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Antibacterial, antifungal, antioxidant, and molecular docking studies of (E)-4-(1-(2-aminophenylimino)ethyl)benzene-1,3-diol

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The Schiff base, (E)-4-(1-(2-aminophenylimino)ethyl)benzene-1,3-diol (APEB) has been synthesized and characterized by different spectroscopic techniques. The Schiff base, APEB shows antibacterial and antifungal activities against a bacterium (*E. coli*) and a fungus (*C. albicans*) with an IC_{50} value of 0.07 µg/mL and 0.06 µg/mL respectively. Clear zones have not been observed in the case of antibacterial activity but are present in the case of antifungal activity. Additionally, antioxidant potential has been evaluated through DPPH, ABTS, FRAP, CUPRAC, SOARSA, RNOSA and HFRSA, with an IC_{50} values of 187.2 µg/mL, 5.967 ± 0.1625 µg/mL, 0.7404 µg/mL, 2.37 µg/mL, above maximum dose limit *i.e.* higher than 1000 µg/mL, 167.6 µg/mL and 9.934 µg/mL, respectively, indicating antioxidant properties. To elucidate the potential mechanisms underlying these bioactivities, molecular docking studies have been performed against the protein: PDB ID 1AI6 and 5AEZ.

Keywords: Schiff base, *o*-Phenylenediamine, Antimicrobial activity, Antioxidant activity, Molecular docking

In 1864, Hugo Schiff laid the groundwork for the synthesis of Schiff bases, leading to the creation of a diverse array of compounds containing an azomethine or imine group (H–C=N-). These compounds are typically formed through a nucleophilic reaction between a primary amine and a carbonyl group with an R-substituent. The presence of the R group enhances the functional properties of Schiff bases, increasing their range of biological and chemical activities[1,2]. Schiff base ligands have been widely utilized in coordination chemistry research due to their growing diversity and the flexibility to design them according to specific needs and preferences[3]. Schiff base forms complexes having demonstrated versatility in a variety of industrially significant transformations, such as the oxidation or epoxidation of alkenes, alkene metathesis, alkene hydrosilylation, and the polymerization of ethene. The oxidation or epoxidation of various functional groups serves as an intermediate step in the production of chemicals and pharmaceuticals, making these Schiff base complexes highly valuable for industrial applications[4]. The biological activities of this class of chemicals includes antibacterial, antioxidant, and anticancer characteristics. Schiff bases are well proven and are important for the growth of novel medicinal treatments. The antibacterial qualities of Schiff bases are especially important in tackling the rising issue of antibiotic

Abbreviations: 1AI6: Penicillin acylase with *p*-hydroxyphenylacetic acid, 2,4DA: 2,4-dihydroxyacetophenone, 5AEZ: Crystal structure of *Candida albicans* Mep2, ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), APEB: [(E)-4-(1-(2-aminophenylimino)ethyl)benzene-1,3-diol], APS: Ammonium persulfate, *C. albicans*: *Candida albicans*, CUPRAC: Cupric ion reducing antioxidant capacity, DDM: Disk diffusion method, DMSO: Dimethyl sulfoxide, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, *E. coli*: *Escherichia coli*, EDTA: Ethylenediaminetetraacetic acid, FRAP: Ferric reducing ability of plasma, GSH: Glutathione, HFRSA: Hydroxyl free radical scavenging assay, IC_{50}: Half maximal inhibitory concentration, Ki: Estimated inhibition constant, MHA: Mueller-Hilton agar plates, MIC: Minimum inhibitory concentration, MTCC: Microbial type culture collection and gene bank, NBT: Nitroblue tetrazolium, Nc: Neocuproine, OPDA: *o*-Phenylenediamine, PMS: Phenazine methosulfate, RMSD/LB: Root mean square deviation lower bound, RMSD/UB: Root mean square deviation upper bound, RNOSA: Reactive nitrogen oxide scavenging assay, ROS: Reactive oxygen species, SDA: Sabouraud dextrose agar, SOARSA: Super oxide anion radical scavenging assay, TBA: Thiobarbituric acid, TCA: Trichloroacetic acid, ZIT: Zone inhibition test.

