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Synthesis and evaluation of antimicrobial and cytotoxic activities of some Mannich bases bearing amino acid units and their copper complexes

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ABSTRACT

A series of Mannich bases, namely, *N*-(2-hydroxynaphthalen-1-ylmethyl)-L-amino acids (ligands, I-VII) have been prepared from condensation reaction of 2-naphthol with the proper amino acids including glycine, alanine, valine, leucine, methionine, phenylalanine, and tyrosine in the presence of formaldehyde. The corresponding copper (II) complexes (VIII-XIV) have been synthesized and obtained in a moderate yields. The structures of all new products were confirmed by elemental analyses of C, H, N and spectroscopic methods. All compounds were screened for their antimicrobial activity against some Gram- positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Micrococcus luteus* (ATCC 6635), Gram – negative bacteria: *Pseudomonas fluorescens* (S 97) and *Salmonella typhimurium* (ATCC 14028), Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*. The comparison of antimicrobial activities of the ligands and complexes proved that the presence of copper metal causes more inhibition i.e., more activity, especially against *Staphylococcus aureus* and *Candida albicans*. The cytotoxic activity of selected two copper complexes was also evaluated against the Mammalian cell line: HEpG2.

Keywords: *2-naphthol, amino acids, copper complexes, biological activity.*

1. INTRODUCTION

Nitrogen containing biologically active compounds are of high interests as pharmaceutical products [1, 2]. The Mannich reaction is one of the most basic and useful methods for the synthesis of such compounds and has been widely used to introduce aminomethyl group into a variety of organic compounds. Mannich base derivatives with bridge *N*-atom have been found to possess a broad range of biological activities including antimicrobial [3-8], antitumor [9-11], antiinflammatory [12,13], antipyretic and antimalarial [14-16] and some of them were used as complexing agents [17,18]. Also, Mannich bases of various bioactive compounds have been prepared as prodrugs as a means of overcoming some of their limitations [19-21]. At the present time, metal based drugs are an important source of novel molecules with therapeutic activity [22]. Furthermore, coordination compounds with copper core have been investigated in the last few decades after the discovery of cisplatin because they have shown good antineoplastic activity and less toxicity [23, 24]. Literature survey shows that a large number of copper complexes of amino acids have been found to be associated with diverse pharmacological activities [25-27]. Complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes, such as oxygen conveyer, electron transfer and oxidation. In these processes, the enzymatic active site, which is very specific, forms complexes with divalent metal ions [28]. In view of these points and in continuation of our work on structure-activity relationship (*SAR*) of amino acid and simple peptide derivatives [29, 30], we report herein the synthesis, structural characterization and antimicrobial and cytotoxic activities of some amino acid Mannich base derivatives of 2-naphthol and their corresponding copper (II) complexes.

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2. EXPERIMENTAL SECTION

2.1. General procedure for the synthesis of Mannich bases, *N*-(2-hydroxynaphthalen-1-ylmethyl)-L-amino acids (I-VII). *N*-(2-hydroxynaphthalen-1-ylmethyl)-L-amino acids have been prepared according to a previously reported method [31] with a minor modification as follows: to a solution of 2-naphthol (0.02 mol) in 70 mL ethanol (80%), proper amino acid (0.021 mol) and formaline (40%, 2.8 mL) were added with stirring for 0.5 h, then the reaction mixture was refluxed for 8-10 h. The crude product was collected by filtration, washed several times with hot dist. water, hot ethanol, dried and then recrystallized from DMF. All the products are chromatographically pure and gave negative ninhydrin spot reaction.

2.2. General procedure for the synthesis of Cu(II) complexes of Mannich bases, *N*-(2-hydroxynaphthalen-1-ylmethyl)-L-amino acids (VIII-XIV). $Cu(OAc)_2.H_2O$ (0.001 mol) dissolved in 20 mL abs. ethanol was poured dropwise into a suspension containing Mannich base (ligand, 0.001 mol) in 30 mL abs. ethanol under vigorous stirring for 15 min. and then heated under reflux for 6 h. Precipitation of products take place after heating time of 15-40 min. The colored solid product was collected by filteration, washed several times with hot abs. ethanol until the washing becomes colorless, and then vacuum dried. Purity of the products was detected by TLC and all of them are chromatographically pure.

2.3. Aparatus. Melting points are uncorrected and meassured on electric melting point apparatus SMP1. The infrared spectra (ν_{max} in cm^{-1}) were taken in KBr discs using a Perkin Elmer spectrophotometer 57928 RXIFT-IR system. The absorption in the UV-Vis region was recorded by a Perkin Elmer Lambda 35 Spectrophotometer using DMF as solvent. The mass spectra were performed using Direct Inlet unit (DI-50) of Shimadzu GC/MS-QP5050A. The IR and UV/Vis spectral analysis were carried out at Faculty of Science, Al-Azhar University, Cairo, Egypt. The mass spectra, elemental analyses of C, H, and N, and cytotoxic activity study were carried out at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

2.4. Antimicrobial properties. Microbiology screening was carried out at Department of Botany, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. The standardized disc–agar diffusion method [35, 36] is followed to determine the activity of the synthesized compounds against the tested microorganisms. Test Organisms. Cultures of the following microorganisms are used in the test: Gram- positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Micrococcus luteus* (ATCC 6635), Gram – negative bacteria: *Pseudomonas fluorescens* (S 97) and *Salmonella typhimurium* (ATCC 14028), Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*.

