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Microwave synthesis, Characterization and Biological Activities of N-((4-amino-5-mercapto-4H-1, 2, 4-triazol-3-yl) methyl) Isonicotinamide and Its Complexes with Cu (II), Ni(II), Cd (II) Metal Ions

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Abstract

In the present study includes environment benign synthesis, characterization, and evaluation of biological activities of new ligand N-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)isonicotinamide (NAMTI) and it's metal complexes with copper(II), nickel(II) and cadmium(II). The ligand and its complexes were synthesized using a microwave synthesizer and characterized through various analytical techniques including UV-Vis, FT-IR, NMR spectroscopy, elemental analysis, TGA, and cyclic voltammetry as well as conductivity and magnetic moment data. The synthesized compounds were evaluated for their antibacterial and antifungal activities against selected microbial strains. The antibacterial activity was assessed using the Kirby-Bauer technique, and the results indicated significant activity of $Cu(NAMTI)_2$ and $Cd(NAMTI)_2$ complexes, often comparable to the standard antibiotic Gentamycin. The antifungal activities were evaluated using Mueller Hinton agar plates, with notable inhibition zones observed for the synthesized complexes. The study highlights the potential of $Cu(NAMTI)_2$ as a promising antibacterial agent and provides insights into the redox behavior of the metal complexes.

Keywords: Environment benign synthesis, biological activities, microwave synthesizer, metal complex

1.0 Introduction: In heterocyclic ligands, azole based ligands are promising complex forming agents with metal ions and are exhibited potential microbial activities [1, 2]. Recently, demand of newer antimicrobial agents in controlling drug resistance diseases is get up steam in medical field [3]. Due to environmental issues, scientist and researchers are focused on environment benign synthesis of drug or chemical compounds based on green chemistry principal [4]. Various azoles based medicines are available in market for treatment of microbial caused diseases and incomplete treatment by using these medicines cause drug resistivity[5]. For development of new efficient medicine to overcome drug resistivity complexion of newer azole derivative with metal ions may be an alternative way [6]. Present works includes use of environment benign reagents as well as method for synthesis of azole based compound NMATI and its complexes[7]. Microwave synthesis is a green method of synthesis with high efficiency than conventional reflux method.[8] Biological activities of Synthesized ligand and compounds were evaluated on bacterial strains, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus andEnterroccus faecalis*and Candida species ,*Candida glabrata, Candida albican, Candida kefyr and Candida krussei*as fungal strains by using Kirby-Bauer technique [9] andMueller Hinton agar plates method [10] respectively .It was observed that complexes of ligand NMATI, espically $Cu(NMATI)_2$have exhibited potential activities against selected bacterial and fungal species than ligand.

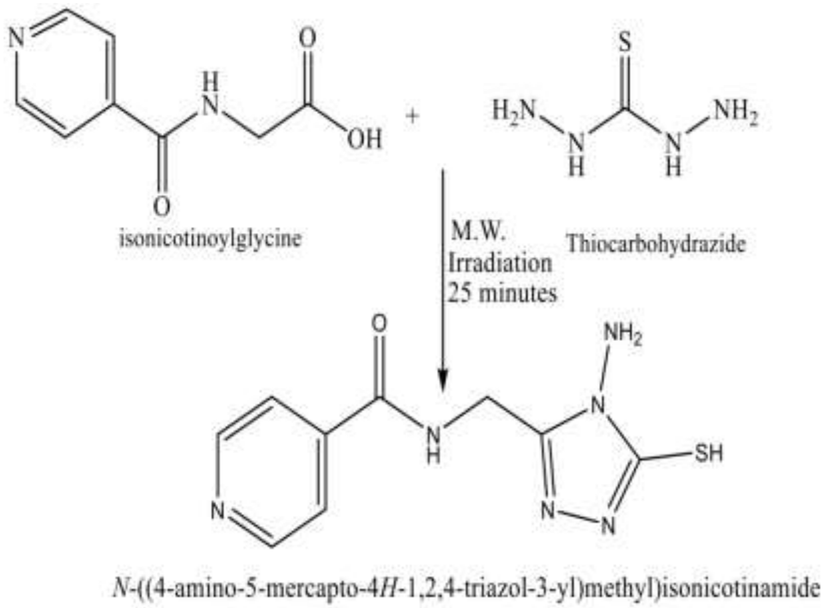
Experimental:

Material and methods: Reactants such as Isonicotinuric acid, thiocarbohydrazide , eNaHCO3 and metal salts as well as solvents have been purchased from Sigma-Aldrich, ECHEM and Merck. These reagents and solvents were utilized without any prurification. Synthesis has been done by microwave synthesizer, Precision-360. Melting point was determined by melting point apparatus M-560. Elemental analysis were carried by Euro Elemental Analyzer[11], UV-Vis spectrum were recorded by Shimadzu-160 while FT-IR were recorded by Agilent Cary 630 FTIR, C13, H1NMR were recorded by Bruker Avance 400. Conductivity and thrmal properties, cyclic volumetric properties were evaluated by using HTLPO82, Leco TGA 701, Ika ElectraSyn 2.0 respectively. Biological activities such as antibacterial,. Antifungal and insecticidal activities for selected biological species of synthesized

Sankatha Prasad Sonkar/Afr.J.Bio.Sc. 6(5)(2024).10201-10215

complexes and ligand were evaluated by appropriate methods discussed in results and discussion section.