resistance. The public's health is seriously threatened by the rising incidence of drug-resistant bacterial strains, which makes the development of innovative antimicrobial medicines with unique modes of action imperative. Apart from their antibacterial qualities, Schiff bases possess strong antioxidant capabilities that are essential in reducing the risk of illnesses related to oxidative stress. Chronic illnesses such as cancer, cardiovascular disease, and neurological disorders are linked to oxidative stress, which brought about by an imbalance between the generation of reactive oxygen species and antioxidant defences. Molecules with improved biological activity are formed by the structural modification of Schiff bases. The goal of this work is to examine the antibacterial and antioxidant activities of synthesised Schiff base molecule. To further clarify the processes of interaction between these Schiff bases and their biological targets, molecular docking experiments are performed. Molecular docking is a potent computational approach that helps with the logical design of more potent medicinal medicines by forecasting the binding capacity and interaction patterns of small compounds with their binding proteins[5-8].

This study focuses mainly on the Schiff bases. Using common *in vitro* experiments, the biological assessment of the antioxidant and antibacterial properties of APEB has been carried out. Using techniques like the disc diffusion test and MIC calculation, the antimicrobial activity has been evaluated against bacterial and fungal species. Utilising tests such as DPPH method, ABTS method, CUPRAC method, FRAP method, super oxide anion radical scavenging method, reactive nitrogen oxide scavenging method, hydroxyl free radical scavenging method antioxidant activity will be assessed. The binding interactions between APEB and their target proteins will be predicted using molecular docking experiments. These investigations will replicate the docking process and analyse the binding conformations using software tools such as AutoDockTools – 1.5.7 and Biovia Discovery Studio 2024 Client. This work has been focused on synthesizing a Schiff base, APEB and their characterizations FTIR, UV, nuclear magnetic resonance (NMR) and assessment of their antimicrobial and antioxidant activities. Molecular docking studies has done to clarify the processes behind their interactions. This work attempts to contribute to define the biological properties, and computer modelling of synthesized Schiff base, APEB.

Experimental Section

The synthesis of Schiff base APEB follows the modified procedure detailed in our previous report[9] as well as others reports[10,11] and was characterized by different spectroscopic techniques. Further, the experimental details, as well as information on material and antimicrobial activity using minimum inhibitory concentration (MIC) method[12,13], zone inhibition test (ZIT)[14,15], Antioxidant activity using DPPH method[16-20], ABTS method[18,21-25], CUPRAC method[26,22,27], FRAP method[19,22,28], SOARSA method[17,18,24], RNOSA method[26,28], HFRSA method[22,24,29,30] and molecular docking[31] are provided in the Supporting Information.

Result and Discussion

The Schiff base, (E)-4-(1-(2-aminophenylimino) ethyl)benzene-1,3-diol (APEB) was prepared by the condensation of *o*-Phenylenediamine (OPD) and 2,4-dihydroxyacetophenone (2,4DA) with ratio of 1:1 by regular reflux method (Scheme S1). The FTIR spectrum of APEB shows key functional group confirmations (Fig. S1). The peaks at 3429 and 3302 cm^{-1} are likely due to O-H stretching vibrations from the phenolic hydroxyl groups, which may also indicate hydrogen bonding in the structure. A peak at 2914 cm^{-1} suggests C-H stretching from aliphatic groups, while a weaker 2590 cm^{-1} peak could be due to minor overtone or combination bands. The strong peak at 1613 cm^{-1} confirms the presence of the C=N (imine) bond, a defining feature of the Schiff base linkage, and the peak at 1579 cm^{-1} corresponds to C=C stretching vibrations in the aromatic rings, indicative of conjugation within the benzene structure. Additional peaks at 1484 cm^{-1} and 1321 cm^{-1} align with C=C and C-N stretching, respectively, supporting the aromatic nature and imine linkage. Peaks at 1256 and 1187 cm^{-1} indicate C-O stretching in the phenolic groups, while 1028 cm^{-1} suggests further C-O or C-N stretching. Out-of-plane C-H bending in the aromatic rings appears at 935, 843, 860, and 750 cm^{-1}, consistent with a benzene ring structure. Finally, the peaks at 653, 574, 509 and 454 cm^{-1} represent bending vibrations of the substituted aromatic rings, further affirming the structural components of this Schiff base. By the above