2.5. Screening for the antimicrobial potential:

2.5.1. Preparation of tested compounds. The tested compounds (I-XIV) were dissolved in dimethyl formamide (DMF) solvent and prepared in one concentration of 50 mg/mL and then 10 µl of each preparation was dropped on disk of 6 mm in diameter, the concentration becaming 0.5 mg/disk.

2.5.2. Testing for anti-bacterial activity. Bacterial cultures were grown in nutrient broth medium at 30°C. After 16 h of growth, each microorganism, at a density of 10^8 cells/mL, was inoculated on the surface of Mueller-Hinton agar plates using sterile cotton swab. Subsequently, uniform size filter paper disks (6 mm in diameter) were impregnated by an equal volume (10 µl) from the specific concentration of dissolved compounds and carefully placed on surface of each inoculated plate. The plates were incubated in the upright position at 36ºC for 24 h. Three replicates were carried out for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of dissolved compound was carried out as negative control. After incubation, the diameters

of the growth inhibition zones formed around the disk were measured with a transparent ruler and expressed in millimeters, averaged and the mean values being then tabulated.

2.5.3. Testing for anti-fungal activity Active inocula for experiments were prepared by transferring many loopfuls of spores from the stock cultures to test tubes of sterile distilled water (SDW), which were agitated and diluted with sterile distilled water to achieve an optical density corresponding to $2.0x10^5$ spores/mL. An inoculum of 0.1 % from the obtained suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes then the same procedure was followed as described above.

2.5.4. Standard references. The Chloramphencol antibiotic was used as standard reference in the case of Gram – negative bacteria, Cephalothin in the case of Gram – positive bacteria and Cycloheximide antifungal was used as standard reference in the case of yeasts and fungi.

2.5.5. Activity index. The activity of tested compounds were classified as follows: Low activity = Mean of zone diameter ≤ 1/3 of mean zone diameter of control; Intermediate activity = Mean of zone diameter ≤ 2/3 of mean zone diameter of control; High activity = Mean of zone diameter > 2/3 of mean zone diameter of control.

2.6. Evaluation of Cytotoxic Effects. Mammalian cell line HEp G2 (human cell line of a well differentiated hepatocellular carcinoma isolated from a liver biopsy of a male Caucasian aged 15 years) was obtained from the American Type Culture Collection (ATCC).

2.6.1. Chemicals Used. Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). DMEM, RPMI-1640, FBS, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from (Biowhittaker Lonza, Belgium). Crystal violet stain (1 %) composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH_2O and filtered through a Whatmann No.1 filter paper.

2.6.2. Cytotoxicity assay. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/mL gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week. Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability.

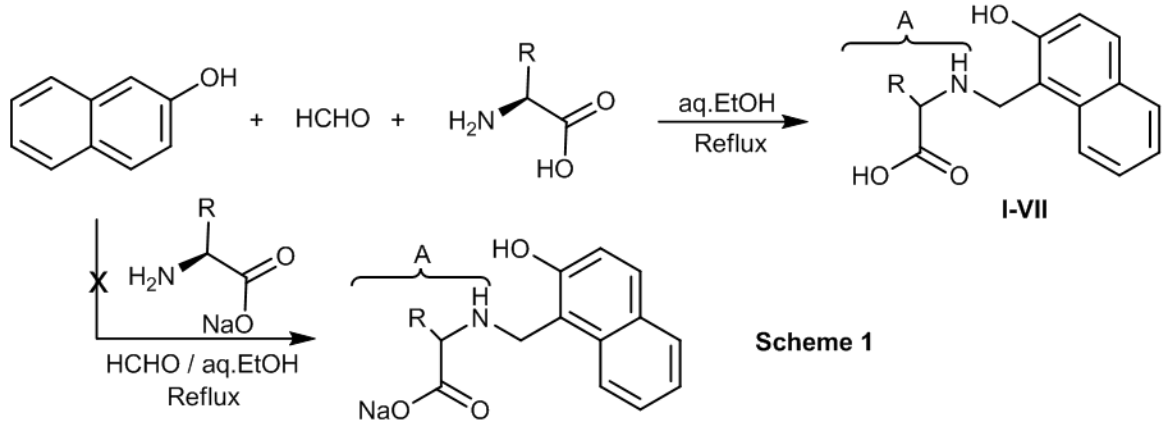
2.6.3. Cytotoxicity evaluation using viability assay. For cytotoxicity assay, the cells were seeded in 96-well plate at a cellular density of $1x10^4$ cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.01%) was found not to affect the experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples

were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compounds was calculated [39, 40].

2.7. Measurement of minimal inhibition concentration (MIC). MIC values of the synthesized compounds were determined using agar dilution technique [37]. Each compound with high or intermediate antimicrobial effect shown in the disk diffusion test was further diluted with DMF to 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 mg/mL respectively. Then 100 µl of each diluted compound was mixed with 10 mL of cooled (50 ºC) melted Mueller-Hinton agar and then plated into 6 cm sterile Petri dish. Each dilution was prepared in duplicate. The concentrations of the compounds became 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL respectively. Each concentration was prepared for 2 dishes. All plates were incubated at 33 ºC for 24 h. MIC of each compound was measured from the plate with the lowest concentration with no visible growth of specific organism.