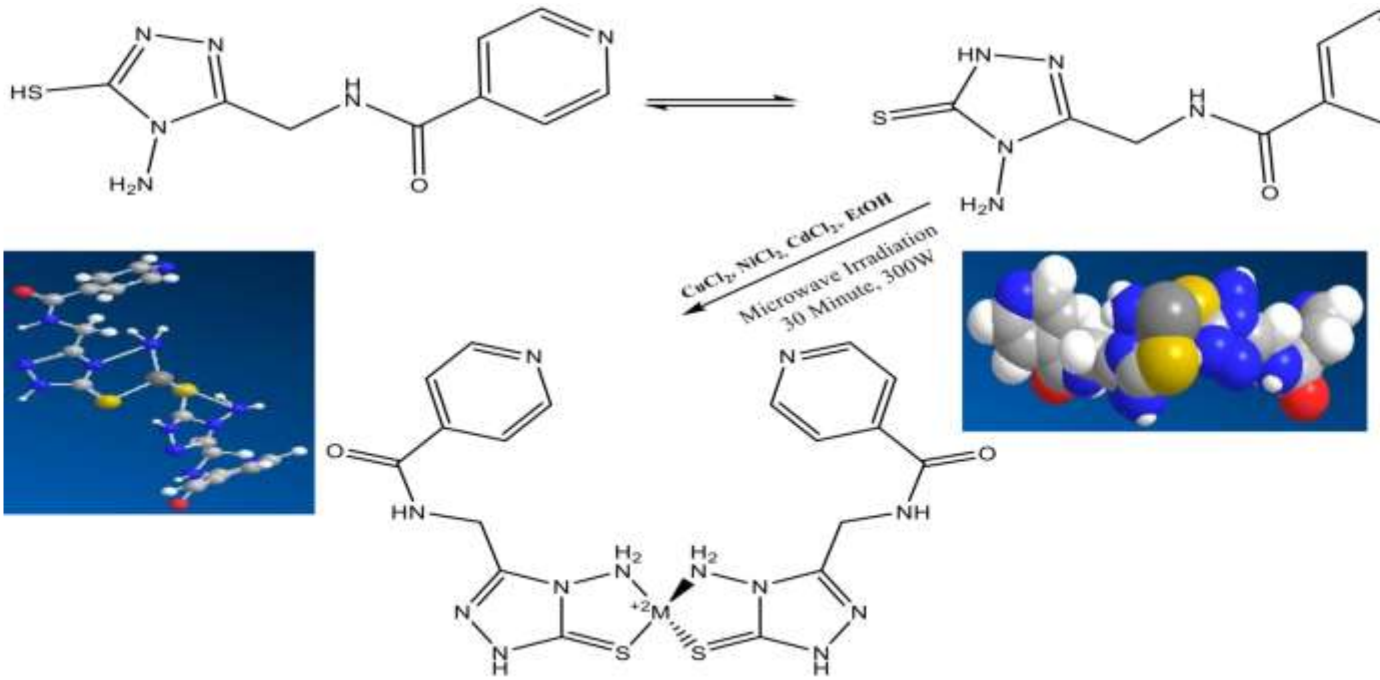
Synthesisof Ligand N-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)isonicotinamide(NAMTI): An equimolar mixture of N-benzoylglycine/N-(p-tolyl)glycine 2a (0.01674 mol) and thiocarbohydrazide 1 (0.01674 mol) were irradiated in microwave synthesizer for 25 minutes at 300W(Scheme-1). Then the reaction mixture was cooled and treated with a cold solution of 5% NaHCO3. The resulted solid was filtered, washed with water and recrystallised from ethanol (%yield = 87%)



Scheme: 1, Synthesis of Ligand {NAMTI)

Synthesis of Complexes of Cu (NAMTI)$_2$, Ni (NAMTI)$_2$, Cd (NAMTI)$_2$: Complexes were synthesized by irradiating ethanolic solution of ligand and metal salts $CuCl_2$, $NiCl_2$, and $CdCl_2$ in 1: 2 molar ratio for 30 minutes. After 30 minutes precipitates of complexes were filtered and recystallized from hot ethanol(%yield= 68%, 76%, 81% respectively) and melting points of each complex were determined.