explanation it confirms the imine formation, phenolic groups, and aromatic character expected from the synthesized Schiff base, APEB[7,32,33]. The UV-Vis absorption spectra of APEB (0.01mmol) in methanol was recorded within 200-1000 nm at room temperature. The UV-Vis spectrum (Fig. S2) shows a strong absorption peak at 238 nm is caused by the π-π* transition and peak at 299 and 349 nm is attributed to n-π* transition[32-34]. The ^{1}H NMR spectra of the APEB (Fig. S3) show the signals at δ 7.53 (d, J = 8.7 Hz, 14H), 6.89 (t, J = 7.3 Hz, 14H), 6.77 – 6.52 (m, 43H), 6.24 (d, J = 1.8 Hz, 15H) for CH of Benzene, 4.74 (s, 24H) for NH_2, 3.34 (s, 14H), 3.16 (s, 1H) for OH, 2.21 (s, 41H) for CH_3. This confirms the proposed structure of the Schiff base (APEB) (Scheme S1)[7,33,35]. Furthermore, the ^{13}C NMR signals (Fig. S4) at δ 172.87 (s) for iminic carbon, 164.73 (s), 162.17 (s) for carbon of OH attached benzene, 140.48 (s), 132.31 (s), 112.91 (s) for carbon of Benzene, 131.61 (s), 126.00 (s), 122.18 (s), 116.71 (s), 115.46 (s), 107.02 (s), 103.34 (s) for C-H of Benzene, 17.15 (s) for CH_3 attached to benzene indicates the formation of Schiff base (APEB)[7,33,35].

Based on the study, it was observed that when test organism *E. coli* was exposed with different concentrations of the APEB then APEB (MIC= 0.07µg/mL) exhibited significant anti-microbial activity against *E. coli* (Fig. 1a)[12,13]. It was observed that when test organism *C. albicans* was exposed with different concentrations of the samples then APEB (MIC = 0.06 µg/mL) exhibited significant anti-microbial activity (Fig. 1b)[12]. In case of *E. coli* in APEB, the clear zones were not observed with different concentration in antibacterial activity with respect to positive control. The clear zones surrounding the disc were measured and recorded (Fig. 2a). In sample APEB, after treatment the clear zones were observed at size of 4 mm at 250 µg/disc, 7 mm at 500 µg/disc and 8.33 mm at 1000 µg/disc with respect to positive control in antifungal activity (Fig. 2b)[14,15]. In the MIC assay, APEB showed significant antibacterial activity, with an MIC value of 0.07 µg/mL, indicating its ability to inhibit bacterial growth in a liquid medium. However, the zone inhibition test did not show clear zones against *E. coli*, suggesting limited or no antibacterial activity on solid agar. Similarly, the compound exhibited strong antifungal activity in the MIC assay, with an MIC value of 0.06 µg/mL, demonstrating its effectiveness in inhibiting fungal growth in a liquid medium. Contrarily, the zone of inhibition test produces visible inhibition zones *C. albicans*, indicating antifungal activity on solid agar.

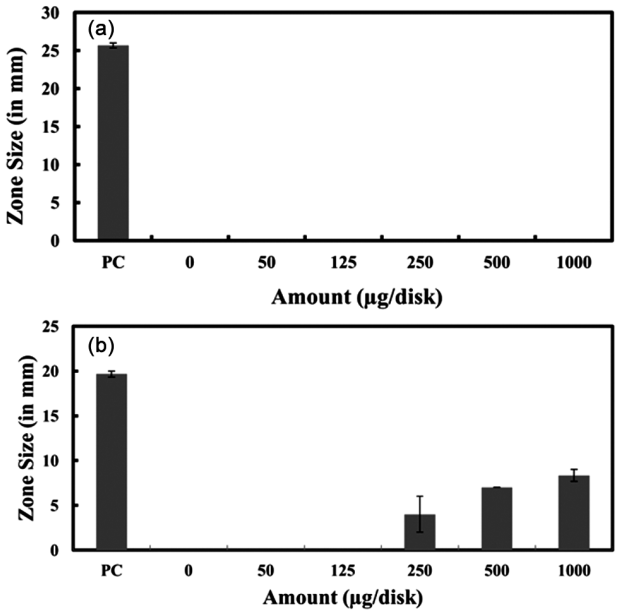

Fig. 2 — (a) Plot of ZIT (DDM) of APEB against *E. coli* (b) Plot of ZIT (DDM) of APEB against *C. Albicans*

