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Pyridine-Based Schiff Bases as Promising Scaffolds for Antioxidant Drug Development

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Abstract: Pyridine-based heterocyclic compounds are well known for their various biological activities and possible impact on the antioxidant behaviour of organic molecules. In this study, four Schiff bases derived from 2-aminopyridine and substituted salicylaldehydes, namely salicylidene-2-aminopyridine (L1), 5-methoxy-salicylidene-2-aminopyridine (L2), 5-nitro-salicylidene-2-aminopyridine (L3), and 5-bromo-salicylidene-2-aminopyridine (L4), were evaluated for their in vitro antioxidant activity. The compounds were studied utilising melting point measurement, elemental analysis, infrared spectroscopy, and nuclear magnetic resonance spectroscopy. Their antioxidant activities were evaluated using DPPH radical scavenging, nitric oxide scavenging, ferric reducing antioxidant power, and total antioxidant capacity tests. The results demonstrated that the compounds' radical scavenging and reduction actions were concentration-dependent. Among the examined Schiff bases, the nitro-substituted derivative, L3, had the highest antioxidant efficacy across all testing. This better performance indicates that the nitro substituent increased the antioxidant capacity of the pyridine-based Schiff base. As a result, L3 could be a good candidate for future research into the development of effective antioxidants.

Keywords: Pyridine Schiff bases; 2-aminopyridine; antioxidant activity; DPPH scavenging assay; nitro-substituted salicylidene.

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Introduction

Many types of ailments such as cancer, heart problems, diabetes, neurological ailments, malarial and inflammatory diseases (Babalola, Jonathan & Michael, 2020) have all been attributed to oxidative stress, where there is an imbalance between the antioxidant capabilities of the body and the amount of reactive oxygen species being produced. Free radical generation leads to such consequences as DNA damage, lipid peroxidation, and protein oxidation, causing harm to tissues and their functions (Pizzino *et al*., 2017). This makes drug discovery efforts concentrate on developing powerful antioxidants for scavenging free radicals.

Antioxidants play a crucial role in protecting the body against oxidative stress by neutralizing reactive oxygen and nitrogen species before they can attack biological molecule (Kurutas, 2016; Altanam, Darwish & Bakillah, 2025; Manful *et al.*, 2025; Mukherjee *et al.*, 2025). Even though there have been many studies conducted on natural antioxidants, limitations such as lack of stability and bioavailability led to the formation of artificial antioxidants with better properties (Zehiroglu & Ozturk Sarikaya, 2019; Tauchen *et al.*, 2025; . Shahidi & Samarasinghe 2025). Among the various classes of bioactive molecules, heterocyclic compounds have attracted considerable attention due to their broad spectrum of biological activities (Islam *et al*., 2023; Rani & Reddy, 2018) and structural versatility. Pyridine, a six-membered aromatic heterocycle with a nitrogen atom, is one of the most important heterocyclic frameworks in medicinal chemistry (Marinescu and Popa, 2022; Vankar *et al.*, 2022). Many biologically active compounds and drugs (De *et al*., 2022; Yousef *et al*., 2023) contain a pyridine framework, which increases their pharmaceutical action owing to its unique electrical properties, aromatic character, and ability to form complexes with metals as well as hydrogen bonds.

Certain structural features of pyridine-based molecules have been identified as responsible for their antioxidant properties (Mojarrab, Soltani & Aliabadi, 2013; Koparir *et al*., 2022; Kishore Sahu, Mahajan, & Chaudhary, 2024; Benkirane *et al*., 2025; Al-Bahrani *et al*., 2025; Muhammed, Fazwi & Abd El-Hamid., 2026). The radicals formed in the free radical scavenging reactions can be stabilized due to the electron withdrawing nature of the nitrogen atom in the aromatic ring. Moreover, the pyridine core is known to enhance the general redox properties, provide pathways for electron transport, and favor the delocalization of

unpaired electrons. Modification of the electron donating/withdrawing capabilities of the molecules through substitution with additional electron donating/withdrawing groups in the pyridine ring could further fine-tune antioxidant properties. Pyridine compounds were also found to reduce oxidative stress caused by metal ions by forming chelates with transition metals that form reactive oxygen species.