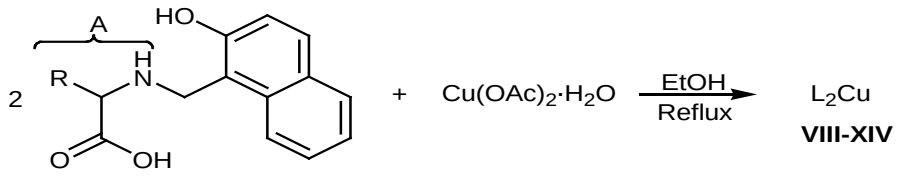
3. RESULTS SECTION

3.1. Synthesis and spectral characterization: In this work, the synthesis of seven Mannich bases (I-VII, Scheme1), three of them are new, has been achieved by following the procedure previously described [31], using longer reaction time. This slightly modified procedure involved the condensation reaction of 2-naphthol with free amino acid in presence of formaldehyde in an aqueous ethanol under reflux conditions for 8-10 h. Washing the crude products repeatedly with hot water followed by hot ethanol is necessary to complete the removal of the unreacted amino acid and 2-naphthol respectively. It has been observed that the yield obtained by this modified procedure is better than that previously obtained. On the other hand, an attempt to improve the overall yield of the reaction products and simplify its purification step by using the sodium salt of amino acids instead of free amino acids was unfruitful. All synthesized *N*-(2-hydroxynaphthalen-1-ylmethyl)-L-amino acids using the procedure described above were summarized in Scheme 1.



Scheme 1

A simple amino acid anion is a potential bidentate ligand which may coordinate to a transition metal ion through the amino lone pair of electrons and the carboxylate oxygen atom. The metallic complexes with amino acids as ligands are deeply investigated due to their capacities of forming chelates, which are used in various domains like ; medicine, chemistry, pharmacy, biology, nutrition and physics. Thus, reaction of Cu(II) acetate with amino acid Mannich bases (I-VII, LH) in 1:1 molar ratio in refluxing ethanol for 6 h, afforded the complexes (VIII-XIV, Scheme 2). The general reaction can be represented by the following equation, in which LH represents the ligands incorporated with the amino acids units.



Scheme 2

All the synthesized complexes are stable at room temperature and are not hygroscopic. On heating, they decompose at high temperature. They are insoluble in water, but soluble in DMF and DMSO. Several analytical techniques were used to characterize the complexes including microanalysis (CHN), IR, UV-Vis and mass spectra. The elemental chemical analyses confirmed the metal ion: ligand of 1:2 for composition of all complexes and this can be represented by the general formula CuL_2. Additional information about the copper ion coordination were obtained by comparing the IR frequencies of the ligands with those of the copper complexes. In the Table 2, the most important absorption bands and their assignments were listed. In spectra of the ligands, the ν(N-H) stretching vibrations appear at 3136-3184 cm^{-1}. These bands were shifted toward higher frequencies in the spectra of the corresponding complexes to 3156-3266 cm^{-1} proving the involvement of the –NH- group in the complex formation [32]. Furthermore, the absorption bands obtained at 1568-1614 cm^{-1} attributed to ν(C=O) stretching vibrations in the spectra of the ligands were shifted toward higher frequencies in the spectra of the complexes to 1609-1630 cm^{-1} indicating the participation of the carboxylate oxygen atom in covalent bonding to the copper ion [33]. The appearance of ν(O–H) stretching vibrations in the spectra of both ligands and complexes at 3394-3492 cm^{-1} revealed also the involvement of the phenolic oxygen atom in the covalent bonding to the metal ion. The non ligand bands occurring in the 494-503 cm^{-1} regions were assigned to *ν*(Cu–O). The IR data were summarized in Table 1.

Table 1: Fundamental infrared bands (in cm^{-1}) of the synthesized compounds (I-XIV)

Compd No.	OH	NH	C=O	CH, ali	CH, aro	Compd No.	OH	NH	COO^-	CH, ali	CH, aro
I	3413	3178	1576	2952	3041	VIII	3431	3266	1609	2942	3070
II	3422	3184	1614	2948	3062	IX	3424	3232	1628	2966	3087
III	3446	3154	1568	2920	3073	X	3412	3158	1630	2952	3068
IV	3434	3146	1576	2958	3058	XI	3396	3156	1628	2952	3060
V	3492	3154	1614	2985	3056	XII	3454	3226	1624	2914	3064
VI	3406	3168	1614	2966	3053	XIII	3413	3413*	1622	2967	3072
VII	3394	3136	1610	2964	3022	XIV	3419	3229	1620	2919	3063

*The ν(N-H) stretching vibration interfered with broad OH band.