Sankatha Prasad Sonkar/Afr.J.Bio.Sc. 6(5)(2024).10201-10215



Scheme:2, Synthesis of complexe

Results and Discussions:

Characterization of Synthesized Ligand and Complexes: Synthesized ligand and metal complexes were characterized by physical parameters, conductivity measurement, magnetic moment, TGA as well as UV-visible, FT-IR , NMR spectral techniques.

Physical characteristics and elemental analysis: Physical characteristics are given in table 1

Physical Properties				Elemental Analysis Calculated (Observed)					
Compounds	**M. F.**	**Colour**	**M.P. $_0$C**	**C**	**H**	**N**	**S**	**O**	**M**
NAMTI	C9H10N6OS (250.28)	Cream White	222	43.19 (43.10)	4.03 (4.00)	33.58 (33.59)	6.39 (6.33)	6.39 (6.33)	-
Cu(NAMTI)$_2$	$C_{18}H_{20}CuN_{12}O_2S_2$ (564.11)	Brown	224	38.33 (38.29)	3.57 (3.55)	29.80 (29.76)	11.37 (11.36)	5.67 (5.59)	11.26 (11.23)
Ni(NAMTI)$_2$	$C_{18}H_{20}N_{12}NiO_2S_2$ (559.25)	Dark Green	235	38.66 (33.56)	3.60 (3.59)	30.06 (30.10)	11.47 (11.45)	5.72 (5.71)	10.49 (10.45)
Cd(NAMTI)$_2$	$C_{18}H_{20}CdN_{12}O_2S_2$ (612.97)	White	251	35.27 (35.24)	3.29 (3.26)	27.42 (27.41)	10.46 (10.45)	5.22 (5.21)	18.34 (18.35)

Table 1:Physical characteristicsand elemental analysis of ligand and complexes

IR, NMR spectral data of ligand and complexes

Sankatha Prasad Sonkar/Afr.J.Bio.Sc. 6(5)(2024).10201-10215

NAMTI: Characteristic I.R. bands (KBr disc): 3273cm-1,3204cm^{-1} 2958cm^{-1} (NH,CHstr.),1653cm1,1637^{-1}, 1532cm^{-1},1491cm^{-1} (C=O,C=C,C=N str.)1286 cm^{-1}, 1142cm^{-1}, 1080cm^{-1}**H^{1}NMR:: (**300 MHz, DMSOd6, TMS, delta, ppm**):** 13.79 (SH), 8.71 (CH), 8.66(NH),7.73(CH) 7.00, 5.66 (NH_2) , 4.22(CH_2) **C^{13}HNMR:**167.8, 166.8,152,149.7, 149.7,140,121.7, 121.7, 32.

Cu(NAMTI)₂, Characteristic I.R. bands (KBr disc):3110cm^{-1},3229cm^{-1}3000cm^{-1} (NH,CHstr.),1656cm1,1638^{-1}, 1538cm^{-1},1499cm^{-1}(C=O,C=C,C=N str.)1256 cm^{-1}, 1137cm^{-1}, 1100cm^{-1}, 525 cm^{-1} (M-L)**H^{1}NMR:(**300 MHz, DMSOd6, TMS, delta, ppm**):**13.83(NH), 8.04(NHCO),8.70(CH) 7.73(CH), 4.2(NH2), 3.92(CH_2) **C^{13}HNMR:**(62.5MHz, DMSOd6, TMS,ppm):181.1,181.1,167.6,167.6,156.2,156.2,149.7,149.7,149.7,149.7,140.7,140.7,121.7,121.7,121.7121.7, 45,45

Ni(NAMTI)₂Characteristic I.R. bands (KBr disc): 3130cm^{-1},3129cm^{-1}3040cm^{-1} (NH,CHstr.),1686cm1,1658^{-1}, 1548cm^{-1},1489cm^{-1}(C=O,C=C,C=N str.)1259 cm^{-1}, 1139cm^{-1},

1110cm^{-1}, 510 cm^{-1} (M-L) **H^{1}NMR:: (**300 MHz, DMSOd6, TMS, delta, ppm**):**13.72(NH),8.75(CH),(8.07(NHCO), 7.73(CH),4.7(NH_2), 4.0(CH_2) **C^{13}HNMR:**(62.5MHz, DMSOd6, TMS,ppm):181.0,181.0,166.6,166.6,156.1,156.1,149.6,149.6,149.6,149.6,140.5,140.5,121.6,121.6,121.6,121.6, 45.5, 45.5.

