Synthesis, Characterization and Microbiological Activities of a new Schiff Base Derived from Hydralazine Hydrochloride

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ABSTRACT

The work describes the synthesis, characterization, and assessment of the biological activity of two novel Schiff bases, PHMP and BPHMP. The condensation process between hydralazine hydrochloride and terephthalaldehyde, facilitated by p-toluenesulfonic acid as a catalyst, resulted in their production. The compounds were studied by spectroscopic techniques, including FTIR, proton nuclear magnetic resonance (1H-NMR), carbon-13 nuclear magnetic resonance (13C-NMR), and mass spectrometry. The antibacterial and antifungal efficacy of the Schiff base compounds assessed against the bacteria S. aureus and E. coli, as well as the fungus C. albicans and A. niger, yielded varying outcomes in comparison to hydralazine hydrochloride as the standard drugs.

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1. Introduction

1-Hydrazinophthalazine, also referred to as hydralazine, is a vasodilator medication that has been prescribed since the 1950s to treat hypertension. Its highly active hydralazine group causes it to undergo a variety of reactions, including the production of Schiff bases with aldehydes and ketones[1]. Schiff bases are interesting organic compounds that consist of an azomethine group ($RCH=NR^{\backslash}$) or an imine group ($RR^{\backslash}C=NR^{\backslash\backslash}$), where each of $R,R^{\backslash},R^{\backslash\backslash}$ is either an aromatic or aliphatic group[2]. In 1864, German chemist Schiff Hugo created Schiff bases for the first time. This was accomplished by condensing primary amines (aliphatic or aromatic) with carbonyl compounds (aldehydes or ketones) in the presence of a weak acid as a catalyst, which resulted in the loss of water [3].

This group of compounds uses as important precursors and can be found within natural or synthe tic chemicals. The biological activities of Schiff bases, which include anthelmintic [4], analgesic, anticonvulsant, anti-inflammatory[5], antimicrobial [6], [7] antitubercular, antioxidant [8], and many more, making them a significant class of organic compounds with a variety of application. Schiff bases are important in pharmacology and medicine, but they are also widely used as corrosion-inhibiting agents, pigments, catalysts, and stabilizers in polymer formulations [9], [10], [11]. In our research, we aim to synthesize novel Schiff bases derived from hydralazine and determine their in vitro antibacterial and antifungal properties.

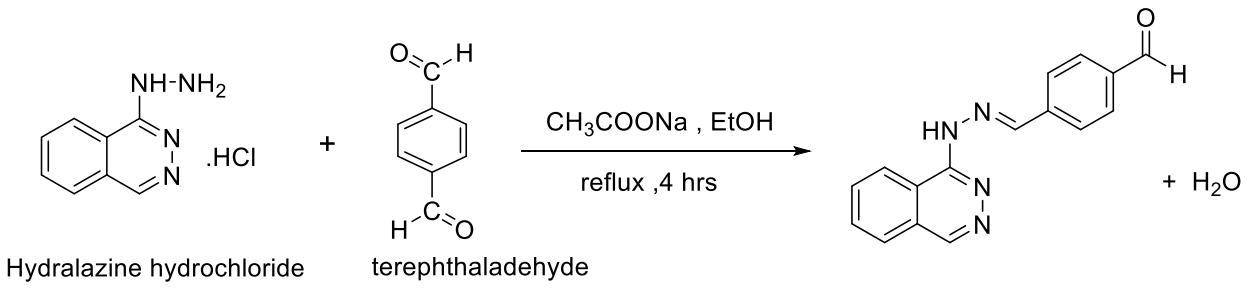
2. Experimental

2.1 General

Hydralazine hydrochloride, terephthaldehyde, sodium acetate and solvent were purchased through commercial sources and used without extra a purification. Melting point were determined via UKSA. The FTIR spectra (400-4000) cm^{-1}were recorded on a Bruker alpha II spectrometer (Japan). The NMR spectra of ^{1}H-NMR and ^{13}C-NMR (400 MHz: δ in ppm and J in Hz) were obtained in DMSO-d_6 using a Bruker spectrometer (Japan) in using internal stander of tetramethyl silane (TMS). The mass spectra were performed using a device SHIMADZUTQ 8040(Japan), at 70eV using a Triple-Axis Detector. This layer chromatography (TLC) was used for following the reactions progress and the produced nitrones using Merk chromatography sheet (GERMANY). Toluene and Ethyl acetate (8:2) were used as the developing solvent. The spot was visualized by exposing the dry plate to UV light.

