight loss of 22 wt%. The maximum DTG values were about 426 oC and the residual weight was 11.6 wt% [34].

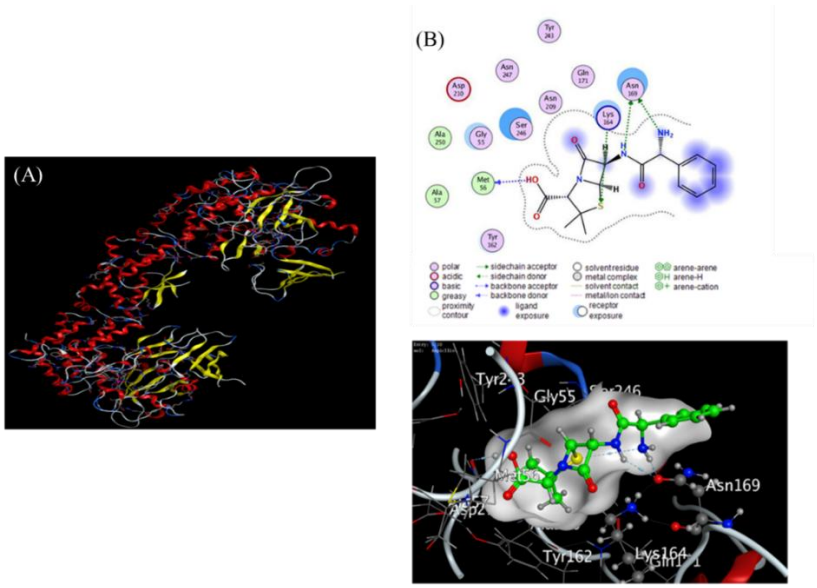

Fig. 5: [A] 3D of Escherichia coli (PDB code:7AB3), [B] 2D and 3D interaction diagram of Ampicillin with E- coli protein (7AB3).

3.2. Molecular Docking

Docking modeling was used to investigate how target substances interacted with certain proteins and to reduce the gap between computational models and practical applications. The crystal structure of Escherichia coli (PDB code: 7AB3) (Fig. 5 (A)) was delivered from the RCSB Protein Data Bank [35]. *E. coli* was selected for this study due to its status as a well-established model organism extensively used in molecular biology and biochemistry. The bacteria are an excellent choice for this kind of study because of their easily manipulable genetics and well-characterized genetics. Additionally, *E. Coli* systems are very effective at producing and purifying recombinant proteins, which enables us to access adequate amounts of the target protein for our docking investigations [36]. Furthermore, some strains of *E. coli* are pathogenic and can cause serious infections, making it important to study how polymers interact with their proteins for potential therapeutic treatments [37]. The amount of structural

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data available for *E. coli* proteins also facilitates accurate and reliable molecular docking studies [38]. The availability of high-resolution crystal structures of these proteins ensures the precision of our docking simulations [39]. Using *E. coli* as a model organism also allows for comparative studies with other bacteria, providing insights that can often be extrapolated to understand similar mechanisms in other pathogenic bacteria [40]. Ampicillin, as a derivative of penicillin used to treat a range of infections brought on by both Gram +ve and Gram -ve bacteria, as well as various anaerobes [41], is the basis for molecular docking. The new synthesized compounds (BPHS, Cl-BPHS, N-BPHS and S-BPHS) were decided to dock and compare with ampicillin at the binding site of Escherichia coli protein (Table 4). The resulted data of ampicillin strongly proved the interaction of the target ligand sites: nitrogen (N15, N 41) formed two hydrogen bonds with the target protein through asparagine (ASN 169), oxygen (O 30) with methionine (MET 56) amino acid, and sulfur (S 24) with lysine (LYS 164), both of which formed one H-bond. However, hydrophobic (Arene-H) bonding is also present. hydrogen bonds are the most prevalent form of contact bonds. Fig. 5 (B) shows the physical forms with the highest binding energy, which was -6.29 kcal/mol.