Cd(NAMTI)₂Characteristic I.R. bands (KBr disc):3140cm^{-1},3128cm^{-1}3045cm^{-1} (NH,CHstr.),1666cm1,1647^{-1}, 1538cm^{-1},1461cm^{-1}(C=O,C=C,C=N str.)1220 cm^{-1}, 1151cm^{-1}, 1095cm^{-1}, 509 cm^{-1} (M-L) **H^{1}NMR:: (**300 MHz, DMSOd6, TMS, delta, ppm**):**13.75(NH),8.77(CH),(8.09(NHCO), 7.75(CH),4.9(NH_2), 4.2(CH_2) **C^{13}HNMR:**(62.5MHz, DMSOd6, TMS,ppm):181.1,181.1,165.6,165.6,154.1,154.1,149.5,149.5,149.5,149.5,140.3,140.3,121.5,121.5,121.5,121.5, 45.4, 45.4.

Conductivity and Magnetic moment of ligand and complexes: NAMTI forms complexes with Cu, Ni, and Cd with varying degrees of ionization and magnetic properties. The copper complex shows low conductivity and a lower-than-expected magnetic moment, indicatingsome electronic interaction or spin-pairing. The nickel complex has the highest conductivity anda slightly higher magnetic moment, suggesting less spin-pairing compared to the copper complex. The cadmium complex is diamagnetic with moderate conductivity, indicating no unpaired electrons and some degree of ionization in solution

Compound	Conductivity(µS/cm)	Magnetic moment (BM)
NAMTI	-	-
Cu(NAMTI)₂	1.0	0.8
Ni(NAMTI)₂	3.0	1.08
Cd(NAMTI)₂	2.1	0

Table2: Conductivity and magnetic moment of complexes

UV-Visible Spectrum of ligand and complexes:

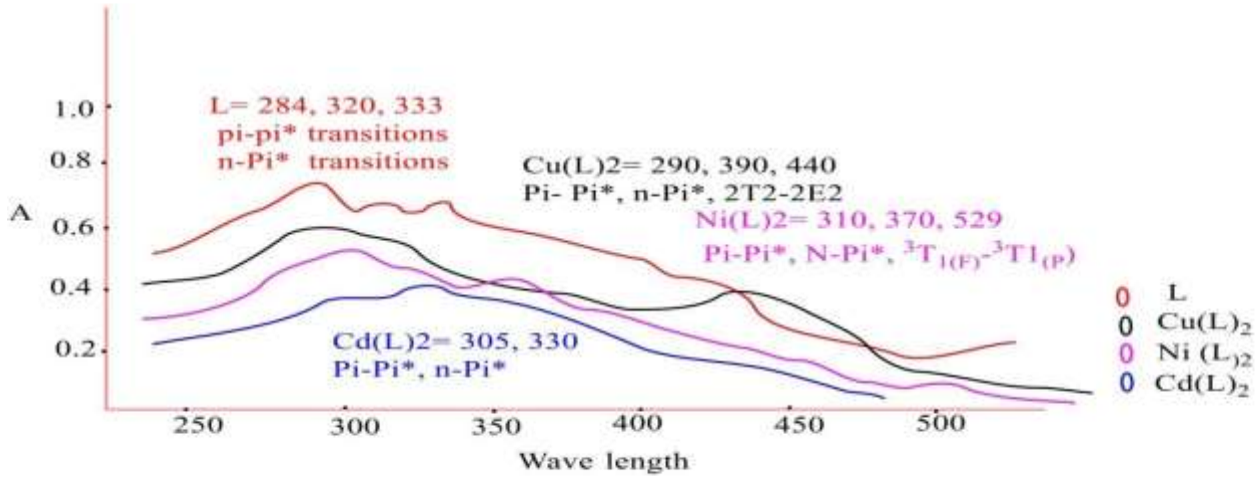


Fig:1 Comparable UV- Visible Spectra of Ligand and complexes

The ultraviolet- visible bands of ligand and complexes were scanned and significant bands were obtained in 230-550nm range. In ligand molecule significant band appeared at 284, 320 and 333 due pi-pi* transition and n-pi* transitions. But in the complexes d-d transition were observed as mentioned in figure.