2.2 Synthesis of 4-((2-(phthalazin-1-yl) hydrazineylidene) methyl) benzaldehyde (PHMB)

The compound is formed by the condensation of Hydralazine hydrochloride and terephthaldehyde. After dissolving (2 mmol, 0.4 g) of Hydralazine hydrochloride and (2 mmol, 0.16 g) of sodium acetate (a buffering agent) in 20 ml of ethanol Abs, (2 mmol, 0.26 g) of terephthaladehyde was also measured by adding (1mg) of para-toluene sulfonic. The two solutions were added into a 250 ml round-bottom flask, refluxed for four hours with constant stirring, and then left to cool overnight. After filtering to isolate the orange precipitate (PHMP), which yielded 87% yield, the mixture was washed with diethyl either way and left to air dry before being recrystallized with ethanol Abs [12]. (Scheme 1) shows the PHMB synthesis pathway. From terephthaldehyde, m.p =248, R_f =0.54, FTIR bands (**U**/cm^{-1}): 3289(N-H), 3025(Ar-H), 1665(C=O), 1610(C=N_{ring}), 1581(C=N), 1526, 1458(C=C), 1209(C-N). ^{1}H NMR(DMSO-d_6,400MHz): δ_H 12.52(S, 1H, NH), 10.05(s, 1H, CHO), 8.6 (s, 1H, H-C=N_{ring}), 8.1(s, 1H, H-C=N), 8.28-8.26(d, J=8.5Hz, A 9,9\), 7.97-7.95(d, J=8.5Hz, 2H, A 8,8\), 7.88-7.80(m,4H, Ar-H). ^{13}C NMR (101 MHz, DMSO) δ_C 193, 153, 153, 149.82, 149.06, 136,134, 133,130, 129, 128, 127.7, 127.2, 124. MS: m/z [M$^+$]276.

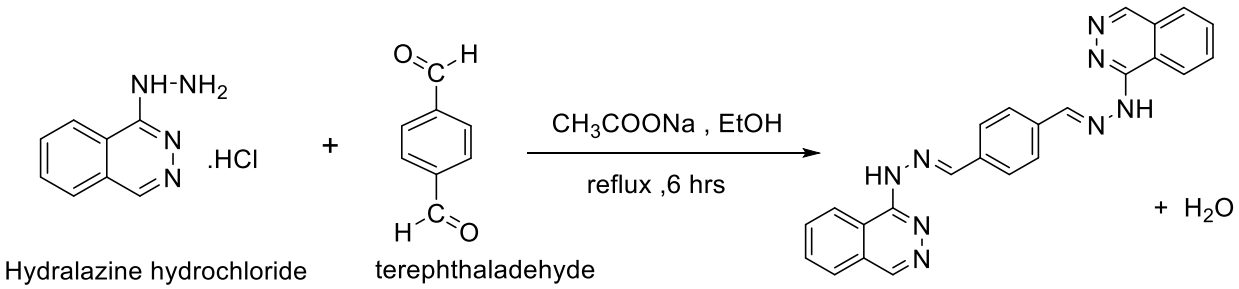


Scheme 1: Synthesis of PHMB

2.3 Synthesis of 1,4-bis((2-(phthalazin-1-yl) hydrazineylidene) methyl) benzene (BPHMB)

Hydralazine hydrochloride (4 mmol, 0.78 g) and sodium acetate (4 mmol, 0.328 g), a buffering agent, were dissolved in 20 ml of ethanol Abs, the mixture was combined with (2 mmol, 0.26 g) of terephthalaldehyde and (1 mg) of para-toluene sulfonic. After being moved into a (250 ml) round-bottom flask, the mixture was refluxed for six hours while being stirred, and it was left to

cool overnight. After filtering and washing with diethyl ether, the orange precipitate (BPHMB) was left to air dry (yield = 80%). and ethanol Abs. recrystallized[12]. (Scheme 2) shows the synthesis pathway for BPHMB. From terephthaldehyde, m.p=286, R_f =0.30, FTIR bands (**U**/cm^{-1}): 3301(N-H), 3008(Ar-H) , 1610(C=N_{ring}),1573(C=N), 1565, 1472(C=C), 1253(C-N). ^{1}H NMR (DMSO-d_6, 400MHz): δ_H 12.18(S,1H, NH), 8.45(s, 1H, H-C=N_{ring}), 8.11(s,1H, H-C=N), 8.30-8.28(d, J=7.8Hz, A 9,9\), 8.06-8.04(d, J=7.8Hz, 2H, A 8,8\), 7.78-7.69(m, 4H, Ar-H). ^{13}C NMR (101 MHz, DMSO) δ_C 152, 149.16, 149.10, 139, 136, 132, 130, 129.66, 129.65, 128, 127, 123. MS: m/z [M^+]418.



Scheme (2): Synthesis of BPHMB

2.4 Evaluation of Anti-Bacteria and Anti-Fungal antibacterial and antifungal

Compounds PHMB and BPHMB were screened against four different types of microorganisms by using disc diffusion technique. The first organism was a fungus represented by Aspergillus niger ATCC16404 and the second organism was Candida albicans ATCC2091. While the third and fourth organisms were Gram-negative bacteria represented by E. colli ATCC25922 and Gram-positive bacteria represented by S. aureus ATCC25923. Hydralazine hydrochloride was used as a standard drug. Agar diffusion methods were used to determine the inhibition zones in mm at 100 mg/ml concentration of each compound.

3. Results and discussions

The condensation reaction between terephthalaldehyde and hydralazine hydrochloride in equimolar quantities, facilitated by p-toluenesulfonic acid, yielded PHMB (Figure 1). Conversely, when the moles of hydralazine hydrochloride were doubled, BPHMB was produced (Figure 2).

3.1 FTIR characterization

Figure 3 shows infrared spectra of PHMB's, which was recorded in the range of 4000-400 cm^{-1}. The IR spectra showed (N-H) stretching vibrations at 3298 cm^{-1}. At 1526 cm^{-1}, a band was observed that corresponded to the (C=N) stretch. Additional strong bands were found at 1667 cm^{-1}, which is where the (C=O) stretching was observed, and at 907 cm^{-1}, which is where the (N-N) stretching was found. This showed these functional grouping are present in PHMB.