UV-Vis spectra further confirmed the anticipated structures. The UV spectra of ligands in DMF showed two absorption bands at ~228 and 255-259 nm assigned to intraligands transitions n→π* and π→π*. In the spectra of complexes, these bands were shifted to ~ 238 and ~277nm respectively due to coordination. The absorption spectra in the visible domain displayed a wide band centered at 664-676 nm, observed only at higher concentrations of the complexes, was assigned to the d-d transition characteristic of the distorted octahedral geometry of the copper(II) in the complexes [34]. The mass spectra of ligands and their copper (II) complexes were recorded and they also used to compare their stoichiometry composition. The general feature of the mass spectra is that all compounds, except XII, give the molecular ion peaks, which are further decomposed *via* different routes to give different fragments in support of their proposed structures. For example, the ligand containing the L-tyrosine residue (VII) showed a molecular ion peak at m/e 337, while the molecular ion peak for its

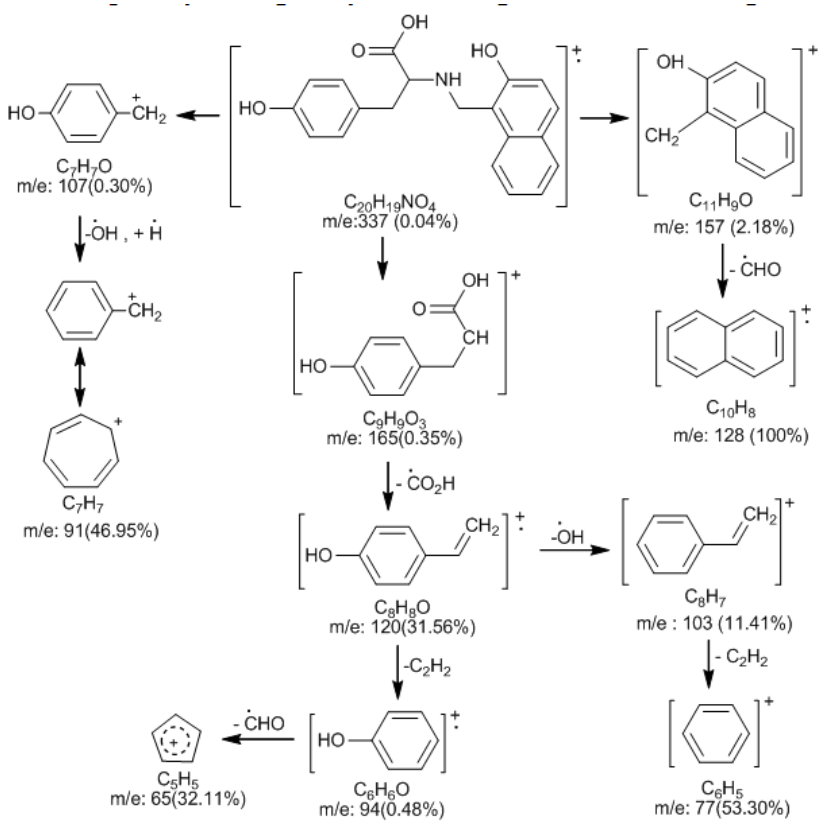
corresponding copper complex (XIV) was observed at m/e 735.5. These data confirmed the stoichiometry of metal chelates as [CuL_2] type. This composition was also supported by the data of mass spectra of other complexes which were collected in Table 2 and some of them presented in Schemes 3-6. They are in good agreement with the microanalytical data. Moreover, it was observed that for some copper complexes, two characteristic peaks obtained at m/e 281 and 292 attributed to $[C_{12}H_{12}CuNO_3]^+$ and $[C_{13}H_{11}CuNO_3]^+$ respectively were noticed confirming their proposed structures.

Table 2: Mass spectra data of the synthesized compounds (I-XIV)