As reported in recent studies, it is observed that the pyridine derivatives exhibit promising antioxidant activity (Al-Bahrani *et al*., 2025; Benkirane *et al*., 2025) that makes them suitable for the design of new drugs for diseases associated with oxidative stress. The addition of a pyridine ring in any molecule results in enhancing its stability and bioactivity and also provides opportunities for making structural modifications to maximize antioxidant activity. The pyridine nucleus is frequently incorporated into Schiff base (Kumar, Padmini & Ponnuvel, 2017; Al-Atbi, Jaraf & Sabu, 2022) ligands because of its aromatic character and nitrogen donor site, which facilitate coordination with metal ions.

In view of the growing interest in pyridine-containing compounds as potential antioxidants, the present study investigates the antioxidant properties of 2-aminopyridine-based Schiff bases and their particular emphasis is placed on evaluating the contribution of the pyridine nucleus to the observed antioxidant activity and establishing structure–activity relationships that may guide the design of more potent antioxidant agents.

Materials and Methods

Commercial chemicals of Analytical grade were purchased from Zayo-Sigma Chemicals Ltd and utilized without additional purification. Melting points were calcualted on a Gallenkemp England melting point apparatus. A Perkin-Elmer 2400 CHNS/O analyzer was used to perform the elemental studies. A FT-IR Perkin-Elmer 1600 spectrometer was used to record infrared spectra as nujol mulls. Tetramethylsilane (TMS) served as the internal standard during the NMR spectra, which were conducted in CDCl3 on a Bruker AMX 400 spectrometer. Ascorbic acid (99%), trichloroacetic acid (TCA), hydrogen peroxide, potassium ferricyanide, iron(II) chloride, methanol, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (ferrozine), iron(III) chloride, and hydrochloric acid (Germany).

Typical procedure for preparation of Schiff bases (L1-L4)

Synthesis of salicylidene-2-aminopyrdine based Schiff bases (L1-L4): An ethanolic solution of 2-aminopyridine (0.10 mmol) and the corresponding aldehyde (0.10 mmol) was agitated in a round-bottom flask for ten minutes. 0.3 mL of formic acid was added to the solution and heated under reflux for six hours at 60°C and yielded a product as a precipitate, which after filtering and recrystallization yielded the desired products (L1-L4).

Salicylidene-2-aminopyridine (L1)

Yield: 26.12 mg (66%); mp: 62–64°C; R_f : 0.52. HNMR (300 MHz, $CDCl_3$) δ_H: 6.91–8.49 (m, 8H), 9.41 (s, 1H), 13.40 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 117.21, 118.93, 119.19, 120.44, 122.54, 133.44, 133.81, 138.45, 148.92, 151.51, 161.82, 164.70.

IR (cm^{-1}): 3058, 2365, 1603, 1586, 1554, 1496, 1465, 1450, 1428, 1350, 1276, 1184, 1142, 1110, 1044, 1030, 993, 958, 913, 787, 623, 562; Anal. calcd. for $C_{12}H_{10}N_2O$: C, 72.71, H, 5.08, N, 14.10.

Found, C, 72.33, H, 5.03, N, 14.00

5-methoxy-salicylidene-2-aminopyridine (L2)

Dark-orange crystal, Yield: 34.20 mg (75%); mp: 82–84°C; R_f: 0.45. IR (cm^{-1}): 3021, 1598, 1580, 1557, 1519, 1454, 1394, 1356, 1334, 1316, 1271, 1169, 1137, 1061, 1029, 915, 875, 701, 641, 610; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 3.39 (s, 3H), 6.93–8.48 (m, 7H), 9.37 (s, 1H), 12.93 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 55.78, 115.79, 118.05, 118.44, 120.46, 121.50, 122.52, 138.42, 148.88, 152.20, 156.16, 157.49, 164.41. Anal.calcd for $C_{13}H_{12}N_2O_2$:C, 68.42, H, 5.26, N, 12.28. Found: C, 68.32, H, 5.28, N, 12.14.