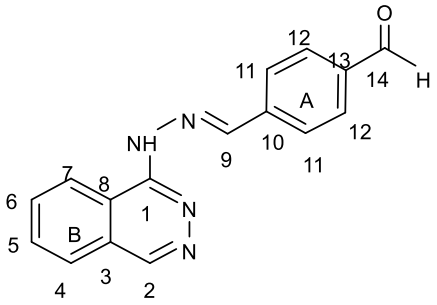


Figure 1: PHMB's structural features

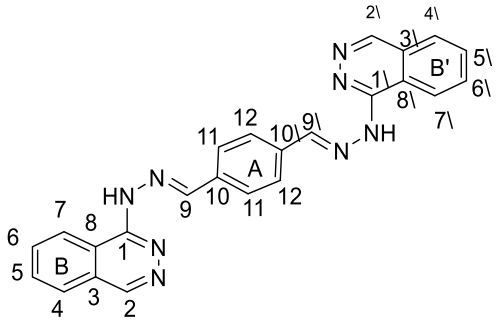


Figure 2: BPHMB's structural features

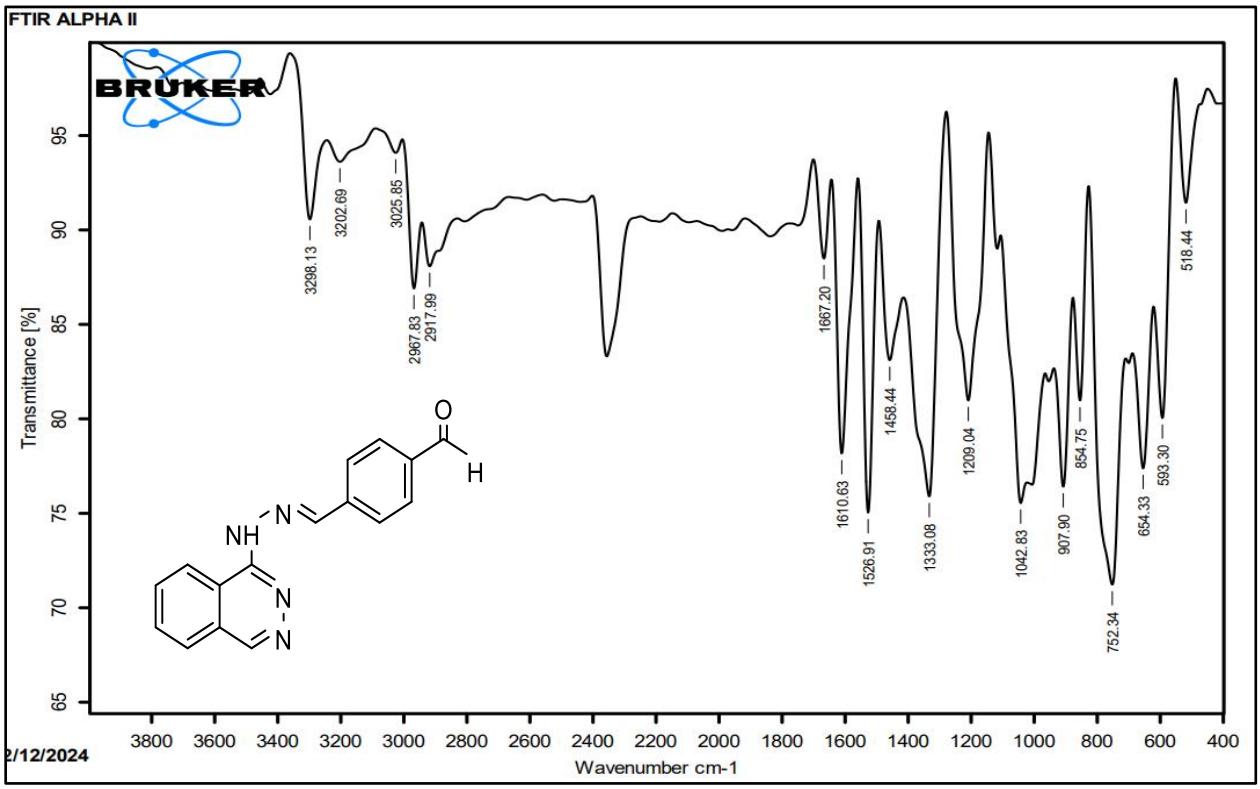


Figure 3: Infrared spectrum of PHMB

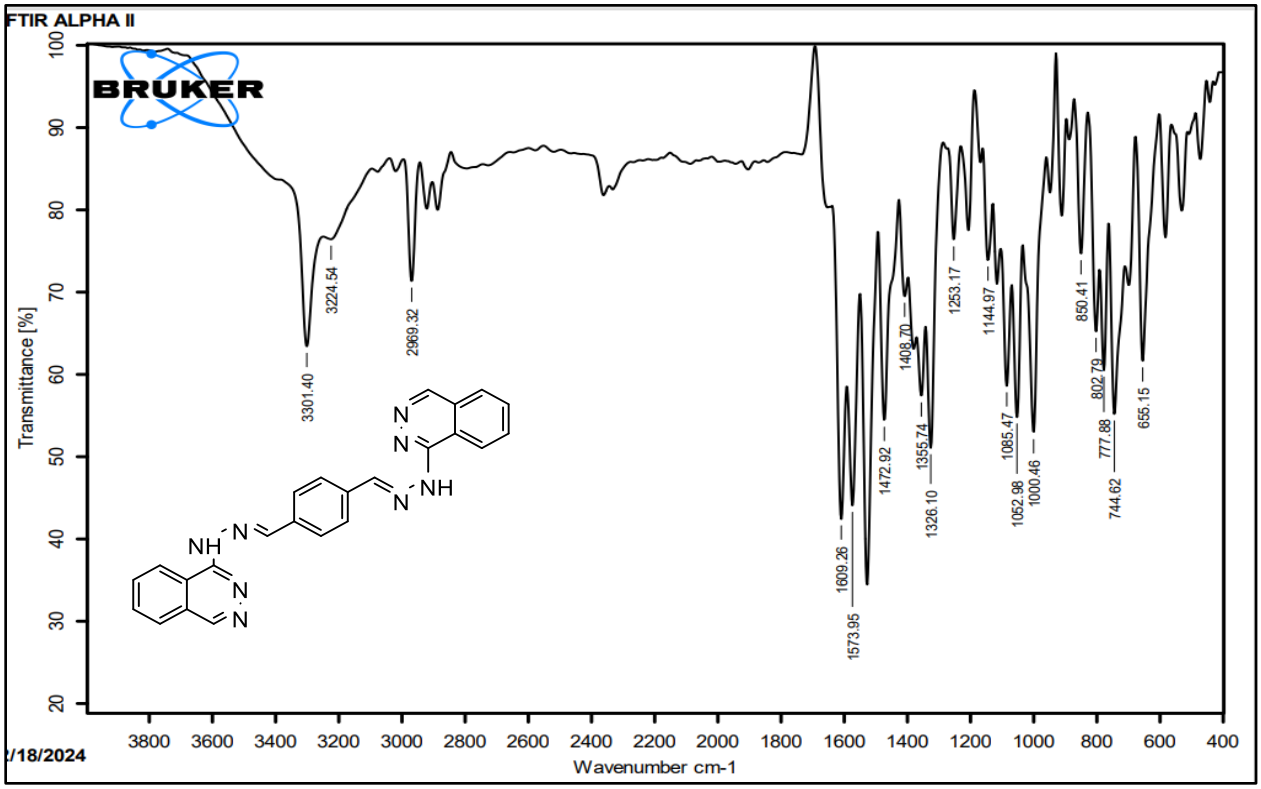


Figure 4: Infrared spectrum of BPHMB

The IR spectra of BPHMP in Figure 4, showed strong band at 3301cm^{-1} and 1573cm^{-1}, respectively, which related to the (N-H) stretching and azomethine (C=N). At 1000cm^{-1}, strong band were seen, which were related to the (N-N) stretch. The (C=N) stretch of the phthalazine ring was observed at 1609 cm^{-1}. The very intense bands in the range (1550-1472) cm^{-1} due to (C=C) vibration. And band at 1253 cm^{-1} due to (C-N) stretrching.

3.2 NMR Spectra

NMR spectra of PHMB is shown in Figure 5. The signal at 12.52ppm (s, 1H) is ascribed to the (N-H) and is highly deshielded because of its tautomeric nature, whereas the signal at 10.05ppm (s, 1H) is ascribed to the proton of aldehyde group (CHO). At carbon 9, the azomethine (-N=C-H) proton was found as the source of a signal observed at 8.12 ppm (s, 1H).The aromatic protons of ring (A)of ring (A) in positions (11,11$^{\backslash}$) shown doublet signals within the range (8.28-8.26) ppm due to interaction with one proton in (12,12$^{\backslash}$) position, two