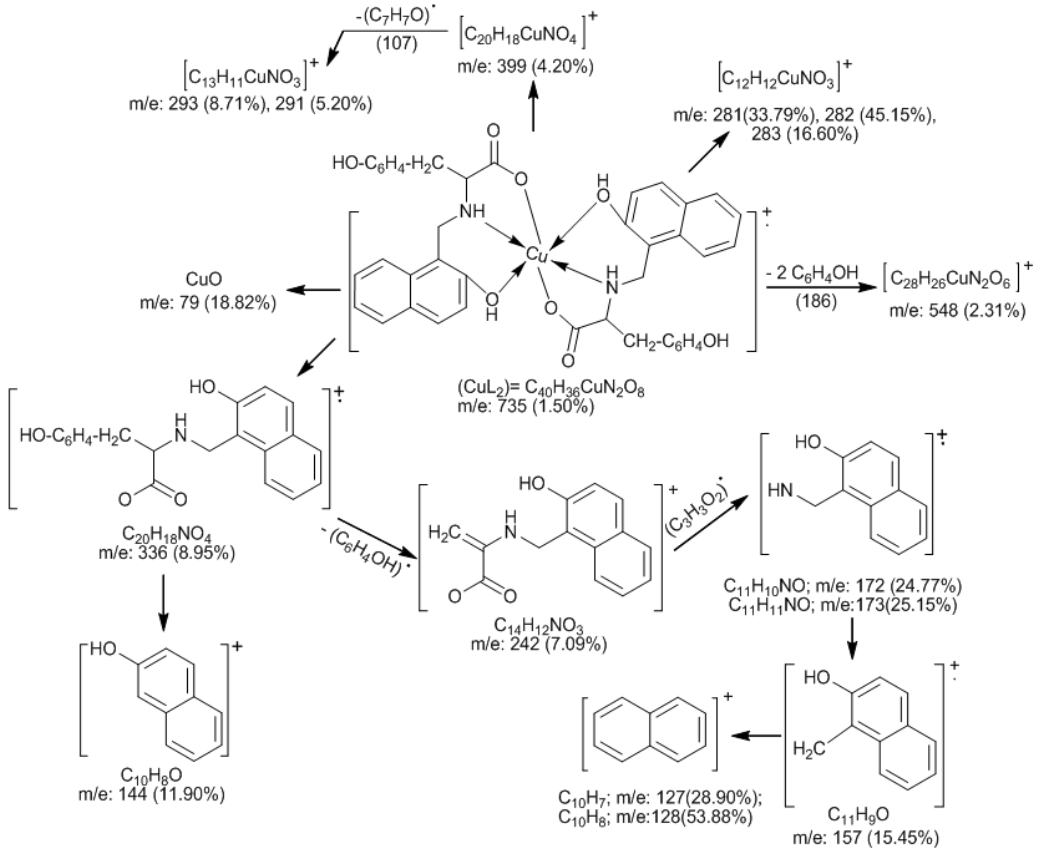
Compd. No.	fragments, m/e (%)
III	273(M^+, 17.74), 258(8.97), 257(26.27), 231(10.47), 214(10.64), 157(15.45), 128(22.60), 115(13.89), 102(15.04), 87(35.55), 86(11.99), 50(100).
IV	288(M+1, 0.35), 158(1.18), 156(18.58), 145(1.07), 144(0.56), 128(100), 102(20.59), 130(0.55), 129(7.14), 86(9.48), 85(8.71), 59(0.87).
V	305(M^+,20.02), 307(M+2,4.95), 288(4.87), 257(6.97), 240(17.37), 231(48.91), 212(29.80), 187(6.86), 186(5.01), 144(11.24), 127(11.42), 65(4.55), 49(100).
VI	321(M^+, 2.87), 178(4.20), 158(2.56), 157(3.77), 144(5.70), 134(16.60), 128(18.56), 91(57.38), 77(100), 65(21.55), 51(59.29).
VII	337(M^+, 0.04), 165(0.35), 157(2.18), 128(100), 120(31.56), 107(0.30), 103(11.41), 94(0.48), 91(46.95), 77(53.30), 65(32.11), 50(65.16).
VIII	523 (M^+,1.33), 352(CuL_2-$C_{11}H_9NO$, 1.47), 351(CuL_2-$C_{11}H_{10}NO$, 8.99), 156(1.47), 151(0.84), 128(13.69), 127(12.71), 79(0.84), 78(10.39), 77(100), 75(7.30), 57(9.06), 51(49.44).
IX	551 (M^+, 1.53), 293($C_{13}H_{12}CuNO_3$, 1.13), 292($C_{13}H_{11}CuNO_3$,2.48), 283($C_{12}H_{13}CuNO_3$,11.99), 282($C_{12}H_{12}CuNO_3$,85.40), 280($C_{12}H_{11}CuNO_3$, 2.89), 245(L, 1.01), 201($C_{13}H_{15}NO$, 1.15), 200 ($C_{13}H_{14}NO$, 2.00), 158 (4.55), 144(65.78), 128(66.56), 127(80.41), 79(12.44), 57(94.97), 55(100).
X	607 (M^+, 1.20), 577 ($C_{30}H_{30}CuN_2O_6$, 0.03), 292 (1.10), 282(23.42), 272 (3.45), 257(2.03), 245 (3.86), 158 (8.30), 157(4.16), 144 (0.09), 128(100), 116 (6.31), 87(1.86), 51(12.34).
XI	636 (M+1, 1.66), 292(1.25), 282(2.73), 281(2.68), 257(1.95), 244 ($C_{14}H_{14}NO_3$, 2.84), 200(2.06), 199(1.18), 173 ($C_{11}H_{11}NO$, 1.80), 158(1.48), 157(8.34), 151(100), 144(1.93), 130($C_6H_{12}NO_2$, 10.34), 128(14.93), 127(12.40), 79(10.32), 57(52.86).
XII	407 ($C_{18}H_{20}CuN_2O_5$, 0.50), 367 ($C_{15}H_{16}CuN_2O_5$, 0.67), 366 ($C_{15}H_{15}CuN_2O_5$, 0.44), 303(L, 0.73), 292 (0.60) , 282(49.26), 257(0.59), 256(1.08), 240(3.74), 213(3.64), 157(29.86), 144(100), 128(51.17), 79(1.20).
XIII	701 (M-2, 0.02), 626 ($C_{34}H_{31}CuN_2O_6$, 0.02) , 551($C_{28}H_{28}CuN_2O_6$, 0.02), 321 (L, 0.04), 211 ($C_4H_8CuN_2O_4$, 0.03), 164 (0.6), 128(1.29), 127(6.23), 105(96.75), 91(7.17), 79(0.04), 78(8.69), 77(100), 65(2.65), 51(44.50).
XIV	735 (M^+, 1.50), 548 ($C_{28}H_{26}CuN_2O_6$, 2.31) , 399 ($C_{20}H_{18}CuNO_4$, 4.20), 336($C_{20}H_{18}NO_4$,8.95), 293(8.71), 291(5.20), 283(22.78), 282(45.15), 242 (7.09), 281(33.79), 157(14.37), 128(53.88), 127(28.90), 79(18.82), 54(100).

According to the above-mentioned data and those of elemental analyses, the structure of the metal complexes can be proposed as illustrated in the Fig.1, in which the amino acid Mannich bases are acting as a tridentate ligands with coordination to Cu (II) ion involving the carboxylate oxygen and the nitrogen atom of amino group and phenolic oxygen atom. The analytical data and physical properties of the ligands and their corresponding complexes were summarized in Table 3.

