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RESEARCH ARTICLE

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Synthesis, characterization, anti-microbial activity studies of salicylic acid and 2-aminopyridine derivatives salts and their Cu(II) complexes

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Abstract

Four salts (**1-4**) obtained between salicylic acid (H_2salic) and 2-amino-Xpyridine {X = (2ap), 3-methyl (2a3mp), 4-methyl (2a4mp) and 5-methyl (2a5mp)} and the Cu(II) complex of H_2salic (**5**) by methods available in the literature and new Cu(II) complexes (**6-9**) of the salts (**1-4**) has been prepared. The Cu(II) complexes (**6-9**) were suggested by elemental analysis, FT-IR, AAS, UV-Vis and magnetic susceptibility techniques. The spectroscopic research results indicated that complexes **6-9** have tetrahedral geometries. Additionally, antimicrobial activities of free ligands (H_2salic, 2ap, 2a3mp, 2a4mp and 2a5mp), **1-9** were studied against *Candida albicans* (F89) yeast, *Staphylococcus aureus* (NRRL B-767), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis*, *Listeria monocytogenes* (ATCC 7644), *Escherichia coli* (ATCC25922) and *Enterococcus faecalis* (ATCC 29212) bacteria. The results were comparisoned with the control compounds (Fluconazole, Vancomycin, Cefepime and Levofloxacin). All compounds showed activity against bacteria and yeasts.

Keywords: 2-Aminopyridine, salicylic acid, salt, Cu(II) complex, anti-bacterial and anti-fungal activities.

1. Introduction

2-Aminopyridines have garnered particular interest due to their diverse pharmacological properties linked to their inclusion in certain compounds. Studies have demonstrated that the presence of a tiny 2-aminopyridine molecule enhances the target molecule's therapeutic qualities, regardless of how complex the molecule is more heterocycles present in its structure or a simple molecule with a few groups on it. Numerous medications, including piroxicam, tenoxicam, sulfasalazine with anti-inflammatory qualities, delavirdine as an anti-HIV medication, sulfapyridine as an antibacterial medication, and tripelenamine as an antihistaminic medication, are currently available on the market and include traces of 2-aminopyridine. The antitumoral, anti-alzheimer, antidiabetic, antimicrobial, antiviral, analgesic, anti-inflammatory, antiparasitic, antimalarial, antihistamine, anticonvulsant, Renin, n-NOS, CXCR1/2, JNK1, PKC, and Syk inhibitors have thus been shown to be present in both simple and complex compounds

containing grafted 2-aminopyridine moiety [1].

Many studies are carried out with salicylic acid (H_2salic) and its derivatives with electron-donating oxygen atoms (-COOH and OH) and its protonated forms ($Hsalic^-$ and $salic^{2-}$). Salicylic acid are one of the well-known hydroxybenzoic acids that have antimicrobial, anti-inflammatory, anti-cancer, anti-tumor, anti-proliferative, anti-viral and analgesic properties [2]. Salicylic acid can bind to metal ion from both carboxylic and hydroxyl group in various modes such as monodentate, bidentate, tridentate, pentadentate and bridging [3-10]. In addition, copper(II), manganese(II) and zinc(II) complexes of salicylic acid and its derivatives have potential for treating cancer [11,12]. Proton transfer salts and metal complexes containing salicylic acid and organic bases have been synthesized in the literature [13-18]. The salts of 2-aminopyridine derivatives and salicylic acid have been synthesized, but the metal complex has not been synthesized [19-27].