5-nitro-salicylidene-2-aminopyridine (L3)

Yellow solid, Yield: 22.10 mg (46%); mp: 182–184°C; R_f: 0.68. IR (cm^{-1}): 3331, 1595, 1556, 1526, 1426, 1373, 1285, 1195, 1147, 1099, 1023, 930, 863, 728, 646, 552; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 7.10–8.54 (m, 7H), 9.53 (s, 1H), 14.56 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 117.79, 118.49, 120.51, 123.60, 128.90, 129.52, 131.63, 138.76, 149.25, 155.78, 162.89, 167.63.

Anal. calcd. for $C_{12}H_9N_3O_3$: C, 59.26, H, 3.73, N, 17.28. Found: C, 59.14, H, 3.56, N, 16.96.

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5-bromo-salicylidene-2-aminopyridine (L4)
Light-orange, Yield: 45.40 mg (81%); mp: 138–140^{0}C; Rf: 0.50. IR (cm^{-1}): 1608, 1586, 1550, 1461, 1428, 1381, 1341, 1276, 1184, 1144, 1100, 990, 918, 870, 814, 782, 700, 628, 607; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 6.89–8.49 (m, 7H), 9.34 (s, 1H), 13.42 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 110.60, 119.28, 120.35, 120.65, 122.99, 135.26, 136.36, 138.56, 149.05, 156.98, 160.86, 163.40. Anal. calcd. for $C_{12}H_9N_2OBr$: C, 52.01, H, 3.27, N, 10.10. Found: C, 51.96, H, 3.21, N, 9.88

Evaluation of Antioxidant Activity

DPPH radical scavenging assay : The ability of the synthesized compounds to scavenge free radicals was measured using a reduction in the absorbance of DPPH with slight changes in the methods of Mensor *et al*, (2001) and Gbasemi *et al*, (2009). The samples' serial solutions (25–100 µg/mL) were made with distilled water. 1 mL of a methanolic solution of DPPH (0.01 mM) was mixed with 4 mL of each solution. After shaking the combination, it was left in a dark closet for 30 minutes. Spectrophotometry was used to detect absorbance at 517 nm. Serially diluted ascorbic acid solutions was utilized as the standard and methanol as the blank. Every test was carried out in triplicate. The samples' scavenging effect was shown as a percentage of inhibition. Scavenging Effect (%) = $D_o - D_1/D_0$ x 100; where D1 is the absorbance when the extract and standard sample are present, and Do is the absorbance of the control.

Nitric oxide scavenging activity: With few modifications, the nitric oxide scavenging activity was measured using spectrophotometry as described by Green *et al*, (1982). It has been shown that nitrite ions are produced by aqueous sodium nitroprusside at physiological pH (Ebrahimzadeh, *et al*., 2010). A pH 7.4 phosphate buffered saline was made using sodium nitroprusside (5 mM L-1). The samples were serially diluted to a concentration of 25–100 µg/mL. 50 µL of each sample solutions were combined with the buffer. For 30 minutes, the mixture was incubated at 25°C. After that, 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride) was added to 1.5 mL of the incubated mixture. At 546 nm, absorbance was measured. The positive standard was ascorbic acid, while the negative control was phosphate buffered saline and 10 mM sodium nitroprusside.

Ferric reducin antioxidant Power (FRAP): The modified approach of Yen & Chen (1995) was used to determine the samples' reducing power. Phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%) were combined with an aliquot of the samples (1.0 mL) at different serially diluted values (25, 50, 75, and 100 ug/mL). For 20 minutes, the mixtures were incubated at 50 °C. Each combination was mixed with 2.5 mL of 10% trichloroacetic acid and centrifuged for 15 minutes at 1000 rpm. Each of the resultant supernatants was combined with 2.5 mL of distilled water and 0.1% of freshly made iron (III) chloride (0.5 mL). Methanol was used as the blank when measuring absorbance at 700 nm. The reducing power of the samples or substance is indicated by an increasing absorbance value. Ascorbic acid (vitamin C), which was likewise synthesized in serial form, served as the study's reference medication. Every experiment was conducted in triplicate.