TGA Curves of ligand and Complexes

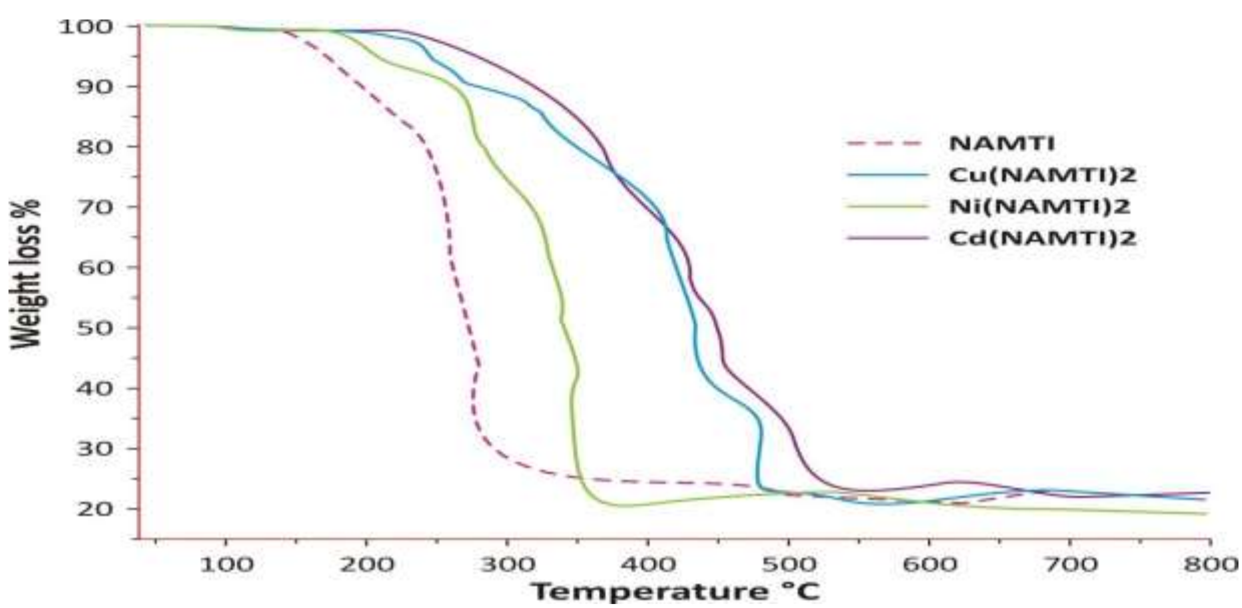
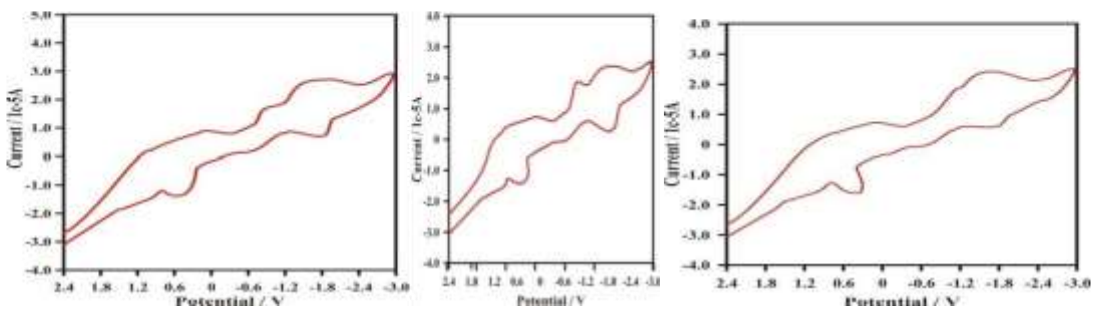


Fig. 2: TGA Curve of Ligand and complexes

In above TGA diagram NAMTI decomposes the earliest, starting around 200°C near to melting point, while Cu(NAMTI)$_2$ starts decomposing at a hihger temperature near about 250°C. The transition metal complexes (Cu, Ni, Cd) tend to decompose over a range of higher temperatures compared to NAMTI.Each compound exhibits a different pattern of weight loss, indicating distinct thermal stability profiles.NAMTI shows the fastest and most significant weight loss early in the temperature range, indicating lower thermal stability.Cu(NAMTI)$_2$ shows a steadier weight loss pattern, indicating a more gradual decomposition process.Ni(NAMTI)$_2$ and Cd(NAMTI)$_2$ show a similar pattern of rapid weight loss within a narrower temperature range, suggesting comparable thermal stability and decomposition processes.The TGA diagram indicates that NAMTI has the least thermal stability among the compounds analyzed, decomposing completely by 500°C. The metal complexes, Cu(NAMTI)$_2$, Ni(NAMTI)$_2$, and Cd(NAMTI)$_2$, exhibit higher thermal stability, decomposing over a broader range of temperatures and completing the process around 600°C. These differences highlight the impact of metal coordination on the thermal stability of these compounds.

3..1.6 CV curves of Complexes:



A B C

Fig 3: CV Curves of Complexes (A)Cu(NAMTI)$_2$ (B) Ni(NAMTI)$_2$ (C)Cd(NAMTI)$_2$

The CV curve of Cu(NAMTI)$_2$ shows characteristic oxidation and reduction peaks, indicating the redox behavior of the copper complex. The observed oxidation peak at +1.8 V and reduction peak at -1.2 V provide insights into the electrochemical properties of the complex. The significant peak separation suggests that the redox process is not fully reversible, pointing to slower electron transfer kinetics or possible structural changes during the redox cycle. The CV curve of Ni(NAMTI)$_2$, the potential is swept back in the negative direction (from +2.4 V to -2.4 V), the current decreases, indicating the reduction of the oxidized species of Ni(NAMTI)$_2$. The reduction peak occurs at approximately -1.2 V, indicating the potential at which the oxidized form of Ni(NAMTI)$_2$ is reduced back to its original state.The separation between the oxidation and reduction peaks provides information about the reversibility of the redox process. significant peak separation suggests a quasi-reversible or irreversible redox process, while a small separation indicates a reversible process. The potential is swept in the positive direction (from -2.4 V to +2.4 V), the current increases, indicating the oxidation of the Cd(NAMTI)$_2$ complex. The oxidation peak is observed at around +1.8 V. This peak represents the potential at which