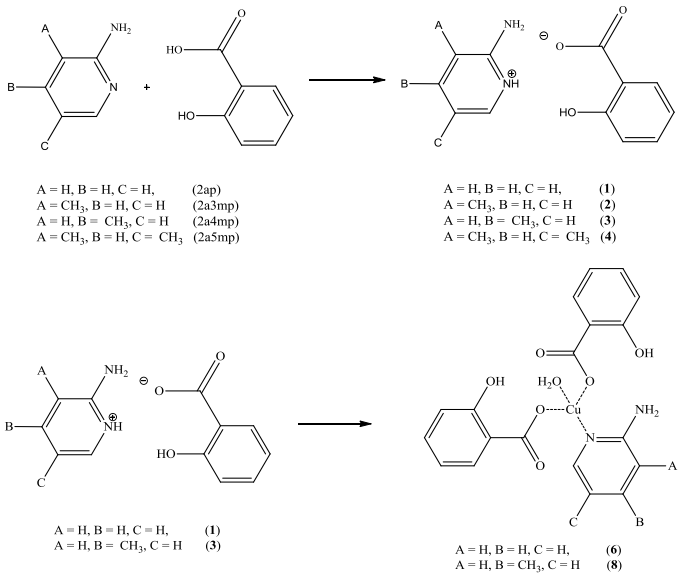
2. Experimental

*2.1. Preparation of **1-4** and **6-9**.*

The Cu(II) complex of H_2salic ($[Cu(Hsalic)_2(H_2O)]$, **5**) was obtained by the method available in the literature [11].

5 mmol H_2salic (2.3123 g) and 5 mmol 2-aminopicoline (**1** for 2ap, **2** for 2a3mp, **3** for 2a4mp and **4** for 2a5mp) dissolved in 100 mL of ethanol. The white amorphous solids were procured by stirring for three days (70% yield for **1**, 80% yield for **2**, 75% yield for **3** and 80% yield for **4**) (Fig. 1).

5 mmol Copper(II) acetate monohydrate and 5 mmol salt {**1** for **6**, **2** for **7**, **3** for **8** and **4** for **9**) was dissolved in ethanol:water solution (2:1) (75 mL) with stirring one week. Green amorphous solids (75% yield for **6**, 65% yield for **7**, 70% yield for **8** and 60% yield for **9**) were obtained from the mixtures (Fig. 1).



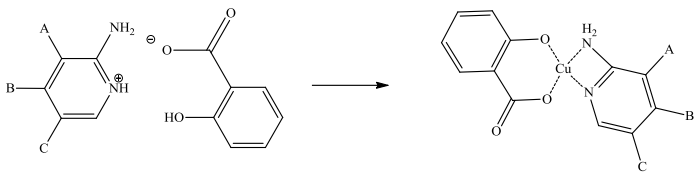


Fig. 1. The structures of compounds **1-4** and **6-9**.

2.2. Antimicrobial study

The antimicrobial properties of the substances were evaluated using a microbroth dilution susceptibility test. Dimethyl sulfoxide was used in the preparation of stock solutions. 4 mg of all compounds were taken and each dissolved in 2 mL of dimethyl sulfoxide. 10^8 Colony Forming Units/mL in double-strength Mueller-Hinton broth. Subsequently, 100 µL of each microbial suspension was added to the wells. A control well without any microorganisms was included for comparison. The growth medium and sterile distilled water served as positive controls. Following 18-24 hours of incubation at 37 °C, the well displaying no turbidity first was identified as the Minimum Inhibitory Concentration (MIC).

3. Results and discussion

3.1. Elemental analysis and AAS results

According to the elemental analysis and AAS results of **6-9**, the metal:H_2salic:aminopyridine ratio was found to be 1:1:2 for **6** and **8** and 1:1:1 for **7** and **9** (Table 1).

Table 1. Elemental analysis and ICP-OES results of the studied substances.

Compound	**Formula**	**Found% Anal. Cald.%**			
		C	**H**	**N**	**Cu^{2+}**
1	$C_{12}H_{12}N_2O_3$	62.10(62.06)	5.20(5.21	12.10(12.06)	-
2	$C_{13}H_{14}N_2O_3$	63.45(63.40)	5.70(5.73)	11.41(11.38)	-
3	$C_{13}H_{14}N_2O_3$	63.42(63.40)	5.71(5.73)	11.39(11.38)	-
4	$C_{13}H_{14}N_2O_3$	63.44(63.40)	5.75(5.73)	11.35(11.38)	-
6	$C_{19}H_{18}CuN_2O_7$	50.75(50.72)	4.05(4.03)	6.20(6.23)	14.10(14.12)
7	$C_{13}H_{12}CuN_2O_3$	50.75(50.73)	3.90(3.93)	9.15(9.10)	20.60(20.65)
8	$C_{20}H_{20}CuN_2O_7$	50.76(51.78).	4.30(4.35)	6.00(6.04)	13.75(13.70)
9	$C_{13}H_{12}CuN_2O_3$	50.78(50.73)	3.96(3.93)	9.08(9.10)	20.60(20.65)