other protons in the position (12,12$^{\backslash}$) shown doublet signals within the range (7.97-7.95) ppm due to the interaction with proton in (11,11$^{\backslash}$) position. The benzene protons of the phthalazine ring (B) contribute for the multiplet signals between 7.88 and 7.80 ppm. The PHMB's ^{1}H-NMR is shown in Figure (5). The proton NMR spectra of BPHMB Figure (6) was obtained. The signal at 12.18ppm is ascribed to the (N-H). A signal seen at 8.11ppm (s,1H) was observed to the (-N=C-H) proton. The aromatic protons at ring(A) in the position (11,11$^{\backslash}$) shown doublet signals within the range (8.30-8.20) ppm due to interaction with proton in (12,12$^{\backslash}$) positions. While the protons in (12,12$^{\backslash}$) position shown doublet signals in the range (8.04-7.84) ppm due to interaction with proton in (11,11$^{\backslash}$) position. The benzene protons of the phthalazine ring have been identified as the multiplet signals between 7.78-7.69 ppm. The BPHMB's ^{1}H-NMR is shown in Figure (6). The ^{13}C-NMR spectra of PHMB Figure (7). The signals at 193,153,149.82 ,149.06 were respectively regraded to carbon 14, 1, 2 and 9. The ^{13}C-NMR spectra of BPHMB Figure (8). Signals ranging from 124ppm to 153ppm were shown. The signals at153,149..16 ,149 were respectively due to carbon 1,2 and 9. [13]