Total antioxidant capacity: Using modified method (Prieto, Pineda & Aguilar, 1999), the samples' total antioxidant capacity was measured. Each sample was serially diluted with ethanol/methanol at 25, 50, 75, and 100 ug/mL. An aliquot (0.4 mL) of the diluted sample solution was combined with 4 mL of a reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) in a vial. For ninety minutes, the vial was incubated at 95 °C in a water bath. The absorbance was measured at 695 nm against ethanol/methanol as a blank once the vial had cooled to room temperature. Every sample's serially diluted solution was tested in triplicate.

Results and Discussion

Synthesis : The Schiff base ligands were isolated in good yield from the reaction of 2-aminopyridine with the corresponding aldehyde to readily afford, salicylidene-2-aminopyrdine (L1), 5-methoxy-salicylidene-2-aminopyridine (L2), 5-nitro-salicylidene-2-aminopyridine (L3) and 5-bromo-salicylidene-2-aminiopyridine (Figure 1).

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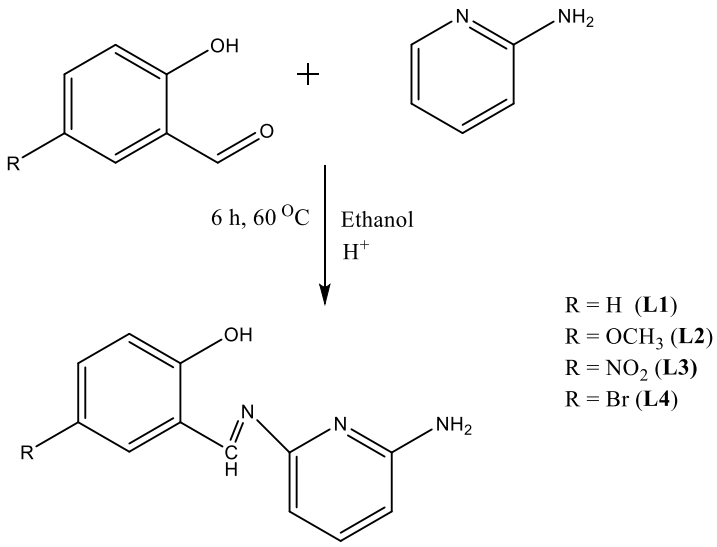

Figure 1: Scheme for the reaction of Schiff bases **L1 – L4**

All of the compounds have sharp melting points that indicate their purity and are air stable. The compounds' elemental analyses support the composition that has been proposed for them. The IR of each compound confirms the formation of imine bond (–C=N-) showing a band at 1607-1615 cm $^{-1}$ due to the stretching vibration of the imine group υ (C=N). All the compounds displayed a band corresponding to different functional groups present in the compounds.

Table 1: Physical and analytical data of 2-aminopyridine Schiff bases

Ligand code	Molecular formular (M.wt(g/mol))	Color	mp (°C)	Yield(%)	Microanalysis: % Calculated (Found)		
					C	H	N
L1	$C_{12}H_{10}N_2O$ (198)	Yellow-orange	62-64	66	72.71 (72.33)	5.08 (5.03)	14.10 (14.00)
L2	$C_{13}H_{12}N_2O_2$ (228)	Dark-orange	82-84	75	68.42 (68.32)	5.26 (5.28)	12.28 (12.14)
L3	$C_{12}H_9N_3O_3$ (243)	Yellow	182-184	46	59.26 (59.14)	3.73 (3. 56)	17.28 (16.96)
L4	$C_{12}H_9BrN_2O$ (277)	Light orange	138-140	81	52.01 (51.96)	3.27 (3.21)	10.10 (9.88)

The NMR in $CDCl_3$ showed all relevant peaks which further confirmed the formation of –C=N- bonds and the Schiff base ligands as a whole.