*3.2. Thermal analyses of **6-9.***

TG-DTG and DTA curves and values of **6-9** are given in Figs 3-5, respectively, and Table 2. Results of thermal analyses are similar to Cu(II) complexes of salicylic acid in the literature [28].

Compounds **6**, **7** and **9** thermally decomposed in two steps. The endothermic first stage corresponds to the loss of H_2O, C_3H_3 and 2a5mp units and the exothermic second stage corresponds to the loss of the 2Hsalic+2ap,

$C_{13}H_{12}N_2O_3$ and Hsalic units, respectively.

Compound **8** thermally decomposed in three steps. The endothermic first stage corresponds to the loss of one mole of water molecules. The endothermic second stage is consistent with the loss of 2a4mp. The exothermic third stage shows the loss of Hsalic. The final product left undecomposed is CuO for **6-9**.

3.3. FT-IR results

The IR data of **1-9** are given in Table 3. There are broad vibration peaks between 3500 and 3437 cm^{-1} attributed to the ν(O-H) vibrations of **1-5**, **6** and **8**. Bands appearing at 3285 and 3202 cm^{-1} for **1**, 3377 and 3325 cm^{-1} for **2**, 3316 and 3294 cm^{-1} for **3**, 3320 and 3293 cm^{-1} for **4**, 3401 and 3341 cm^{-1} for **6**, 3405 and 3320 cm^{-1} for **7**, 3322 and 3291 cm^{-1} for **8** and 3420 and 3330 cm^{-1} for **9**, are assigned to NH_2 vibrations. The $\nu(N^+–H)$ peaks observed in the range 3529-2745 cm^{-1} for **1-4** were not observed in the complexes (**6-9**). The difference (Δυ) between the extensions of the asymmetric/symmetric vibrations of the COO^- group shows how it coordinates to the metal ion. The differences of (**5-9**) were calculated 199 (1601 and 1437 cm^{-1}), 208 (1674 and 1466 cm^{-1}), 213 (1662 and 1449 cm^{-1}), 219 (1667 and 1448 cm^{-1}) and 200 (1636 and 1436 cm^{-1}), respectively. These results indicate that the carboxylate group is monodentate bound to the metal ion [29]. The peaks at the range of **1-9**, 3046-3104 cm^{-1}, 2783-2981 cm^{-1}, 1408-1647 cm^{-1}, 1075-1390 cm^{-1}, 748-757 cm^{-1}, 533-593 cm^{-1} and 426-450 cm^{-1} are assigned $\nu(C–H)_{ar.}$, $\nu(C–H)_{alp.}$, ν(C=N)/ ν(C=C) (except **5**), ν(C-O), ν(py) (except **5**), ν(M-O) (except **1-4**) and ν(M-N) (except **1-5**), respectively.

Table 2. Thermal analyses results of compounds **6-9**.

Compound	Temperature (ºC)	DTG_{max} (ºC)	Leaving Group	Found (%)	Calculated (%)
6	30-148	131	H_2O	4.00	4.00
	148-550	205, 239, 322	2Hsalic+2ap	81.88	81.80
	-	-	Cu	14.12	14.20
7	30-275	255	C_3H_3	13.02	13.00
	275-650	288	$C_{13}H_{12}N_2O_3$	66.33	66.30
	-	-	Cu	20.65	20.70
8	30-150	120	H_2O	3.88	4.10
	150-215	201	2a4mp	23.29	23,30
	215-700	259, 304, 340	2Hsalic	59,13	58,70
			Cu	13.70	13.90
9	30-317	267, 289, 314	2a5mp	35.13	35.20
	317-600	440	Hsalic	44,22	44.1
			Cu	20.65	20.70