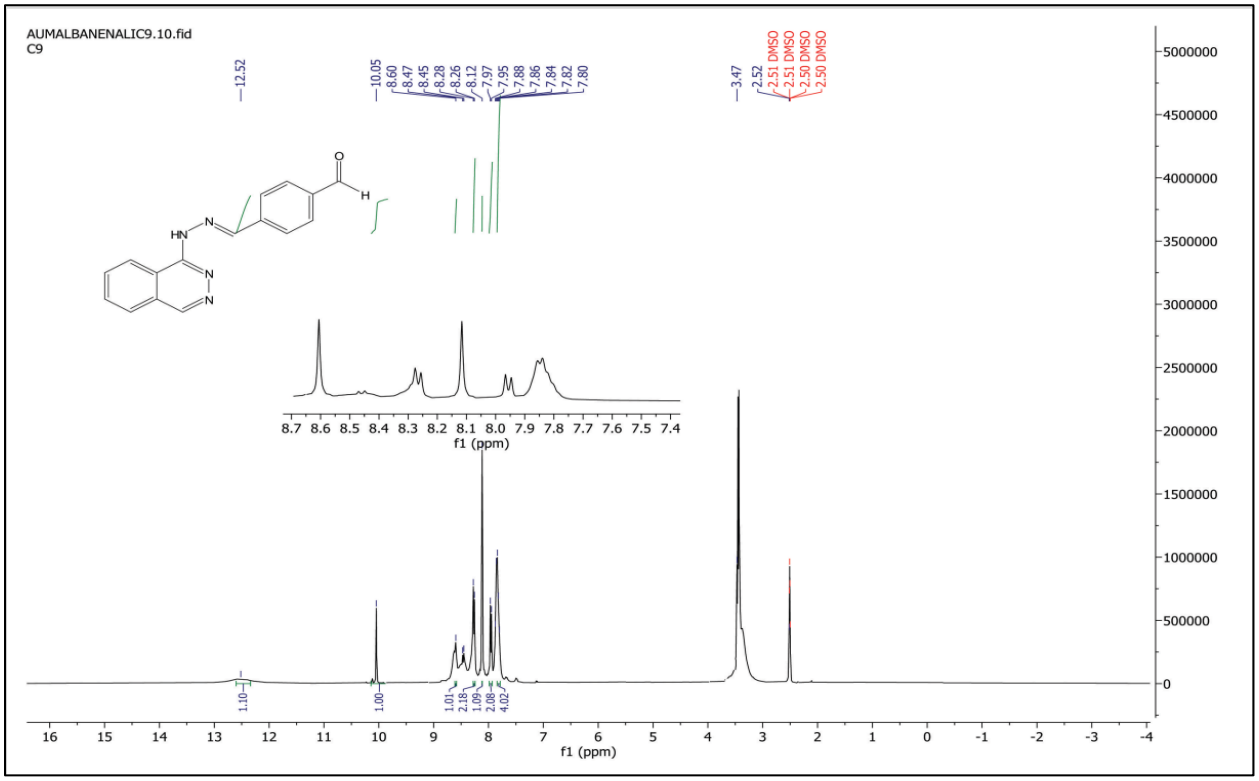


Figure (5): ^{1}H-NMR of PHMB

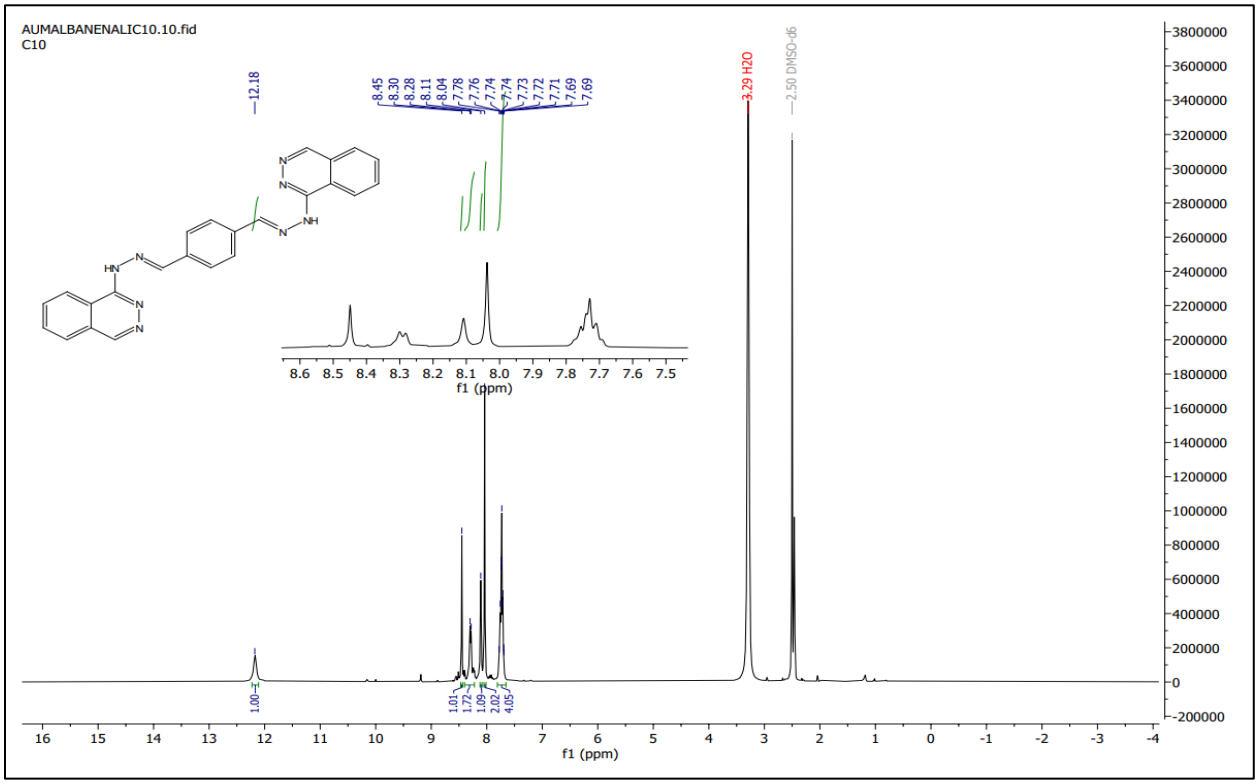


Figure 6: ^{1}H-NMR of BPHMB

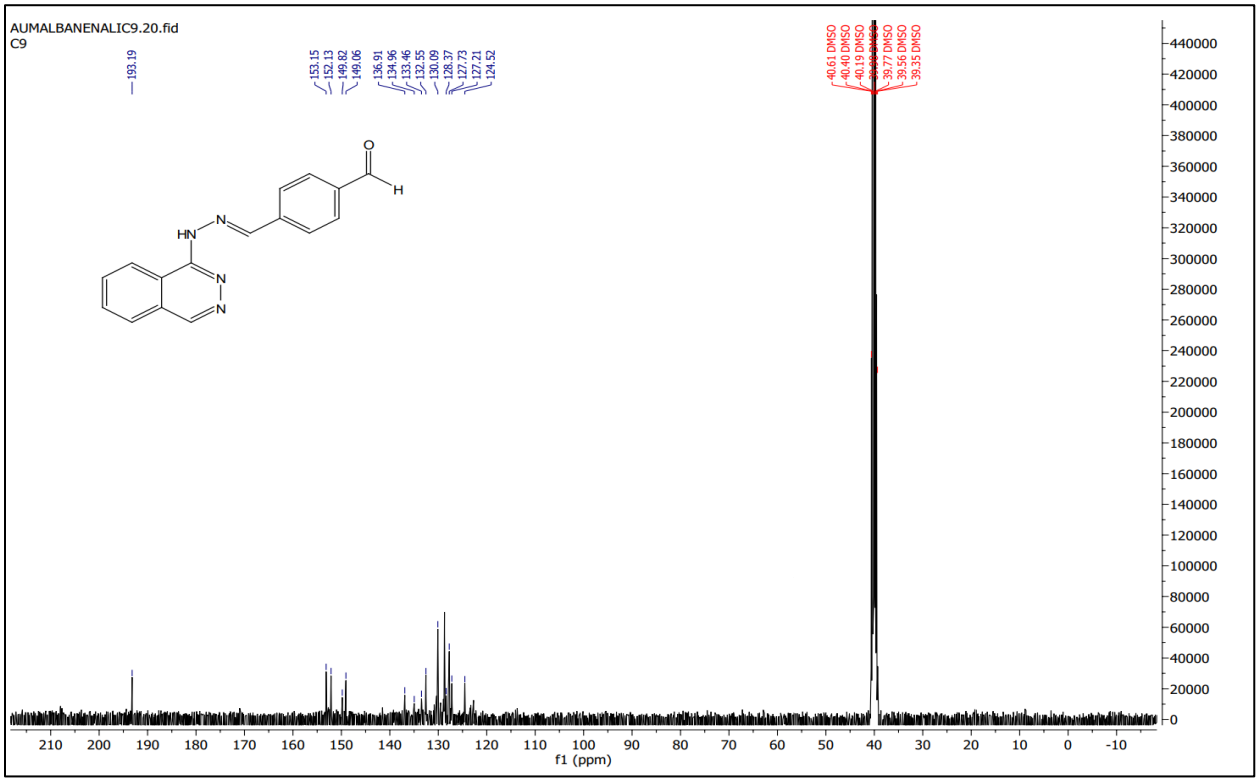


Figure 7: ^{13}C-NMR of PHMB

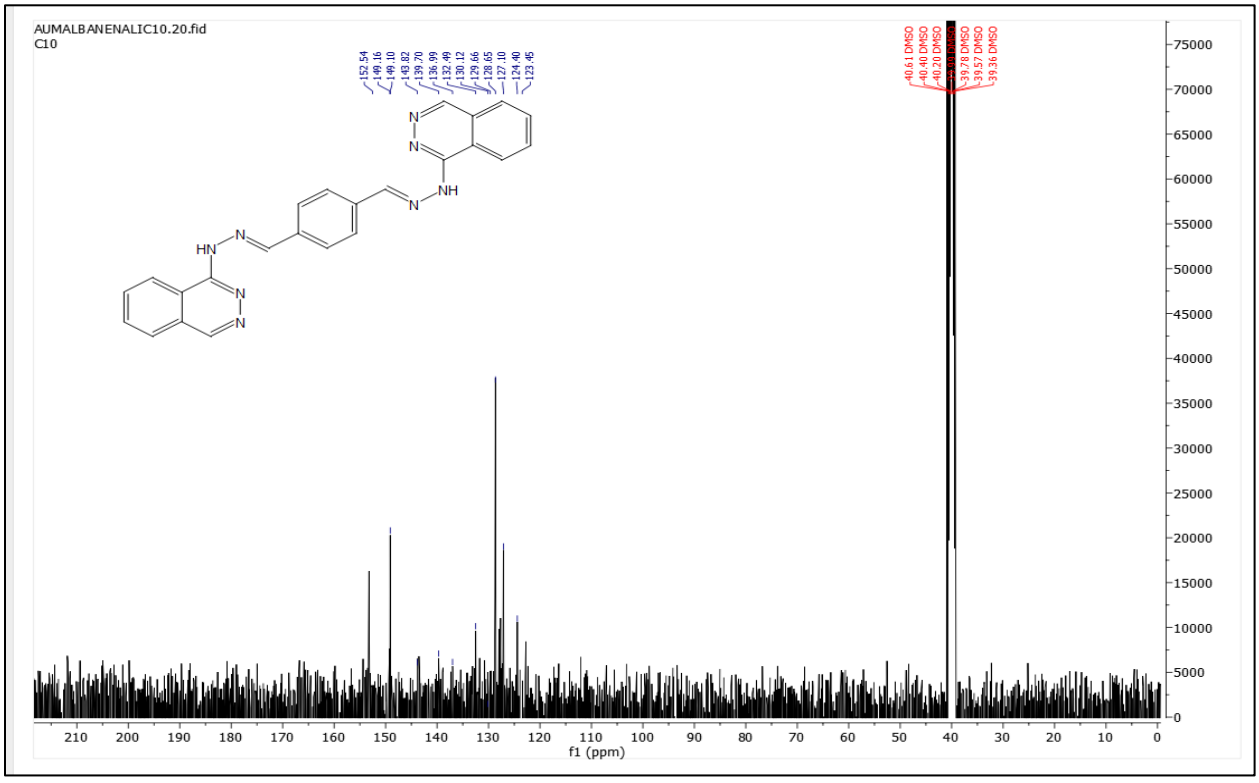


Figure 8: ^{13}C-NMR of BPHMB

3.3 Mass spectra

The mass spectra of Schiff bases shown in Figures 9 and 10 demonstrate that the molecular ion (M+) band is present with other fundamental ion bands in the spectra of compounds PHMB and BPHB, confirming the accuracy of the molecular formulas of the synthesized compounds. Schemes 2 and 3 depict the fragmentation behavior seen by these compounds.