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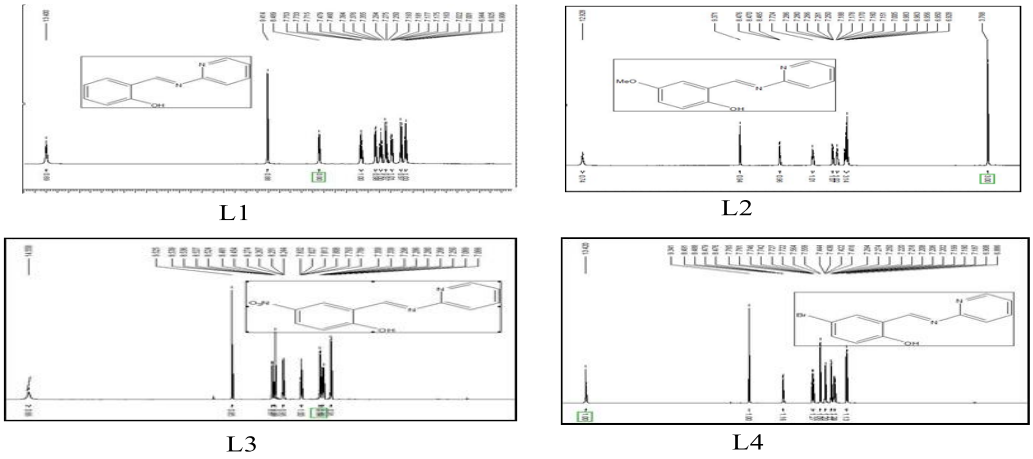

Figure 2: ^{1}HNMR spectra of the Schiff bases L1-L4

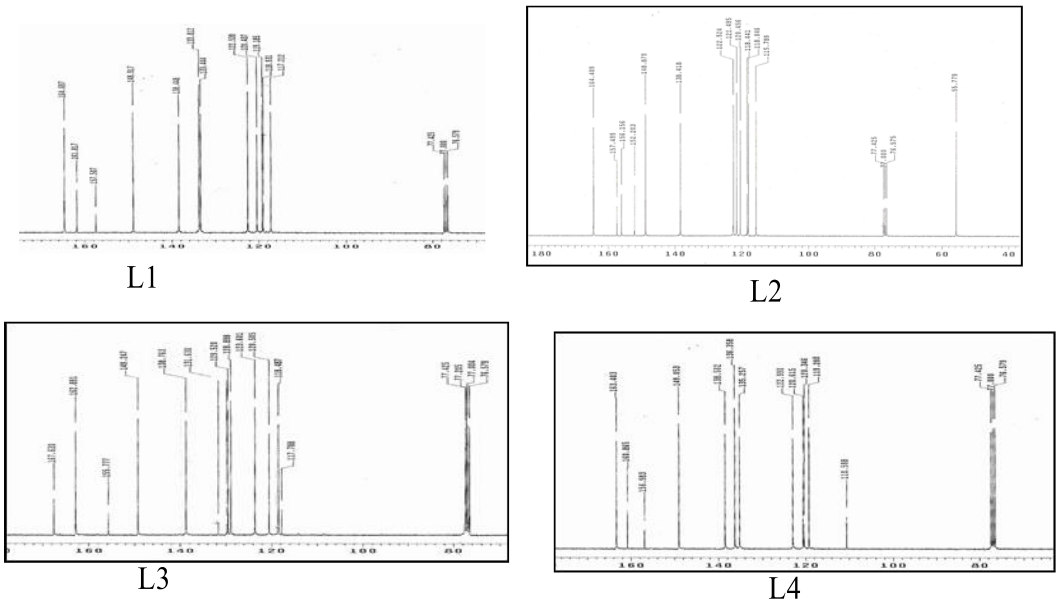

Figure 3: ^{13}CNMR spectra of Schiff bases L1-L4

Antioxidant Assay: All the compounds inhibited the radical in direct proportion to their concentrations, and there were variations between the substituted compound (L2-L4) and the unsubstituted ligand (L1).