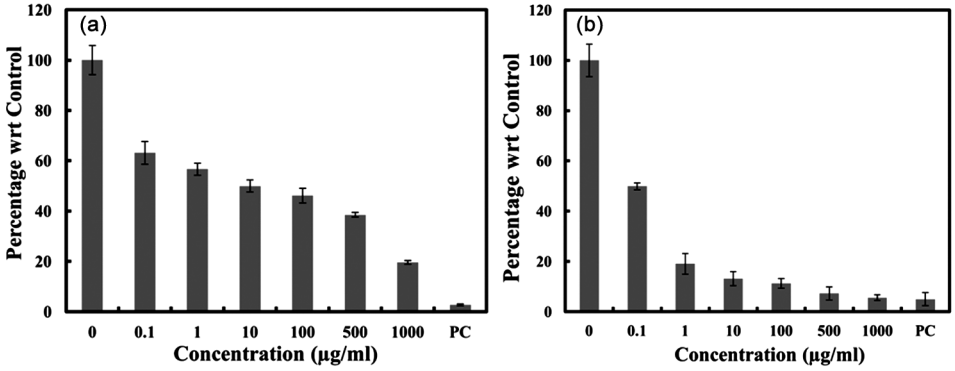

Fig. 1 — (a) Plot of MIC of APEB against *E. coli* (b) Plot of MIC of APEB against *C. Albicans*

Antioxidant property (DPPH Fig. 3a, b) was observed in APEB with IC_{50} = 187.2 µg/mL, in comparison standard ascorbic acid (IC_{50} = 5.428 µg/mL *i.e.*, 50% inhibition at this concentration)[16,17,20]. From the study outcomes, it was observed that APEB with an IC_{50} value of 5.967 ± 0.1625 µg/mL exhibited antioxidant property (ABTS Fig. 3c, d), in comparison to the standard ascorbic acid, which had an IC_{50} value of 3.407 ± 0.04 µg/mL[21,23,25]. Antioxidant property (CUPRAC Fig. 3e, f) was observed in APEB (IC_{50} = 2.37 µg/mL *i.e.*,50% inhibition at this concentration), and IC_{50} of the standard Trolox was observed as 1.264 µg/mL[26,27]. Very high antioxidant property (FRAP Fig. 4a, b) was

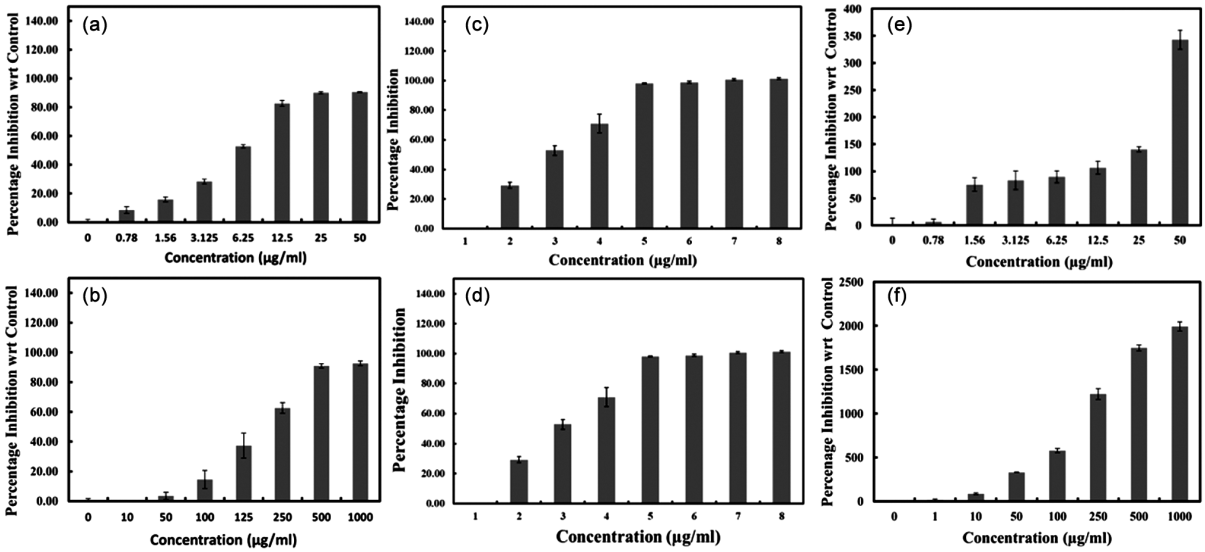

Fig. 3 — (a) Plot of DPPH scavenging assay (Ascorbic acid) (b) Plot of DPPH scavenging assay (APEB) (c) Plot of ABTS radical scavenging ability (Ascorbic acid) (d) Plot of ABTS radical scavenging ability (APEB) (e) Total antioxidant assay - CUPRAC (Trolox) (f) Total antioxidant assay – CUPRAC (APEB)