Table 1: The basic fragmentation mass spectra of prepared Schiff base

Comp	M/Z								
	M^+	1	2	3	4	5	6	7	8
PHMP	276	247	158	130	102	89	76	51	-
BPHMP	418	260	158	130	102	89	76	51	-

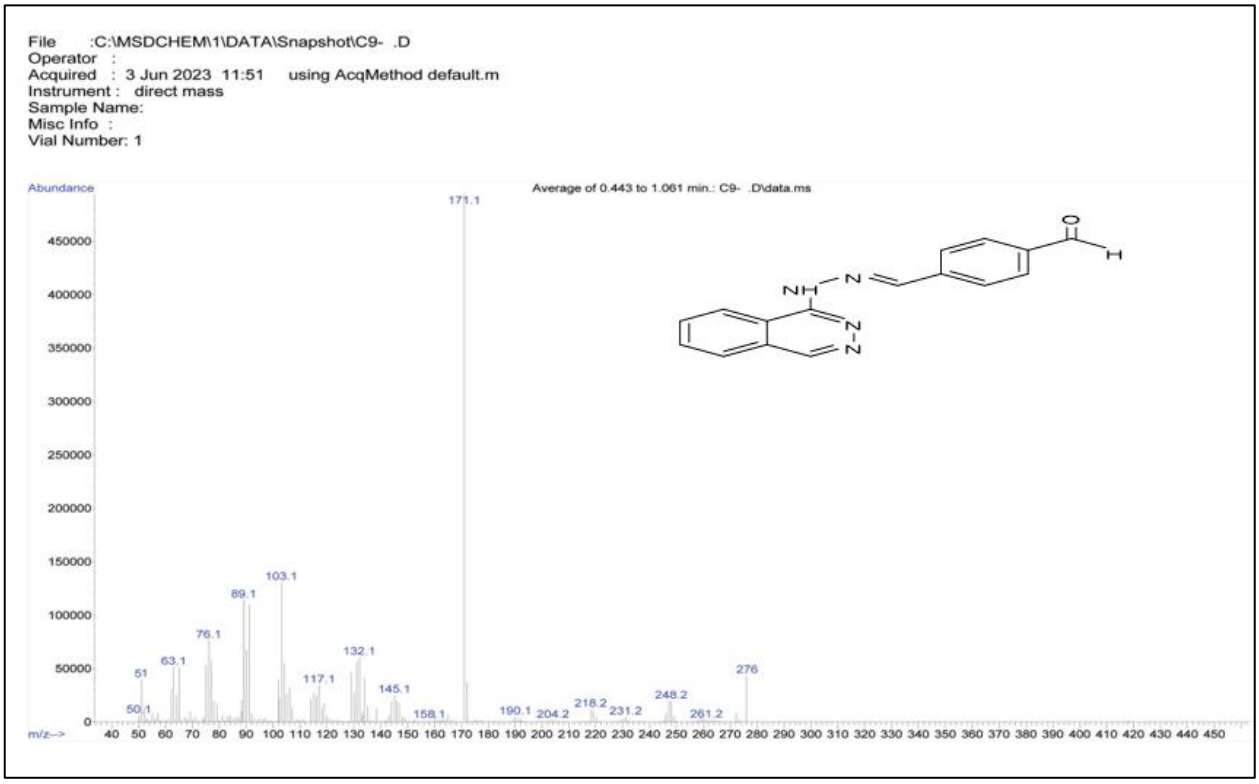


Figure 9: Mass spectra of PHMP

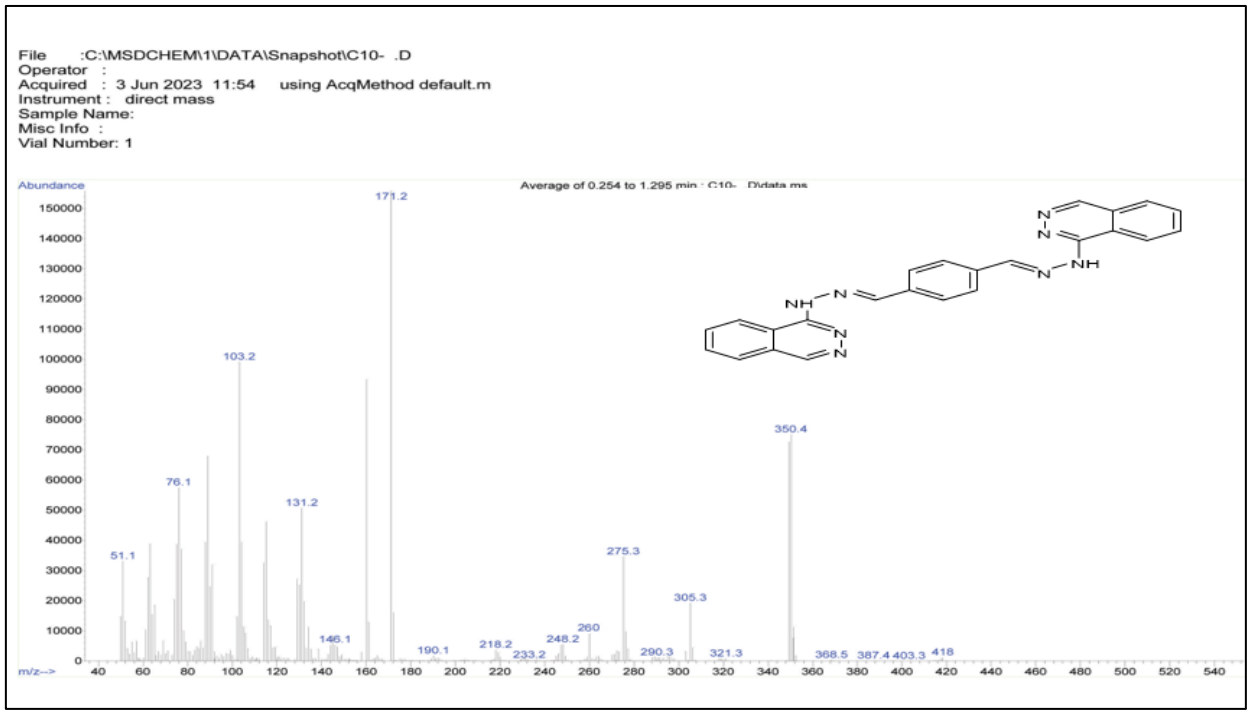


Figure 10: mass spectra of BPHMP

3.4 Result of Anti-bacterial and anti-fungal

Schiff bases have significance in pharmaceutical chemistry. They are potential targets for the suppression of several bacterial infections. In the present investigation, an antibacterial and antifungal agent was examined utilizing a concentration of 100 mg/ml for each compound. According to Table 5, both PHMB and BPHMB exhibited activity against gram-positive bacteria and the fungus A niger.

Table 2: antibacterial and antifungal activity of synthetic Schiff base in various zone (mm)

Comp	Inhibition zone(mm)		Inhibition zone(mm)	
	S. aureus	E. coli	*C. albicans	*A. niger
PHMP	8	-	-	20
BPHMP	17	-	-	20
H	20	30	20	25
(-)= no inhibition , H= Hydralazine Hydrochloride, S= Staphylococcus ,E= Escherichia ,C= Candida , A= Aspergillus				

4. Conclusion