Table 2: DPPH Scavenging Activity (% Inhibition)

Sample	µg/mL			
Code	25	50	75	100
L1	33.47	46.99	54.77	65.57
L2	35.68	55.31	73.81	70.25
L3	45.02	54.43	77.44	91.39
L4	37.30	45.29	55.78	67.99
Ascorbic Acid	45.67	54.71	78.53	92.22

The DPPH result showed that among the ligands, L3 (nitro-substituted) showed the highest DPPH activity, reaching 91.39% inhibition at 100 µg/mL, comparable to ascorbic acid (92.22%). The enhanced activity of L3 may be attributed to the electron-withdrawing effect of the nitro group, which facilitates radical stabilization. The methoxy-substituted ligand (L2) showed better

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activity than the unsubstituted ligand (L1), while L4 (bromo-substituted) displayed moderate activity. The antioxidant activity followed the order: L3 (NO_2) > L2 (OCH_3) > L4 (Br) > L1 (H).

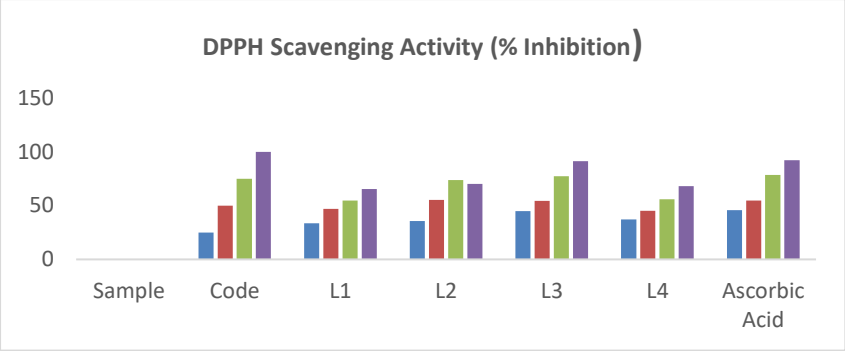

Figure 4: DPPH Scavenging Activity of the Ligands

Nitric Oxide (NO) Scavenging Radical: The nitric oxide scavenging capacity of all Schiff base ligands (Table 3) was found to depend on their concentrations with percentage inhibition increasing from 25 to 100 µg/mL. L3 (with nitro functional group) exhibited the highest activity, exhibiting inhibition of 84.00%, similar to that shown by ascorbic acid (86.70%). The nitro group's electron-withdrawing properties, which promote radical stabilization, may be responsible for increased activity. Order of activity: L3 (NO_2) > L1 (H) > L4 (Br) > L2 (OCH_3).

Table 3: Nitric Oxide Scavenging Activity (% Inhibition)

Sample	µg/mL			
Code	25	50	75	100
L1	31.37	46.33	67.66	72.39
L2	31.27	47.30	65.82	64.67
L3	38.65	56.81	72.32	84.00
L4	32.49	51.36	62.63	69.99
Ascorbic Acid	38.77	58.93	72.71	86.70