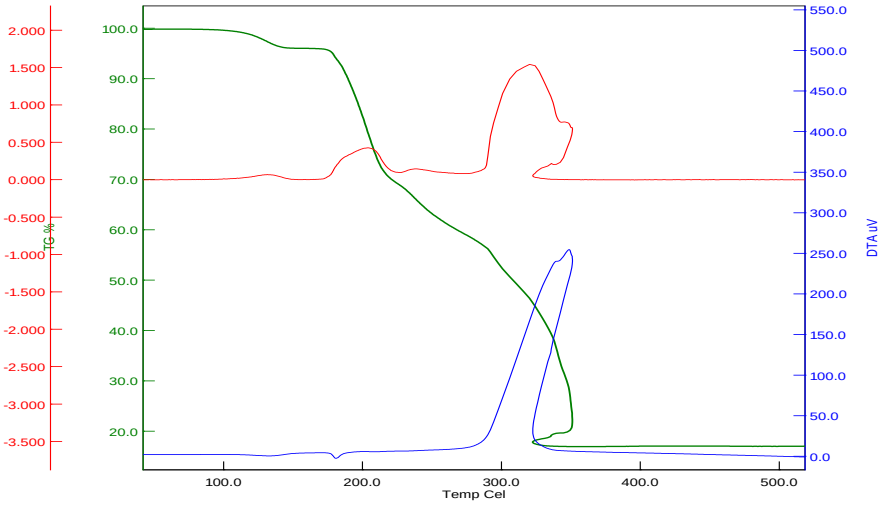


Fig. 2. Thermal analysis results of **6**.

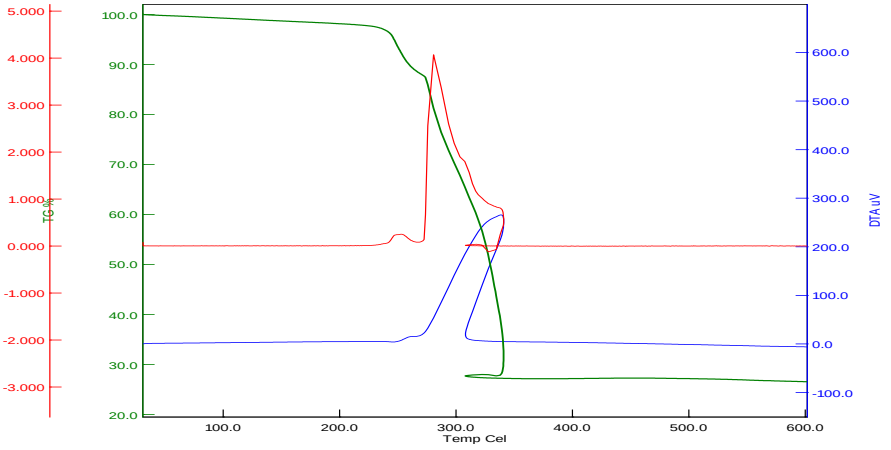


Fig. 3. Thermal analysis results of **7**.

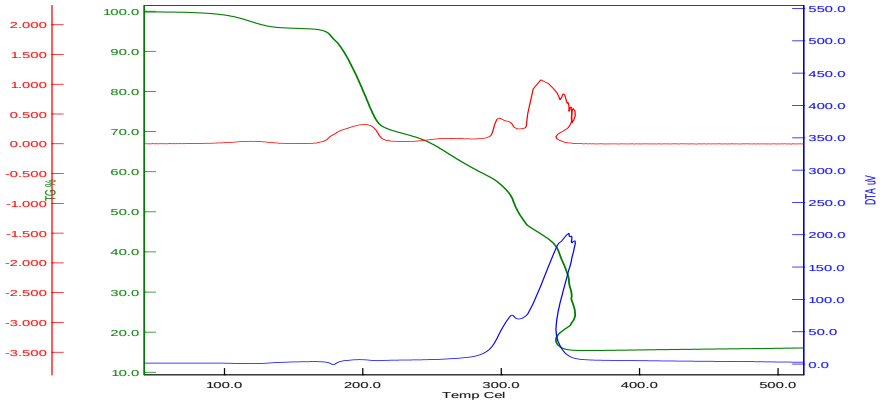


Fig. 4. Thermal analysis results of **8**.