Sankatha Prasad Sonkar/Afr.J.Bio.Sc. 6(5)(2024).10201-10215

Cd(NAMTI)$_2$ undergoes oxidation.When the potential is swept back in the negative direction (from +2.4 V to -2.4 V), the current decreases, indicating the reduction of the oxidized species of Cd(NAMTI)$_2$. The reduction peak occurs at approximately -1.2 V, indicating the potential at which the oxidized form of Cd(NAMTI)$_2$ is reduced back to its original state.

Biological Activities:

Antibacterial Activities: Antibacterial activities of bacterial species *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterroccus faecalis* were evaluated by Kirby-Bauer technique [10]

Bacterial Species	**Compounds Concentration in µg/ml Zone of inhibition in mm**																			
	NAMTI				**Cu(NAMTI)$_2$**				**Ni(NAMTI)$_2$**				**Cd(NAMTI)$_2$**				**Gentamycin**			
	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
Escherichia coli	**6**	**8**	**11**	**16**	**5**	**7**	**9**	**16**	**5**	**6**	**7**	**11**	**6**	**9**	**11**	**14**	**7**	**9**	**13**	17
Pseudomonas aeruginosa	**5**	**7**	**10**	**15**	**6**	**8**	**10**	**17**	**6**	**7**	**8**	**13**	**5**	**7**	**10**	**13**	**8**	**10**	**14**	17
Staphylococcus aureus	**6**	**8**	**12**	**16**	**5**	**9**	**12**	**16**	**4**	**7**	**11**	**14**	**4**	**8**	**12**	**14**	**7**	**11**	**14**	16
Enterroccus faecalis	**7**	**8**	**10**	**14**	**4**	**7**	**11**	**15**	**5**	**8**	**11**	**16**	**6**	**9**	**11**	**15**	**7**	**10**	**15**	17

C1= 100**µg/ml, C2= 150µg/ml, C3= 200µg/ml,C4= 250µg/ml**

Table 3: Antibacterial activities of Ligand and complexes

Bacterial Species	**Minimum Inhibitory Concentration in µg/ml**				
	NAMTI	**Cu(NAMTI)$_2$**	**Ni(NAMTI)$_2$**	**Cd(NAMTI)$_2$**	**Gentamycin**
Escherichia coli	12	10	20	10	10
Pseudomonas aeruginosa	15	12	15	12	12
Staphylococcus aureus	10	12	10	12	10
Enterroccus faecalis	12	10	12	10	11

Table 4: Minimum inhibitory concentration

Zone of Inhibition

The antibacterial activities of the compounds NAMTI, Cu(NAMTI)2, Ni(NAMTI)2, Cd(NAMTI)2, and the standard antibiotic Gentamycin were evaluated against four bacterial species: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis. The zone of inhibition was measured at four different concentrations (100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml).

Escherichia coli:

The inhibition zones for NAMTI ranged from 6 mm at 100 µg/ml to 16 mm at 250 µg/ml..Cu(NAMTI)2 showed inhibition zones from 5 mm at 100 µg/ml to 16 mm at 250 µg/ml, indicating significant antibacterial activity.Ni(NAMTI)2 displayed a lower range of inhibition, from 5 mm at 100 µg/ml to 11 mm at 250 µg/ml. Cd(NAMTI)2 showed increasing inhibition zones from 6 mm at 100 µg/ml to 14 mm at 250 µg/ml.Gentamycin, the control, exhibited the highest inhibition zone, peaking at 17 mm at 250 µg/ml.

Pseudomonas aeruginosa:

NAMTI showed inhibition zones from 5 mm to 15 mm across the concentrations..Cu(NAMTI)2 demonstrated better activity, with zones ranging from 6 mm to 17 mm..Ni(NAMTI)2 showed lower inhibition, with zones from 6 mm to 13 mm.Cd(NAMTI)2 had inhibition zones from 5 mm to 13 mm.Gentamycin had the highest efficacy, with zones from 8 mm to 17 mm.

Staphylococcus aureus:

NAMTI inhibition ranged from 6 mm to 16 mm..Cu(NAMTI)2 showed strong activity with zones from 5 mm to 16 mm. Ni(NAMTI)2 had a range from 4 mm to 14 mm. Cd(NAMTI)2 displayed zones from 4 mm to 14 mm.. Gentamycin was highly effective, with zones from 7 mm to 16 mm.