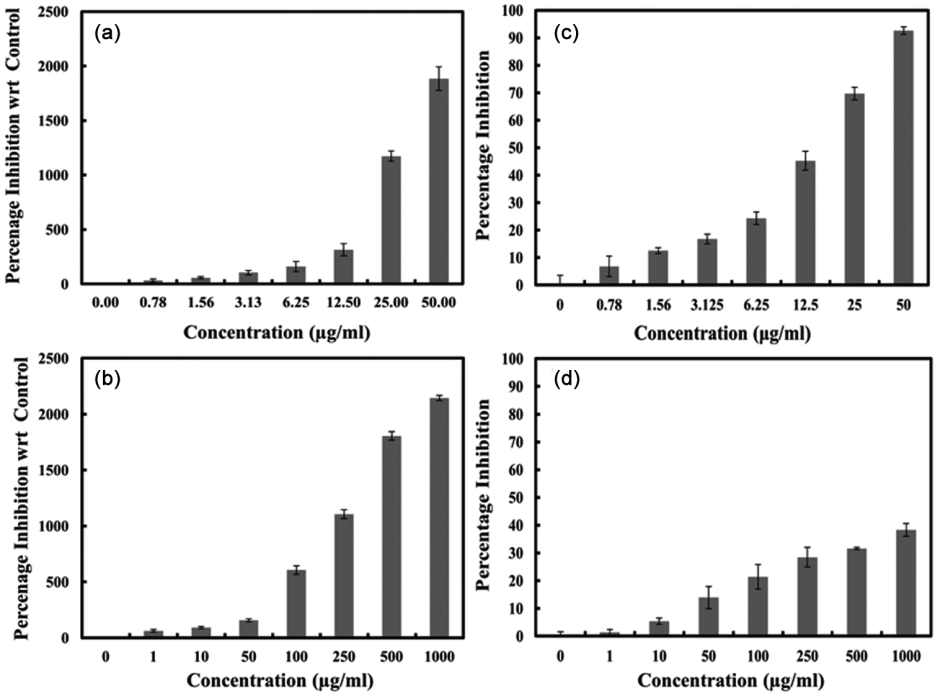

Fig. 4 — (a) Total antioxidant assay – FRAP (Ascorbic acid) (b) Total antioxidant assay – FRAP (APEB) (c) Super oxide anion radical scavenging activity (Gallic acid)) (d) Super oxide anion scavenging activity (APEB)

observed in the APEB (IC_{50} = 0.7404 µg/mL) with respect to standard ascorbic acid (IC_{50} = 1.158 µg/mL)[28]. Very low superoxide anion radical scavenging activity was observed in the APEB (IC_{50} = above maximum dose limit *i.e*. higher than 1000 µg/mL) with respect to standard gallic acid (IC_{50} = 12.94 ± 1.31 µg/mL) (SOARSA Fig. 4c, d)[36]. Reactive nitrogen oxide scavenging ability was observed in APEB and (IC_{50} = 167.6 µg/mL *i.e*. 50% inhibition at this used concentration), as compared to gallic acid (IC_{50} = 12.71µg/mL) (RNOSA Fig. 5a, b)[28]. According to the results obtained from the study, hydroxy free radical scavenging activity was observed in APEB (IC_{50} = ~ 9.934 µg/mL as compared to standard gallic acid (IC_{50} = 1.569 µg/mL) (Table 1) (HFRSA Fig. 5c, d)[29,30]. Table 1 explains the antioxidant activity of APEB compared to standard antioxidants using different assays. For assays like DPPH, ABTS, FRAP, CUPRAC, RNOSA, and HFRSA, APEB exhibited higher IC_{50} values than the standards, indicating lower antioxidant potency. However, for FRAP, APEB showed a comparable value (0.7407 µg/mL) to the standard ascorbic acid. In the SOARSA assay, APEB's activity was beyond the measurable range, indicating very low antioxidant activity in that method.