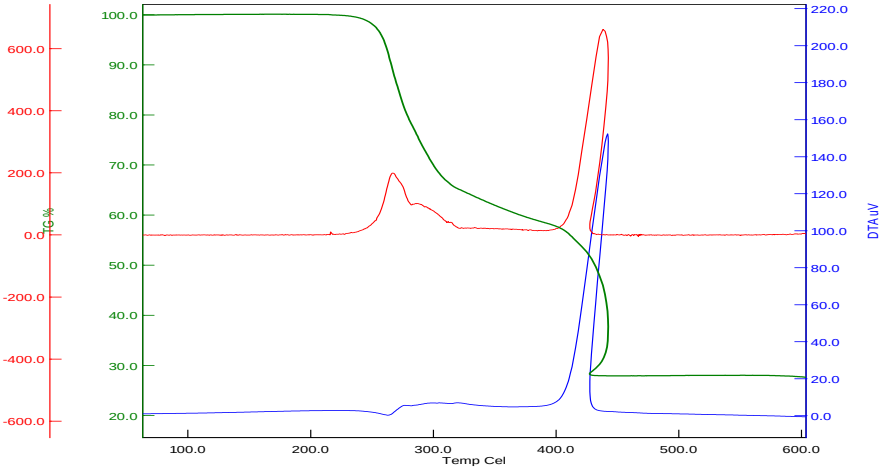


Fig. 5. Thermal analysis results of **9**.

Table 3. IR data of all compounds (cm^{-1}).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
ν(O–H)	3450(br)	3500(br)	3420(br)	3425(br)	3550(br)	3437(br)	-	3437(br)	
ν(N–H)	3285(m)	3377(m)	3316(m)	3320(m)	-	3401(m)	3405(m)	3322(m)	3420(m)
	3202(m)	3325(m)	3294(m)	3293(m)		3341(m)	3320(m)	3291(m)	3330(m)
$\nu(C–H)_{ar.}$	3046(w)	3087(w)	3082(w)	3056(w)	3063(w)	3104(w)	3055(w)	3082(w)	3060(w)
$\nu(C–H)_{alp.}$	-	2977(w)	2981(w)	2956(w)	-	-	2974(w)	2971(w)	2959(w)
		2918(w)	2870(w)	2912(w)			2923(w)	2881(w)	2891(w)
		2876(w)	2821(w)	2878(w)			2855(w)	2794(w)	2783(w)
$\nu(N^{+}–H)$	2706(w)	2709(w)	2717(w)	2745(w)	-	-	-	-	-
	2545(w)	2529(w)	2523(w)	2560(w)					
ν(C=O)	1670(s)	1667(s)	1666(s)	1659(s)	1601(s)	1674(s)	1662(s)	1667(s)	1636(s)
					1402(s)	1466(s)	1449(s)	1448(s)	1436(s)
ν(C=N)	1647(s)	1635(s)	1642(s)	1623(s)	1557(s)	1627(s)	1640(s)	1609(s)	1601(s)
ν(C=C)	1613(s)	1575(s)	1606(s)	1578(s)	1487(s)	1596(s)	1602(s)	1584(s)	1562(s)

	1588(s)	1480(s)	1579(s)	1553(s)	1470(s)	1568(s)	1560(s)	1553(s)	1516(s)
	1553(s)	1451(s)	1552(s)	1478(s)	1434(s)	1526(s)	1505(s)	1525(s)	1501(s)
	1479(s)		1478(s)	1446(s)		1488(s)	1471(s)	1479(s)	1484(s)
	1451(s)		1448(s)			1443(s)	1408(s)		1456(s)
ν(C-O)	1374(s)	1380(s)	1378(s)	1378(s)	1331(s)	1379(s)	1388(s)	1379(s)	1390(s)
	1248(s)	1250(s)	1248(s)	1252(s)	1240(s)	1225(s)	1261(s)	1249(s)	1250(s)
	1105(s)	1075(s)	1138(s)	1135(s)	1155(s)	1143(s)	1140(s)	1138(s)	1153(s)
ν(py)	753(s)	757(s)	756(s)	750(s)	-	748(s)	753(s)	757(s)	753(s)
ν(M-O)	-	-	-	-	582(w)	533(w)	593(w)	551(w)	562(w)
ν(M-N)	-	-	-	-	-	426(w)	435(w)	450(w)	434(w)