Table 4. Apparent interaction parameters of the new synthesized compounds and ampicillin into the binding site of Escherichia coli protein (PDB code:7AB3)

Ligand	**Ligand sites**	**Receptor sites**	**Type of the interaction**	**Distance of bond (Å)**	**Binding energy (kcal/mol)**	**Total free binding energy (kcal/mol)**
Ampicillin	N 15	ASN 169	Side chain donor H-donor	3.10	-2.2	-6.29
	O 30	MET 56	H-donor	2.90	-1.8	
	N 41	ASN 169	Backbone donor H-donor	3.11	-1.3	
	S 24	LYS 164	Side chain acceptor H-acceptor	4.09	-0.7	
BPHS	O 24	ASN 247	Side chain donor H-donor	2.93	-2.6	-4.21
Cl-BPHS	O 26	ASN 169	H-acceptor	3.04	-1.1	-5.57
	6-ring	LYS164	pi-H	3.52	-0.8	
AM-PBHS	N 30	GLN 171	H-donor	2.90	-1.5	-6.28
	O 26	ASN169	H-acceptor	2.94	-1.3	
	N 38	GLN 171	H-acceptor	3.43	-1.3	
	6-ring	LYS 164	pi-H	3.95	-0.6	
	6-ring	SER 246	pi-H	3.73	-0.7	
S-PBHS	N 44	GLN 171	H-donor	3.11	-0.8	-7.21
	O 26	ASN169	H-acceptor	3.06	-0.8	
	6-ring	LYS 164	pi-H	3.57	-0.6	

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Table 5. Inhibition zone for antimicrobial and antifungal properties for BPHS, Cl-BPHS, N-BPHS and S-BPHS

Compounds	*E. coli*		*S. aureus*		*C. Albicans*	
	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index
BPHS	13	50.0	12	49.7	19	70.4
Cl-BPHS	17	42.3	21	77.8	16	59.2
AM-BPHS	11	40.7	NA	----	3	11.1
S-BPHS	15	57.7	18	75.0	21	77.8
Ampicillin	26	100	24	100	----	----
Colitrimazole	----	----	----	----	27	100

The docking result of compound BPHS showed one hydrogen bond: O (24) with the asparagine (ASN 247) amino acid of the target protein (Fig. 6 (A)). The binding energy of compound BPHS with E-coli protein was -4.21 kcal/mol which was less than that of ampicillin (-6.29 kcal/mol) (Table 4). Compound Cl-BPHS showed one hydrogen bond: O (26) with glutamine ASN 169 amino acids of the target protein, one Arene-H bond: phenyl group with LYS 164 (Fig. 6 (B)).The binding energy of B was -5.57 kcal/mol. While compound AM-PBHS showed three hydrogen bonds: two bonds N 30 and N 38 with GLN 171, and O 26 with ASN169 amino acid of the protein (Fig.6 (C)) and formed two Arene-H bonding: 6-ring with LYS 164 and SER 246. The energy between the protein (7ab3) and the ligand AM-PBHS is -6.28 kcal/mol. On the other hand, compound S-PBHS was interacted with protein by two hydrogen bonds: N 44 with GLN 171, O 26 with ASN169 and one Arene-H bonding: 6-ring with LYS 164), and the binding energy of B was -7.23 kcal/mol (Fig. 6 (D).

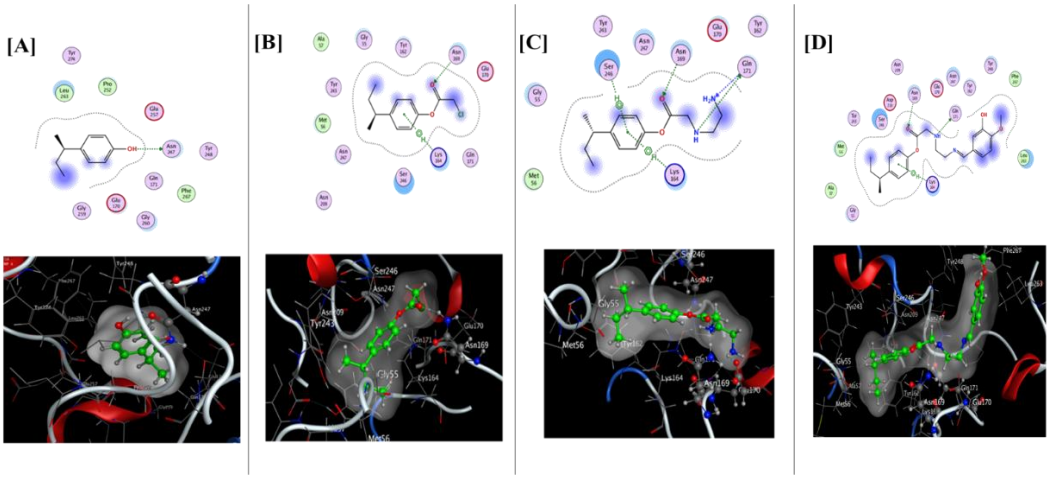

Fig. 6: 2D and 3D interaction diagram of [A] BPHS, [B] Cl-BPHS, [C] AM-BPHS and [D] S-BPHS with E-coli protein (7AB3).