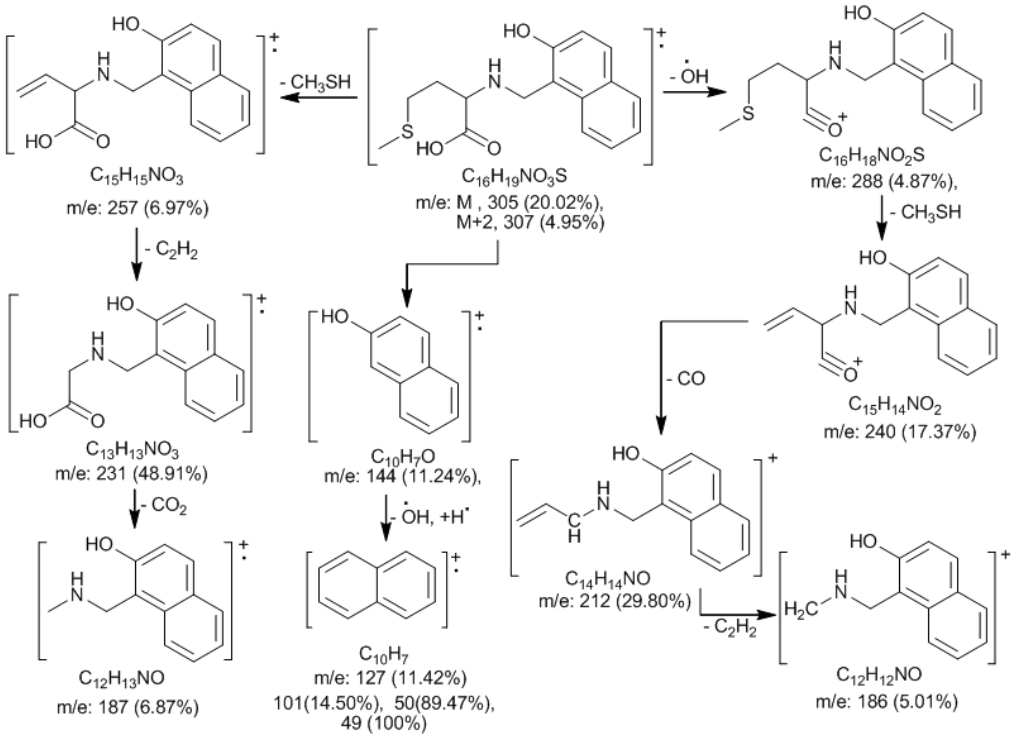
3.2. Antimicrobial activity. This study revealed that the seven amino acid Mannich bases (ligands) displayed activities against only *C. albicans* with an inhibition zone of 4-10 mm and nearly all of them were found to be completely biologically inactive against the remaining tested microorganisms.



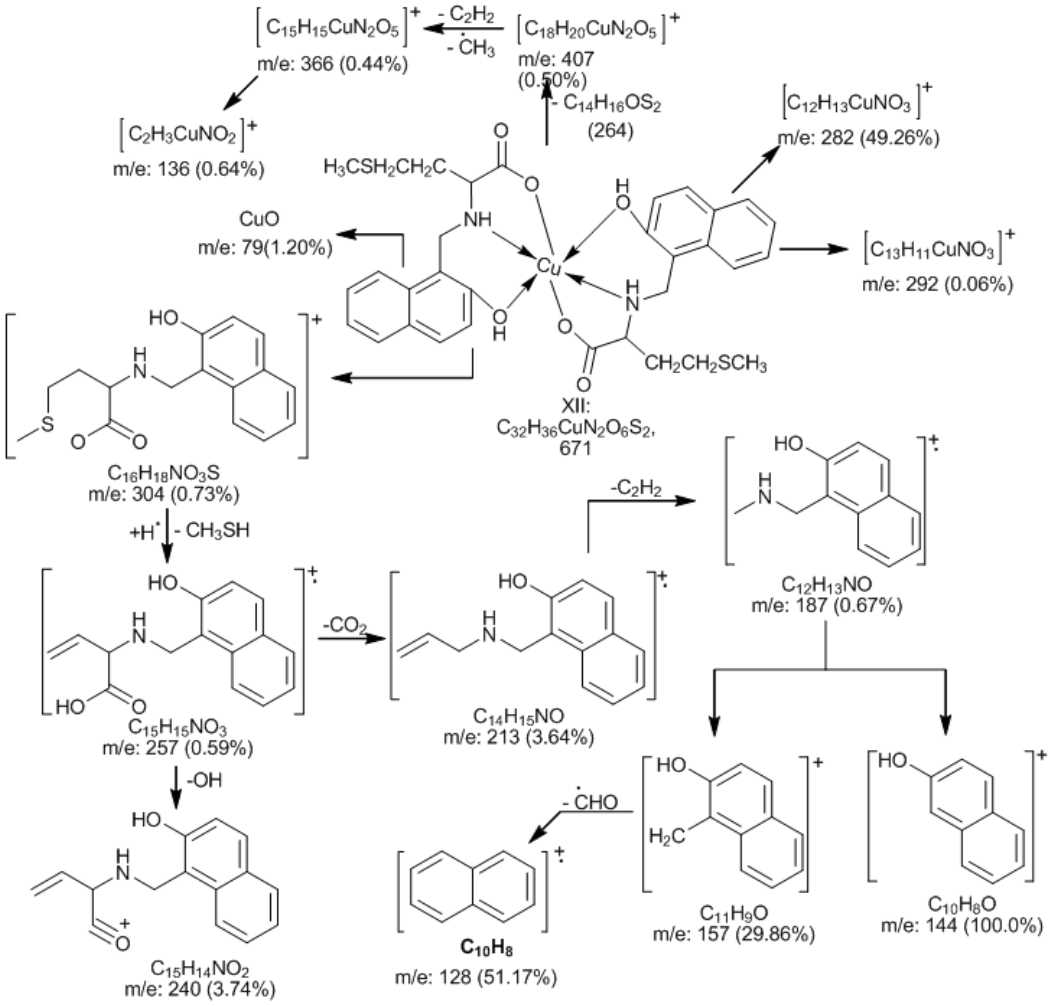
Scheme 3: Mass fragmentation patter of VII