Molecular docking studies are utilized for the prediction of atomic interaction of a ligand molecule and an identified target protein. It gives explanation about the small amount of interaction patterns at the binding sites of target proteins and also explains the key biochemical processes. The molecular docking of the APEB was done against two different active sites with PDB ID: 1AI6 and 5AEZ using two different docking software (AutoDockTools – 1.5.7 and PyRx). The crystal structure of the active sites and APEB was obtained from the protein data bank and ChemDraw 3D respectively. The docking was done using

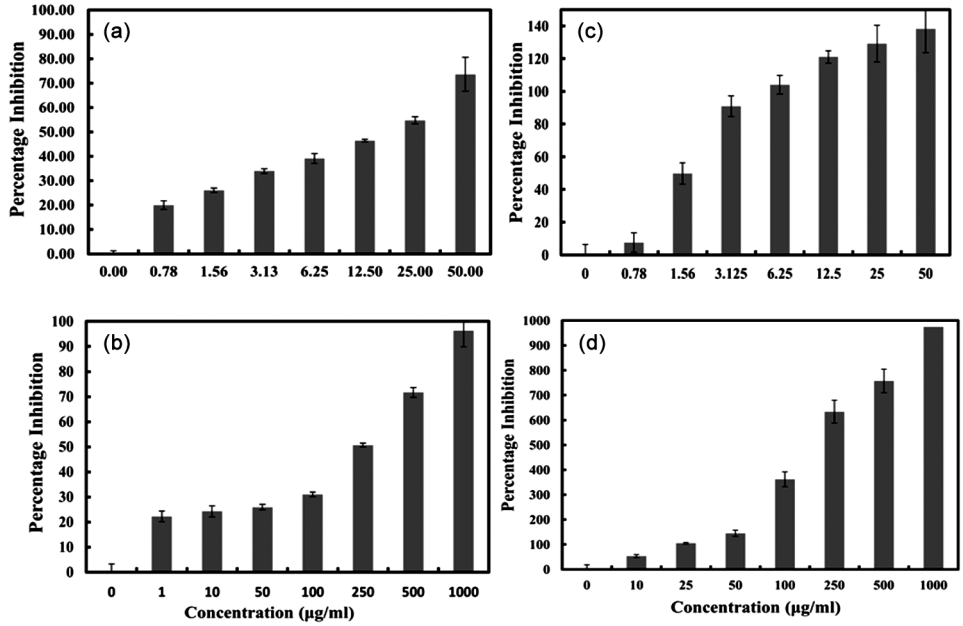

Fig. 5 — (a) Reactive Nitrogen Oxide Scavenging Method Assay (Gallic acid) (b) Reactive Nitrogen Oxide Scavenging Method Assay (APEB) (c) Hydroxyl radical scavenging assay (Gallic acid) (d) Hydroxyl radical scavenging assay (APEB)

Table 1 — Antioxidant activity of APEB

S. N0.	Antioxidant activity	Standards	IC_{50} values (standard)	IC_{50} values (APEB)
1.	DPPH	Ascorbic acid	5.428 µg/mL	187.2 µg/mL
2	ABTS	Ascorbic acid	3.407 ± 0.04 µg/mL	5.967 ± 0.1625 µg/mL
3	FRAP	Ascorbic acid	1.158 µg/mL	0.7404 µg/mL
4	CUPRAC	Trolox	1.264 µg/mL	2.37 µg/mL
5	SOARSA	Gallic acid	12.94 ± 1.31 µg/mL	above maximum dose limit *i.e*. higher than 1000µg/mL
6	RNOSA	Ascorbic acid	12.71 µg/mL	167.6 µg/mL
7	HFRSA	Gallic acid	1.569 µg/mL	9.934 µg/mL