3.4. Results of UV/Vis measurements

The electronic spectra of compounds **1-4** (Fig. 6) and **6-9** (Fig. 7) were registered in DMSO. π-π* and n-π* transitions are shown 317 nm (38640 $Lmol^{-1}cm^{-1}$) and 309 nm (36410 $Lmol^{-1}cm^{-1}$) for **1**, 320 nm (38640 $Lmol^{-1}cm^{-1}$) and 314 nm (37030 $Lmol^{-1}cm^{-1}$) for **2**, 324 nm (43400 $Lmol^{-1}cm^{-1}$) and 315 nm (39720 $Lmol^{-1}cm^{-1}$) for **3**, 308 nm (48170 $Lmol^{-1}cm^{-1}$) and 302 nm (43400 $Lmol^{-1}cm^{-1}$) for **4**, 414 nm (01170 $Lmol^{-1}cm^{-1}$) and 317 nm (40390 $Lmol^{-1}cm^{-1}$) for **6**, 412 nm (01390 $Lmol^{-1}cm^{-1}$) and 318 nm (45160 $Lmol^{-1}cm^{-1}$) for **7**, 413 nm (01320 $Lmol^{-1}cm^{-1}$) and 316 nm (32490 $Lmol^{-1}cm^{-1}$) for **8**, 413 nm (01320 $Lmol^{-1}cm^{-1}$) and 316 nm (32490 $Lmol^{-1}cm^{-1}$) for **9**. The d-d transitions are shown at 769 nm (170 $Lmol^{-1}cm^{-1}$) for **6**, 750 nm (160 $Lmol^{-1}cm^{-1}$) for **7**, 750 nm (180 $Lmol^{-1}cm^{-1}$) for **8** and 750 nm (180 $Lmol^{-1}cm^{-1}$) for **9** [30].

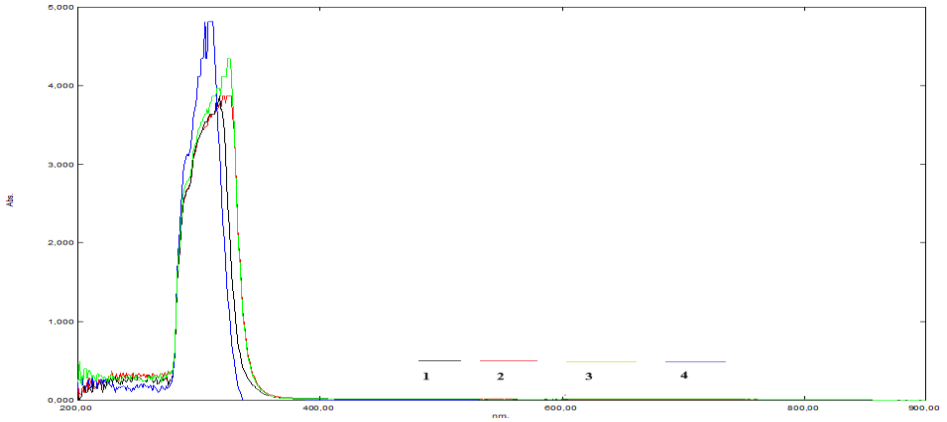


Fig. 6. UV–Vis spectra of compound **1-4**.