The interaction sites and total binding energy of compounds BPHS and Cl-BPHS were less than those of ampicillin. This showed that compounds BPHS and Cl-BPHS have far less inhibitory effect on *E. coli* than ampicillin. The interaction sites and total binding energy of compounds AM-PBHS and S-PBHS were greater than or approximately equal to those of ampicillin. The docking result indicated that the two compounds (AM-PBHS and S-PBHS) are more effective on *E. coli* than ampicillin.

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3.3. The *In Vitro* Antimicrobial Activity of Some Modified Polymers

The antimicrobial and antifungal properties of BPHS and its derivatives were evaluated against *E. coli*, S. aureus and *C. albicans* using the disc diffusion method. Ampicillin and Clotrimazole served as reference medications [42]. The result of inhibition zones of bacterial and fungal growth around the discs in mm was recorded in Table 5. Against *E. coli*, S-BPHS showed the highest activity among the BPHS compounds, with an activity index of 57.7%. This was followed by BPHS (50.0%), Cl-BPHS (42.3%) and AM-BPHS (40.7%). For S. aureus, Cl-BPHS demonstrated the strongest antimicrobial effect with an activity index of 77.8%, then closely followed by S-BPHS at 75.0%. BPHS, while AM-BPHS showed the lowest activity. Against the fungal strain *C. albicans*, S-BPHS exhibited the highest antifungal activity (77.8% activity index) among the BPHS derivatives, surpassing even BPHS (70.4%). Cl-BPHS showed moderate activity (59.2%), while AM-BPHS demonstrated low antifungal effects (11.1%). These results suggest that structural modifications of BPHS can significantly alter its antimicrobial and antifungal properties. The results revealed that Schiff base S-BPHS with hydroxyl and methoxy groups had the most powerful antifungal and antibacterial action. One strategy of antibacterial action is the deactivation of multiple cellular enzymes involved in bacterial metabolism, as well as disruption of normal cell functions by building a hydrogen bond between active groups (for example, the azomethine group) and the active centers of cells [43]. Phenolic substances are also famed for their antibacterial properties, as they can form hydrogen bonds with the cell membrane. This interaction can alter membrane permeability, resulting in cell death, as well as disrupt normal cellular pathways due to the denaturation of specific cell proteins. The modified Schiff bases can form H-bonds with cellular components due to imino and hydroxyl groups allowing them to permeate cell walls [44, 45]. Chloride substitution (Cl-BPHS) improved activity against S. aureus but showed varied effects on other strains. This result goes in agreement with Song *et. al*. [46]. This was due to the electron-withdrawing nature of chloride, which could affect the compound's reactivity and interactions with bacterial cell components [47].

3.4. Antioxidant Activities of Some Derivatives

The antioxidant activity was assessed using the DPPH free radical strategy as it can take up electrons or hydrogen from the molecule and then decrease. The test compounds' color changed from purple to yellow after one hour of incubation. The absorbance at 517 nm was decreased after the addition of modified polymers [BPHS, Cl-BPHS, AM-BPHS, and S-BPHS] which validated the existence of the antioxidant constituent in the samples. The DPPH radical scavenging activity increased as the concentration increased, as displayed in Fig. 7. The high capability of the samples to scavenge the DPPH free radical was affirmed by the lower IC50 value, and the results are noted in Table 6.