AutoDockTools – 1.5.7 and PyRx software and structure visualization has done by Biovia Discovery Studio 2024 Client. The docking results obtained from AutoDockTools – 1.5.7 utilized the Lamarckian genetic algorithm. For the first protein (PDB ID: 1AI6), the lowest binding energy was observed in run 3, with a cluster rank of 1, yielding a binding energy of –5.05 kcal/mol and an estimated inhibition constant (Ki) of 198.13 µM at 298.15 K. In contrast, the second protein (PDB ID: 5AEZ) showed a binding energy of –5.51 kcal/mol in run 7, cluster rank 1, with an estimated inhibition constant (Ki) of 91.13 µM at 298.15 K. The docking performed with PyRx yielded more negative binding energies. For the first protein (PDB ID: 1AI6), the lowest binding energy recorded was –6.3 kcal/mol with a rmsd/ub (upper bound) of 0 and rmsd/lb (lower bound) of 0. Similarly, the second protein (PDB ID: 5AEZ) exhibited a lowest binding energy of –7.0 kcal/mol with a rmsd/ub (upper bound) of 0 and rmsd/lb (lower bound) of 0. Docking results indicate that the Schiff base, APEB has a stronger binding affinity and inhibitory potential toward the 5AEZ protein compared to 1AI6, as reflected in both AutoDockTools – 1.5.7 and PyRx results. The more negative binding energies from PyRx further confirm the strong interaction, particularly with 5AEZ. This information can guide experimental validation of the Schiff base, APEB as a potential inhibitor, particularly focusing on 5AEZ for biological activity studies. The Schiff base, APEB exhibited a stronger binding affinity and lower inhibition constant toward PDB ID: 5AEZ, suggesting that this protein may be a better biological target for inhibitory action. The consistency in binding energies across two docking platforms AutoDockTools – 1.5.7 and PyRx supports the reliability of the predicted interactions[37-41]. Docking related 3D and 2D structures are shown following (Fig. 6a–h). Fig. 6a–d are obtained as a result of docking by using AutoDockTools – 1.5.7 and Fig. 6e–h are obtained as a result of docking by using PyRx.

Although the Schiff base (APEB) is not new but our study reveals the significant antimicrobial and antioxidant activities, demonstrated by the results of the assays and molecular docking studies by using two different software, AutoDockTools – 1.5.7 and PyRx. These findings align with previous studies on Schiff base (APEB) but provide unique insights through antimicrobial, antioxidant and molecular docking studies, which shows strong binding affinities to target proteins, suggesting a robust mechanism of action. The implications of this research extend to the development of new therapeutic agents, emphasizing the necessity for further exploration of Schiff bases in