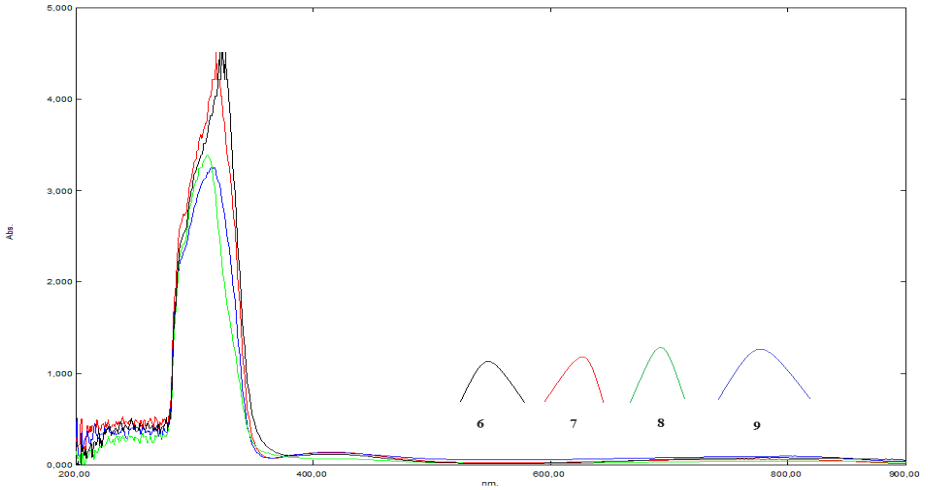


Fig. 7. UV–Vis spectra of compound **6-9**.

3.5. Magnetic susceptibilities

Magnetic susceptibility results of Cu(II) complexes (**6-9**) were found between 1.60, 1.63, 1.61 and 1.65 BM, respectively. These values say that there are unpaired electrons in the complexes. The magnetic moment for the Cu(II) ion obtained in the tetrahedral geometry is also consistent with this value [31,32].

3.6. Antimicrobial results of compounds.

The antimicrobial activity of Fluconazole, Levofloxacin, Cefepime, Vancomycin, copper(II) acetate, free ligands and **1-9** were investigated by microdilution method. All compounds showed activity against bacteria and yeast. MIC values of anti-fungal and anti-microbial agents, all compounds are given in Table 4. Activity values are similar to 2-aminopyridine found in the literature [33-36].

The antifungal drug and substances have activity against *C. albicans* when MIC values are compared; copper(II) acetate observed greater activity than according to Fluconazole while H_2salic, 2ap, 2a3mp, 2a5mp, **2**, **4**, **6** and **7** showed equal effective. Other compounds were found to have a lower degree of action.

All antibacterial drugs and substances have activity against *L. monocytogenes*; when MIC values are compared; all compounds indicated greater activity than (except **2** and **6**) according to Vancomycin {H_2salic, 2a3mp and **7** > copper(II) acetate, 2ap, 2a4mp, 2a5mp, **1**, **3-5**, **8** and **9** > **2** and **6** }. H2salic, 2a3mp and **7** exhibited comparable efficacy in relation to Levofloxacin and Cefepime, while demonstrating lower effectiveness compared to other compounds.

B. subtilis; all compounds showed greater activity than according to Vancomycin {2a5mp > **5** and **8** > copper(II) acetate, H_2salic, 2ap, 2a3mp, 2a4mp, **2**, **4**, **6**, **7** and **8** > **1** and **3** }. 2a5mp, **5** and **8** showed greater activity than according to Levofloxacin and Cefepime while copper(II) acetate, H_2salic, 2ap, 2a3mp, 2a4mp, **2**, **4**, **6**, **7** and **8** showed equally effective. Other compounds were found to have a lower degree of according to Levofloxacin and Cefepime.

E. coli; 2ap, 2a3mp, 2a4mp and **7** indicated greater activity than according to Cefepime while copper(II) acetate, H_2salic, 2a5mp, **4**, **5**, **8** and **9** showed similar effective . Compounds **1-3** and **6** were found to have a lower degree of according to Cefepime. 2ap indicated greater activity than according to Vancomycin and Levofloxacin while 2a3mp, 2a4mp and **7** showed equal effective. The other compounds seen lower degree of according to Vancomycin and

Levofloxacin.