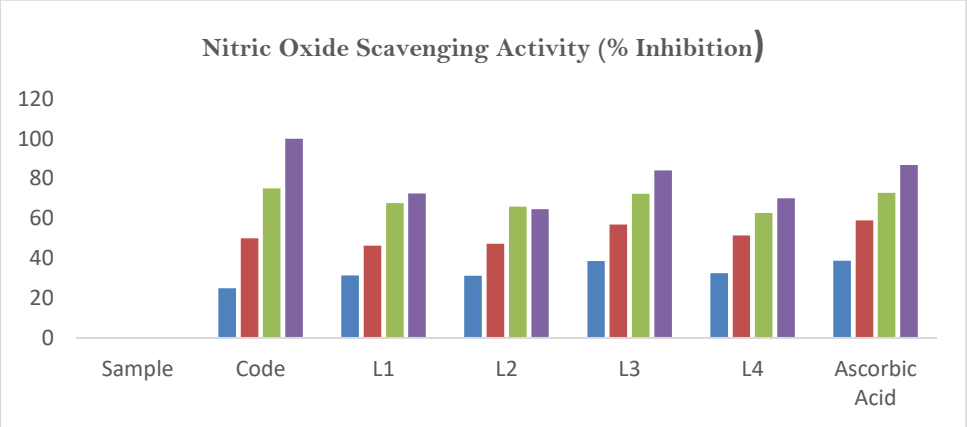

Figure 5: Nitric Oxide Scavenging Activity of the ligands

Ferric Reducing Antioxidant Power: All the Schiff base ligands exhibited concentration-dependent ferric reducing antioxidant activity, as evidenced by the increase in absorbance with increasing concentration. Among the compounds, L3 (nitro-substituted) showed the highest reducing power, with an absorbance of 0.398 at 100 µg/mL, although lower than that of ascorbic acid (0.486). The enhanced activity of L3 may be attributed to the influence of the nitro group on electron transfer processes. The reducing power of the ligands followed the order: L3 (NO_2) > L4 (Br) ≈ L2 (OCH_3) > L1 (H).

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Table 4: Ferric Reducing Antioxidant Power (% Inhibition)

Sample	µg/mL			
Code	25	50	75	100
L1	0.127	0.135	0.321	0.349
L2	0.129	0.147	0.335	0.348
L3	0.141	0.177	0.375	0.398
L4	0.127	0.143	0.331	0.351
Ascorbic Acid	0.162	0.213	0.433	0.486

Total Antioxidant Capacity: The TAC further supported the trend in the antioxidant capacity of the synthesized compounds. The total antioxidant capacity assay offers a comprehensive evaluation of antioxidant capability. Each assay examines distinct antioxidant processes, a direct correlation is not usually seen, even though substances with strong DPPH and nitric oxide scavenging capabilities frequently demonstrate elevated total antioxidant capacity. According to the current study, L3 consistently performed better in all antioxidant experiments, indicating that it has a broad-spectrum antioxidant impact, as evidenced by its high total antioxidant capacity of 97.30% inhibition, which was very close to that of ascorbic acid (98.24%). In contrast, L1 and L2 showed comparable activities, while L4 (bromo-substituted) displayed moderate activity.

Table 5: Total Antioxidant Capacity (% Inhibition)

Sample Code	**% Inhibition**
L1	68.85
L2	67.67
L3	97.30
L4	72.45
Ascorbic acid	98.24

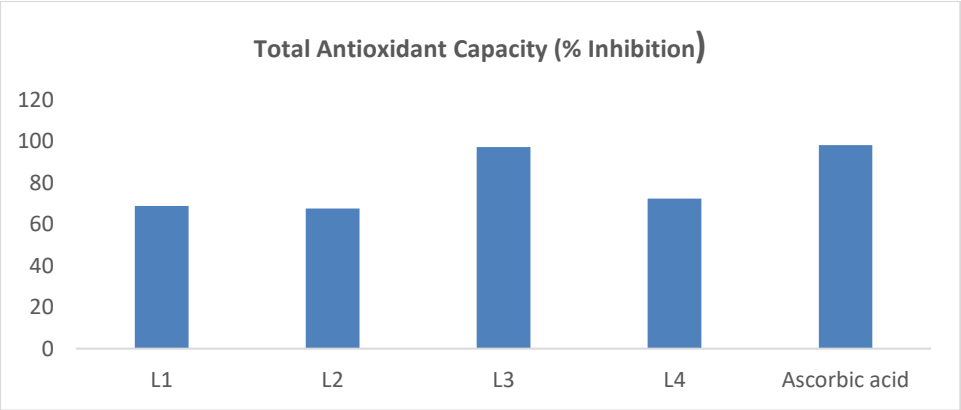

Figure 6: Total Antioxidant capacity of the ligands

Conclusion

The synthesized pyridine Schiff bases showed reasonable antioxidative capacity during the different in vitro tests. Of all the prepared molecules, the nitro derivative (L3) had the highest capability of scavenging DPPH, nitric oxide, ferric, and total antioxidant activity values comparable with those of ascorbic acid. The higher antioxidant activity displayed by the nitro compound L3 indicates the importance of the electronic effect of the substituents on the antioxidant properties of the Schiff base derivatives of pyridine ring. Overall, the findings indicate that Schiff bases with pyridine ring have good potential as an antioxidant agent.

Declarations

Declaration of conflict of interest: The authors declare that no known conflict of interest or personal relationships that could have influence the work reported in this paper.

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