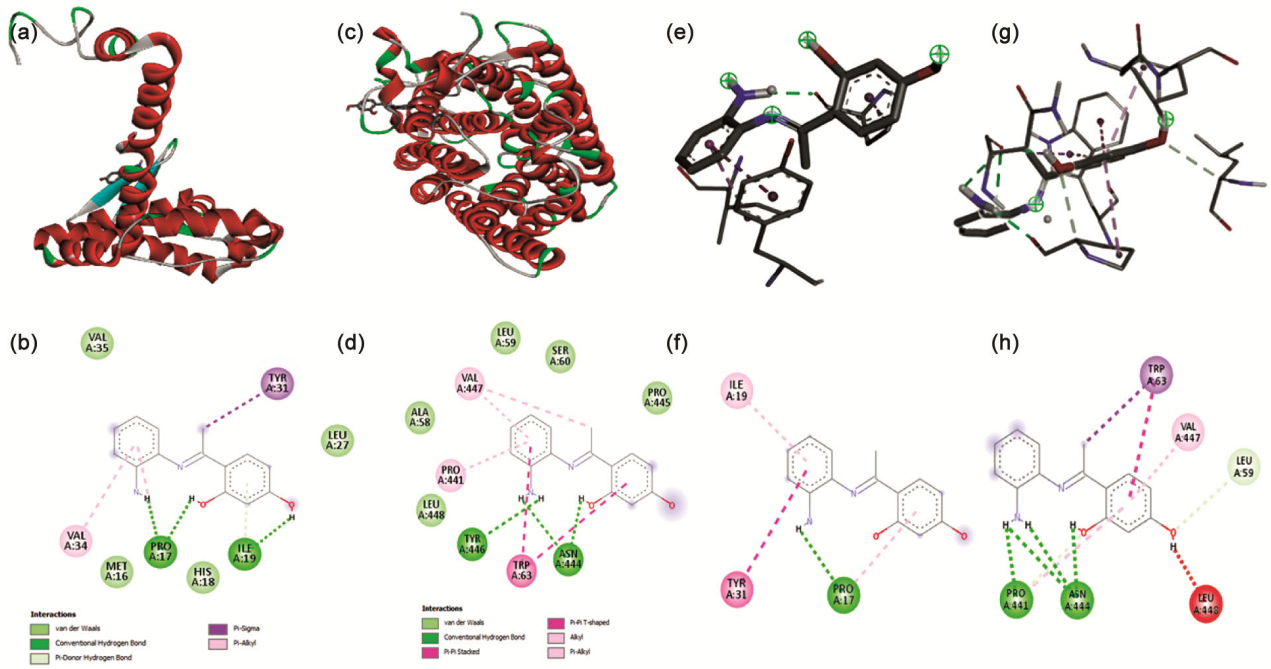

Fig. 6 — (a) 3D structure of APEB docked with 1AI6 (b) 2D structure of APEB docked with 1AI6 (c) structure of APEB docked with 5AEZ (d) 2D structure of APEB docked with 5AEZ (e) 3D structure of APEB docked with 1AI6 (f) 2D structure of APEB docked with 1AI6 (g) 3D structure of APEB docked with 5AEZ (h) 2D structure of APEB docked with 5AEZ

medicinal chemistry. The positive MIC result for antibacterial activity suggests that the Schiff base effectively inhibits bacterial growth in a liquid medium. However, the absence of a zone of inhibition on solid agar indicates that the compound may have limited diffusion ability or altered activity when applied to a solid medium. This could be due to factors such as molecular size, solubility, or interaction with agar components, which may hinder its ability to diffuse and exert its antibacterial effects on the agar surface. The positive MIC and zone inhibition test result for antifungal activity suggests that APEB effectively inhibits fungal growth in a liquid medium as well as solid agar surface. Future research should replicate these results in larger, more diverse populations and include *in vitro* studies to confirm efficacy and safety, as well as explore additional derivatives to optimize their biological activities. The contrasting results observed in the MIC and zone of inhibition assays for both antibacterial and antifungal activities suggest that the Schiff base's antimicrobial efficacy may be influenced by the testing environment. The results emphasize the importance of using multiple testing methods to fully assess the antimicrobial potential of APEB. In case of antibacterial activity, the differential activity observed in liquid *versus* solid media suggests that the APEB's mechanism of action may be more effective in environments where diffusion is not a limiting factor. Future research should replicate these results in larger, more diverse populations and include *in vitro* studies to confirm efficacy and safety, as well as explore additional derivatives to optimize their biological activities. The contrasting results observed in the MIC and zone of inhibition assays for both antibacterial and antifungal activities suggest that the APEB's antimicrobial efficacy may be influenced by the testing environment.

Conclusion

The Schiff base APEB has been synthesized and characterized by FTIR, UV-Vis Spectroscopy and NMR. The antimicrobial behavior of APEB was examined towards bacterial and fungal microorganisms *E. coli* and *C. albicans* respectively. The Schiff base APEB exhibited promising antibacterial and antifungal activity in the MIC assays, indicating its potential as an antimicrobial agent in liquid media. However, the absence of inhibition zones in the solid agar assays for bacterial strains suggests that its effectiveness may be limited in solid media due to poor diffusion or interaction with the medium components. These findings highlight the need for further investigation into the compound's physical properties and potential modifications to enhance its activity in solid media. Additionally, antioxidant properties of the APEB were analyzed in comparison with reference substances by DPPH assay, ABTS assay, CUPRAC assay, FRAP assay, super oxide anion radical scavenging assay, reactive nitrogen oxide scavenging assay and hydroxyl free radical scavenging assay. It was found that the APEB shows antioxidant activity as a result of all the testing methods mentioned above. APEB showed very high antioxidant property (FRAP) and very low superoxide anion radical scavenging activity. This study shows the comparative docking by the use of two different software AutoDockTools – 1.5.7 and PyRx. The highly negative binding energies observed from PyRx analysis further support the strong interaction of Schiff base, particularly with the target protein 5AEZ. This data provides valuable insights for experimental validation, suggesting that Schiff base could be a promising inhibitor, with 5AEZ being a key protein to focus on for biological activity studies. The Schiff base demonstrated a stronger binding affinity and lower inhibition constant against PDB ID: 5AEZ, indicating that this protein may serve as a more effective biological target for inhibitory action.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

Author Contributions

Renuka Sidhanty:- Writing - Original draft, Conceptualization, Data curation, Formal analysis, Investigation, Methodology. Soumyadeep Roy Chowdhury: Investigation, Methodology, Software. Dr. Alekha Kumar Sutar: Data curation, Validation, review and editing. Dr. Tungabidya Maharana: Supervision, Validation;, Visualization, Review and editing. All authors read, reviewed and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Funding Declaration

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