Scheme 4: Mass fragmentation pattern of XIV



Scheme 5: Mass fragmentation pattern of V



Scheme 6: Mass fragmentation pattern of XII

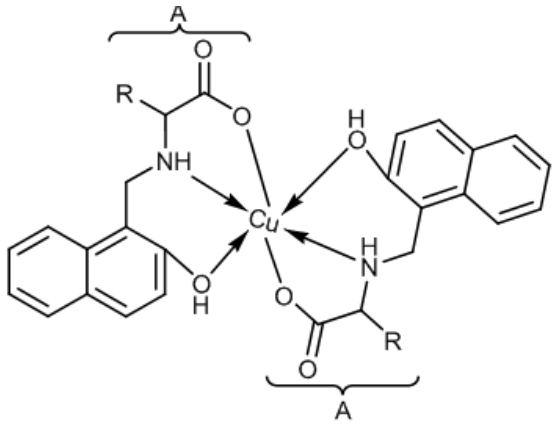


Figure 1: The proposed structure of Cu(II) complexes of amino acid Mannich bases

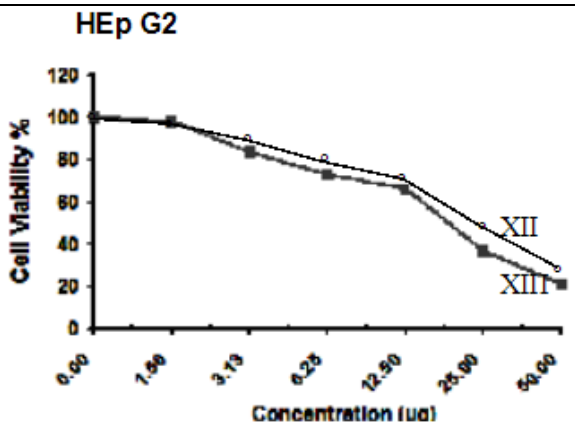


Figure 2: Cell viability (%) of compounds XII, XIII

Table 3: The Physical data of the synthesized compounds (I-XIV)

Compd. No.	A	Yield %	M.P. °C	Color	Mol. Formula	Elemental analyses calc./ found		
						C%	H%	N%
I	Gly	75	228 224-226*	white	$C_{13}H_{13}NO_3$	67.53 67.76	5.63 5.89	6.06 5.97
II	L-Ala	76	239 238-240*	white	$C_{14}H_{15}NO_3$	68.57 68.30	6.12 6.32	5.71 5.63
III	L-Val	73	231	white	$C_{16}H_{19}NO_3$	70.33 70.06	6.96 6.90	5.13 5.25
IV	L-Leu	81	205-207	white	$C_{17}H_{21}NO_3$	71.08 71.27	7.31 7.46	4.88 4.76
V	L-Met	64	247	white	$C_{16}H_{19}NO_3S$	62.95 62.75	6.23 6.14	4.59 4.46
VI	L-Phe	78	213 214-216*	white	$C_{20}H_{19}NO_3$	74.76 74.51	5.92 6.09	4.36 4.29
VII	L-Tyr	63	206 209-210*	white	$C_{20}H_{19}NO_4$	71.21 71.38	5.64 5.82	4.15 4.04
VIII	Gly	43	246d	pale green	$C_{26}H_{24}CuN_2O_6$	59.65 59.76	4.59 4.68	5.35 5.53
IX	L-Ala	57	263d	green	$C_{28}H_{28}CuN_2O_6$	60.98 60.73	5.08 5.15	5.08 5.19
X	L-Val	49	209d	dark green	$C_{32}H_{36}CuN_2O_6$	63.26 63.42	5.93 6.10	4.61 4.43
XI	L-Leu	54	232d	green	$C_{34}H_{40}CuN_2O_6$	64.25 64.31	6.30 6.47	4.41 4.32
XII	L-Met	34	206d	green	$C_{32}H_{36}CuN_2O_6S_2$	57.23 57.37	5.36 5.52	4.17 4.05
XIII	L-Phe	41	228d	pale green	$C_{40}H_{36}CuN_2O_6$	68.28 68.42	5.12 4.99	3.98 4.14
XIV	L-Tyr	39	257d	blue	$C_{40}H_{36}CuN_2O_8$	65.30 65.31	4.90 4.73	3.81 3.88

* reported m.p.