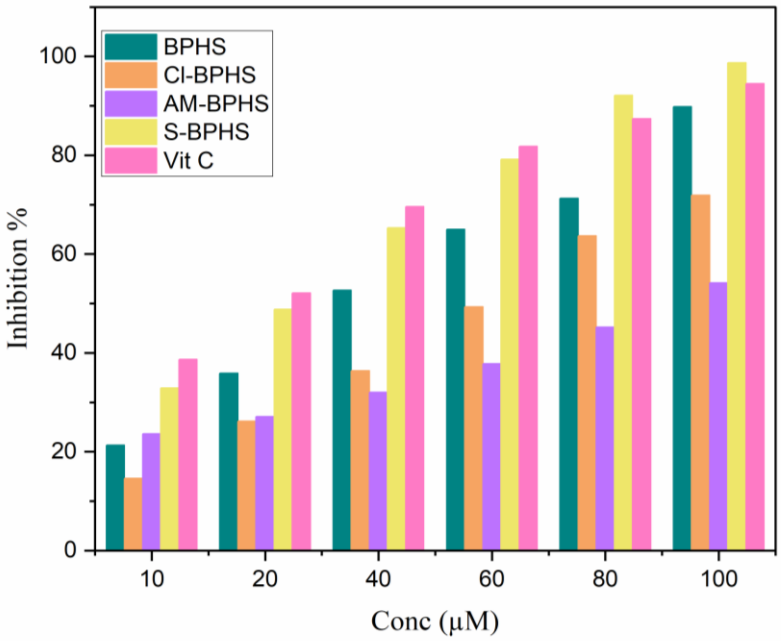

Fig. 7: 2D and 3D interaction diagram of [A] BPHS, [B] Cl-BPHS, [C] AM-BPHS and [D] S-BPHS with E-coli protein (7AB3).

The results showed that S-BPHS was the most active compound with IC50 (19.69±0.12) which is close to vitamin C according to the fact that protons may be transferred to free radical DPPH and transformed into the resonance-stabilized phenoxide [48, 49]. On the other hand, as seen in Fig.8, vitamin C can contribute both protons to the DPPH radical to produce dehydroascorbate. While the aminated polymer (AM-BPHS) showed the lowest antioxidant activity with IC50 (106.30±0.61) this may be attributed to the intramolecular H-bonding in the compound. These results were in agreement with Bora et al. [50].

Table 6. DPPH Radical scavenging activity of some modified polymer

Comp.	Conc (µM)						
	10	20	40	60	80	100	IC50
	% Radical scavenging activity						
Vit.C	38.7	52.1	69.6	81.8	87.4	94.5	16.81±0.10
BPHS	21.3	35.9	52.7	65.0	71.3	89.8	32.84±0.20
Cl-BPHS	14.6	26.2	36.4	49.3	63.7	71.9	53.75±0.29
AM-BPHS	23.6	27.1	32.0	37.8	45.9	54.2	106.30±0.61
S-BPHS	32.9	48.8	65.3	79.2	92.1	98.7	19.69±0.12

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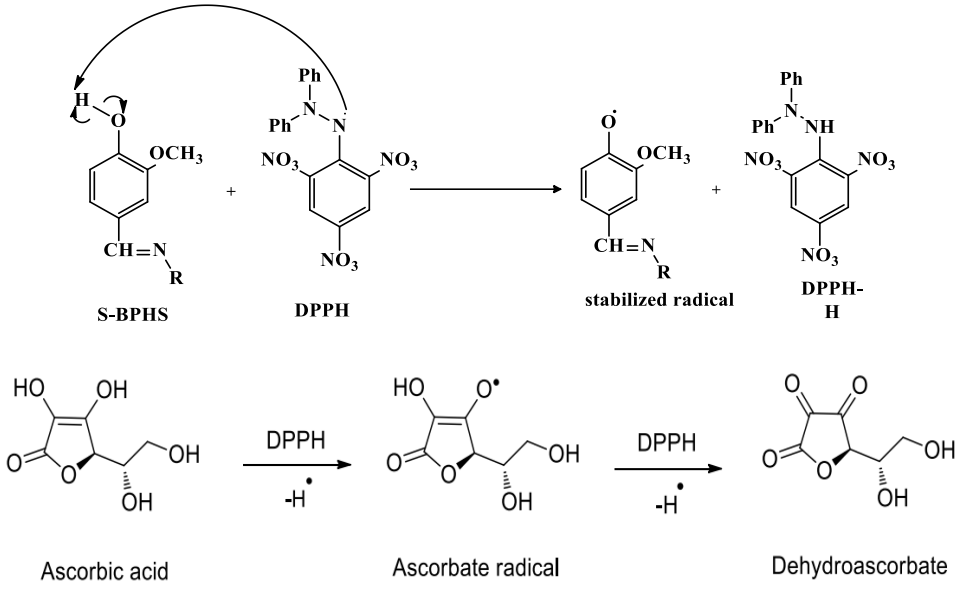

Fig. 8 : Proposed mechanism of action of Schiff base S-BPHS and ascorbic acid as antioxidants.

4. CONCLUSION

In this study, a successful modification of poly(p-hydroxystyrene) was achieved by amination of chloroacetylated form using ethylenediamine, followed by condensation with 3-hydroxy-4-methoxybenzaldehyde to form a Schiff base compound. The synthesized compounds were investigated by FT-IR, elemental analysis, XRD and TGA-DTG techniques. The compounds were detected for their biological activities. The results showed that Schiff base S-BPHS with hydroxyl and methoxy groups had the highest antibacterial, antifungal and antioxidant activities compared to the parent polymer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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