Enterococcus faecalis:

NAMTI showed inhibition zones from 7 mm to 14 mm. Cu(NAMTI)2 had zones from 4 mm to 15 mm. Ni(NAMTI)2 showed inhibition zones from 5 mm to 16 mm. Cd(NAMTI)2 had zones from 6 mm to 15 mm. Gentamycin showed the highest inhibition, ranging from 7 mm to 17 mm.

Overall, Cu(NAMTI)2 and Cd(NAMTI)2 exhibited considerable antibacterial activity across all bacterial strains, often approaching or surpassing the standard antibiotic Gentamycin, particularly at higher concentrations.

Minimum Inhibitory Concentration (MIC)

The MIC values provide further insight into the potency of these compounds:

Escherichia coli:

MIC values for NAMTI, Cu(NAMTI)2, and Cd(NAMTI)2 were all 10 µg/ml, showing comparable effectiveness to Gentamycin.. Ni(NAMTI)2 had a higher MIC value of 20 µg/ml, indicating lower effectiveness.

Pseudomonas aeruginosa:

NAMTI had an MIC of 15 µg/ml. Cu(NAMTI)2 and Cd(NAMTI)2 had MIC values of 12 µg/ml, which were equal to Gentamycin. Ni(NAMTI)2 had an MIC of 15 µg/ml.

Staphylococcus aureus:

NAMTI and Ni(NAMTI)2 both had MIC values of 10 µg/ml, showing high effectiveness. Cu(NAMTI)2 and Cd(NAMTI)2 had MIC values of 12 µg/ml, similar to Gentamycin.

Enterococcus faecalis:

NAMTI and Cd(NAMTI)2 had MIC values of 10 µg/ml. Cu(NAMTI)2 and Ni(NAMTI)2 had MIC values of 12 µg/ml. Gentamycin had an MIC of 11 µg/ml, showing comparable effectiveness.

The study demonstrates that the synthesized compounds, particularly Cu(NAMTI)2 and Cd(NAMTI)2, exhibit significant antibacterial activity against the tested bacterial strains, often comparable to or surpassing the standard antibiotic Gentamycin. Cu(NAMTI)2 consistently showed high efficacy across different concentrations and bacterial species, making it a promising candidate for further investigation as an antibacterial agent.

The MIC values corroborate the zone of inhibition results, further highlighting the effectiveness of Cu(NAMTI)2 and Cd(NAMTI)2. Ni(NAMTI)2 showed lower antibacterial activity compared to the other compounds, suggesting that its potential as an antibacterial agent might be limited or require higher concentrations for effectiveness.

Further research should focus on the detailed mechanisms of action, potential toxicity, and in vivo efficacy of these compounds to fully assess their potential as new antibacterial agents.

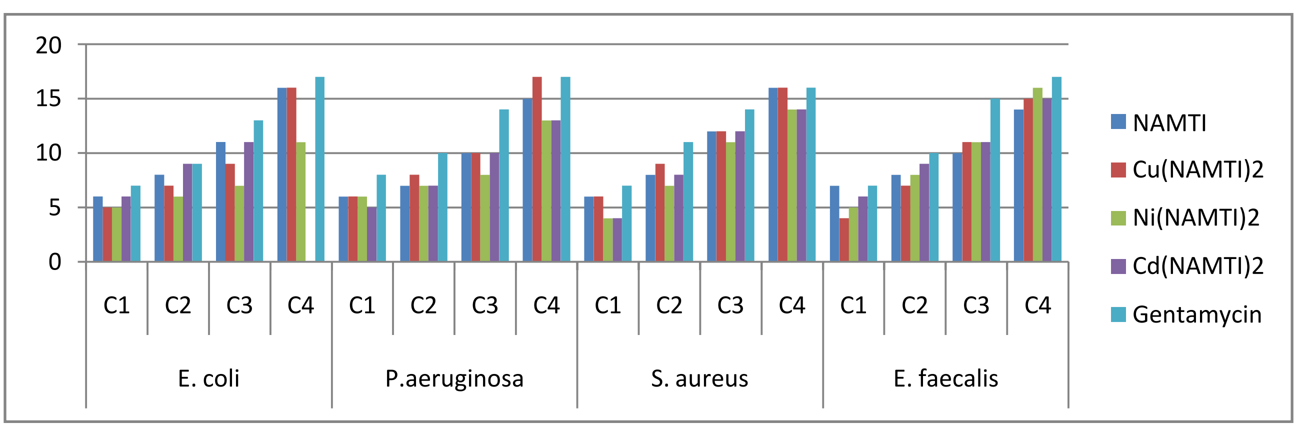


Fig 3: Antibacterial activities of ligand and complexes

Antifungal Activities: Antifungal Activities of all candida species were evaluated by Mueller Hinton agar plates method [11] at 500 **µg/ml concentration**. Minimum inhibitory concentration of each compound is give in bracket for specific fungus.