On the other hand, it was found that the efficiency of copper complexes against Gram positive bacteria is higher than that observed against Gram negative bacteria. All complexes except XIV showed good activities against *S. aureus* (inhibition zone diameter of 18-30 mm) and all of them were found to be nearly inactive against the remaining Gram positive and negative microorganisms.

Furthermore, the results revealed that copper complexes exhibited good activities against *C. albicans* with inhibition zone diameters of 23-30 mm and two of them only (VIII and IX) showed moderate activity against *A. fumigatus* with inhibition zone diameters of 14-18 mm. All the obtained results were summarized in Table 4.

Table 4: Antimicrobial activities of amino acid Mannich bases and their corresponding copper complexes (I-XIV).

Compd. No.	Tested microorganism, Mean of inhibition zone diameter,nearest whole mm*.					
	Gram - positive bacteria		Gram - negative bacteria		Yeasts and Fungi**	
	S. aureus	*M. luteus*	*S. typhimurium*	*P. fluorescens*	*C. albicans*	*A. fumigatus*
I	-	-	-	-	10 I	-
II	-	-	-	-	4 L	-
III	-	-	-	-	2 L	-
IV	-	-	-	-	5 L	10 I
V	-	-	-	-	3 L	6 L
VI	-	-	-	-	4 L	-
VII	4 L	-	-	-	4 L	-
VIII	29 H	-	-	-	30 H	18 H
IX	30 H	-	3 L	-	28 H	14 I
X	25 H	-	-	-	26 H	-
XI	20 H	-	-	-	27 H	-
XII	23 H	-	-	-	34 H	-
XIII	18 H	-	-	-	25 H	-
XIV	-	-	4 L	-	23 H	-
Control	26	25	28	27	28	26

* = Calculated from 3 values.

** = identified on the basis of routine cultural, morphological and microscopical characteristics.

– = No effect.

3.3. Measurement of minimal inhibition concentration (MIC). The minimum inhibitory concentration (*MIC*) values of the compounds were summarized in Table 5. A comparative study of the ligands and their complexes indicated that complexes showed higher activities than those recorded for ligands against *S.aureus* and *C. albicans* with *MIC* values 16-128 μg/mL and 16-64 μg/mL respectively. From the *MIC* values listed, it was found that the two compounds XII and XIII are more potent among the other investigated derivatives. Such increased activity of the complexes can be explained on the basis of Overtone's concept [38]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials due to which liposolubility is an important factor, which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism.

3.4. Evaluation of Cytotoxic Effects of XII and XIII. The cytotoxic activity of the DMSO soluble complexes XII and XIII was determined by calculation of IC_{50} that is the half maximal inhibitory

concentration, represents the concentration of an inhibitor required for 50% inhibition of its target. Calculation of this value allows a direct comparison of the cytotoxicity of each of the test agents.

Table 5: Minimum inhibitory concentration *MIC* (µg/mL) of the synthesized compounds (I-XIV)

Compd. No.	*Tested microorganism, MIC* µg/mL					
	Gram - positive bacteria		Gram - negative bacteria		Yeasts and Fungi**	
	S. aureus	*M. luteus*	*S. typhimurium*	*P. fluorescens*	*C. albicans*	*A. fumigatus*
VIII	≤ 64	ND	ND	ND	≤ 32	ND
IX	≤ 64	ND	ND	ND	≤ 32	ND
X	≤ 64	ND	ND	ND	≤ 16	ND
XI	≤ 128	ND	ND	ND	≤ 32	ND
XII	≤ 16	ND	ND	ND	≤ 64	ND
XIII	≤ 16	ND	ND	ND	≤ 16	ND
XIV	ND	ND	ND	ND	≤ 64	ND

ND= Not determined

The IC_{50} values were calculated using the data tabulated in Table 6 and presented in Fig 2. The values obtained of IC_{50} were 28.3 and 19.5 µg/mL respectively. The results showed that although the activities of XII and XIII do not fall within the accepted activity parameters adopted for in vitro screening (i.e., IC_{50} values not exceeding 4 µg/mL) [41], they refer to the chemotherapeutic potential of these complexes. The cytotoxicity study of the remaining compounds described in the present work is in progress.

Table 6: The cytotoxic activity of XII and XIII

Sample conc. (µg)	Viability %	
	XII	XIII
50	27.88	21.03
25	53.42	37.12
12.5	69.14	66.16
6.25	80.86	73.01
3.125	89.12	83.63
1.56	97.08	97.95
0	100	100
IC_{50}	*28.3*	*19.5*

4. CONCLUSIONS

N-(2-hydroxynaphthalen-1-ylmethyl)-L-amino acids (ligands, I-VII) and their corresponding copper complexes (VIII-XIV) have been synthesized in 2:1 molar ratio of ligand to copper (II) ion. They have been characterized based on the elemental analyses and spectroscopic methods which indicated that the amino acid Mannich bases act as a tridentate ligands with coordination to Cu (II) ion involving the carboxylate oxygen and the nitrogen atom of amino group and phenolic oxygen atom. Biological activity of the synthesized derivatives was studied and the results revealed that all the copper complexes showed stronger antimicrobial activities than the free ligands. The minimum inhibitory concentrations (MIC) of the complexes were found in the range of 16-128 µg/mL.

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