S. aureus: while **7** and **9** showed equally effective, the other compounds were found to have a lower degree of according to Levofloxacin and Vancomycin. **7** and **9** showed greater activity than according to Cefepime while the other compounds (except **3**) showed equally effective. Compound **3** was found to have a lower degree of according to Cefepime.

E. faecalis; **8** displayed analogous performance with respect to Cefepime, whereas other substances showed a diminished level of effectiveness {copper(II) acetate, H_2salic, 2ap, 2a3mp, 2a4mp, 2a5mp, **1**, **3-5**, **7** and **9** > **2** and **8**}. While **8** showed equally effective according to Levofloxacin and Vancomycin, the other compounds (except **2** and **6**) showed equally effective. Compounds **2** and **6** was found to have a lower degree of according to Cefepime.

P. aeruginoa; 2a4mp, H_2salic and **9** showed greater activity than according to Vancomycin while copper(II) acetate, 2ap, 2a3mp, 2a5mp, **4**, **6** and **7** equally effective. Compounds **1**-**3**, **5** and **8** were found to have a lower degree of according to Vancomycin. 2a4mp demonstrated superior efficacy compared to Cefepime and Levofloxacin, while 9 proved to be equally potent. Other compounds exhibited a lesser degree of effectiveness in relation to Cefepime and Levofloxacin.

Table 4. MIC values of compounds (µg/mL).

Compound	***C. albicans***	***L. monocytogenes***	***B. subtilis***	***E. faecalis***	***S. aureus***	***E. coli***	***P. aeruginoa***
Vankomisin	-	125.00	250.00	62.50	31.25	31.25	62.50
Levoflaksasn	-	31.25	62.50	62.50	31.25	31.25	31.25
Sefepim	-	31.25	62.50	31.25	62.50	62.50	31.25
Fluconazole	62.50	-	-	-	-	-	-
copper(II) acetate	31.25	31.25	62.50	62.50	62.50	62.50	62.50
H_2salic	62.50	31.25	62.50	62.50	62.50	62.50	31.25
2ap	62.50	62.50	62.50	62.50	62.50	15.60	62.50
2a3mp	62.50	31.25	62.50	62.50	62.50	31.25	62.50
2a4mp	125.00	62.50	62.50	62.50	62.50	31.25	15.60
2a5mp	62.50	62.50	7.80	62.50	62.50	62.50	62.50
1	125.00	62.50	125.00	62.50	62.50	125.00	125.00
2	62.50	125.00	62.50	125.00	62.50	125.00	125.00
3	125.00	62.50	125.00	62.50	125.00	125.00	125.00
4	62.50	62.50	62.50	62.50	62.50	62.50	62.50
5	125.00	62.50	31.25	62.50	62.50	62.50	125.00
6	62.50	125.00	62.50	125.00	62.50	125.00	62.50
7	62.50	31.25	62.50	62.50	31.25	31.25	62.50
8	125.00	62.50	31.25	31.25	62.50	62.50	125.00
9	125.00	62.50	62.50	62.50	31.25	62.50	31.25

4. Conclusions

New four salts (**1**-**4**) between salicylic acid (H_2salic) and 2-amino-X-pyridine {X = (2ap), 3-methyl (2a3mp), 4-methyl (2a4mp) and 5-methyl (2a5mp)}, the Cu(II) complex of H_2salic (**5**) by methods available in the literature and new Cu(II) complexes (**6**-**9**) of **1**-**4** have been synthesized. The structures of **6**-**9** were suggested by elemental

analysis, AAS, FT-IR, UV-Vis and magnetic susceptibility studies. While the metal:acid:base ratio was 1:2:1 for **6** and **8** 1:1:1 for **7** and **9**. The spectroscopic research results indicated that complexes **6–9** have tetrahedral geometries. All compounds showed activity against bacteria and yeast. Compounds copper(II) acetate for *C. Albicans*, copper(II) acetate, H_2salic, 2a3mp and **7** for *L. monocytogenes*, 2a5mp for *B. subtilis*, **7** and **9** for *S. aureus*, 2ap for *E. Coli*, **8** for *E. Faecalis*, and 2a4mp for *P. aeruginosa* have the best activity.

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