This study developed a novel Schiff base through the condensation reaction of hydralazine hydrochloride with terephthaldehyde in a mixture of p-toluenesulfonic acid and ethanol. The Schiff base compounds were further evaluated using infrared (FTIR), 1H-NMR, 13C-NMR spectroscopy, and mass spectrometry, and the analytical results confirmed the accuracy of the synthesized chemical composition. The Schiff base compounds demonstrated antifungal effectiveness against C. albicans and Gram-positive bacteria utilized in this study, in comparison to the standard (Hydralazine hydrochloride), indicating their potential as future drugs.

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تحضير وتشخيص ودراسة الأنشطة المايكروبايولوجية لبعض قواعد شف جديدة مشتقة من الهايدرالازين هايدروكلورايد

أم البنين علي كاظم, عباس فاضل عباس

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المستخلص

يصف البحث الحالي تحضير وتشخيص ودراسة الفعالية البايولوجية لأثنين من مركبات قواعد شف جديدة PHMP وBPHMP. تم تحضيرها من خلال تكاثف الهايدرالازين هايدروكلورايد والتريفثالديهايد بأستخدام الحامض (بارا-تولوين سلفونك) كعامل محفز. شخصت هذه المركبات بالتقنيات الطيفية, بما فيها الأشعة تحت الحمراء FT-IR ومطيافية الرنين النووي المغناطيسي للبوتون ^{1}H-NMR ومطيافية الرنين النووي للكاربون ^{13}C-NMR ومطيافية الكتلة. وقد أعطت الأنشطة المضادة للبكتيريا والفطريات لقواعد شف التي تم تقييمها ضد البكتيريا: S. aureus و E. col والفطريات: C. albicans و A. niger، نتائج مختلفة عند مقارنتها مع الهايدرالازين هايدروكلورايد كدواء قياسي.