Candida species	**Compounds Concentration in 500 µg/ml (MIC in µg/ml) Zone of Inhibition in mm**				
	NAMTI	**Cu(NAMTI)₂**	**Ni(NAMTI)₂**	**Cd(NAMTI)₂**	**Clotrimazole**
Candida glabrata	**7(80)**	**10(60)**	**11(70)**	**13(80)**	**13(60)**
Candida albican	**9 (70)**	**8(80)**	**10(70)**	**10(60)**	**12(70)**
Candida kefyr	**6 (90)**	**9(80)**	**8 (80)**	**11(70)**	**11(70)**
Candida krussei	**9 (80)**	**10(60)**	**11(90)**	**13(70)**	**14 (60)**

Table 5: Antifungal activities of complexes and ligand

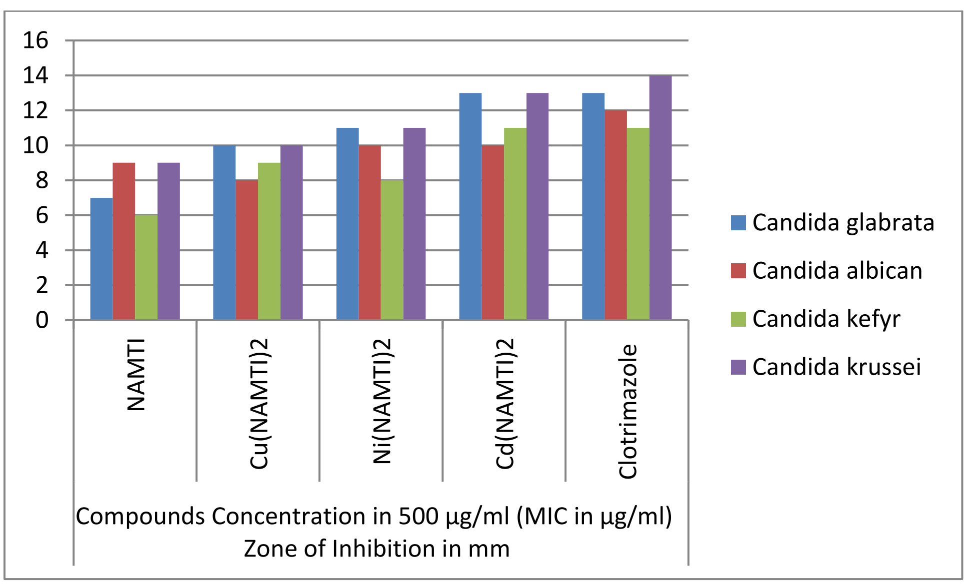


Fig 4: Antifungal activities of ligand and complexes

The study demonstrates that the metal complexes of NAMTI, particularly $Cu(NAMTI)_2$, Ni(NAMTI)$_2$, and Cd(NAMTI)$_2$, generally exhibit better antifungal activity compared to NAMTI alone. Among the tested compounds, Cd(NAMTI)$_2$ showed the highest zones of inhibition across all Candida species, which were comparable to or slightly less than those of the standard antifungal agent Clotrimazole. Clotrimazole consistently showed the highest antifungal activity with the largest zones of inhibition and lowest MIC values.This suggests that while NAMTI and its metal complexes are effective against Candida species, Cd(NAMTI)$_2$ stands out as the most potent among them. The findings highlight the potential of metal complexes in enhancing the antifungal efficacy of organic compounds, providing a promising direction for developing new antifungal agents

Conclusion

The biologically active ligand N-((4-amino-5-mercapto-4H-1, 2, 4-triazol-3-yl) methyl) Isonicotinamide(NMTI) and its metal complexes with Cu(II), Ni(II), and Cd(II) were synthesized by microwave heating and characterized by elemental analysis, UV-Vis, FT-IR and NMR spectral data as well as magnetic moment and conductivity mesurements. TGA confirmed the structural and thermal properties of the synthesized compounds. The cyclic voltammetry studies revealed distinct redox behaviors, particularly for the Cu(NAMTI)$_2$ complex, indicating its potential electrochemical properties. The microbial activities of ligand and the metal complexes evaluated against selected bacterial strains Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis as well as also evaluated for fungal strains of Candida species. It was observed that Cu(NAMTI)$_2$ and Cd(NAMTI)$_2$, exhibit substantial antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis, with zones of inhibition and MIC values often comparable to Gentamycin. The antifungal activities also showed significant inhibition zones for Candida species, suggesting broad-spectrum antimicrobial potential. NMATI is a azole based ligand and exhibited potential microbial activity but their complexes consistently showed high efficacy across different microbial strains, making these a strong candidates for further development as an antimicrobial agents especially Cu(NAMTI)$_2$

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Competing Interests: The authors declare that they have no competing interests

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Disclaimer

The authors alone are responsible for the content and writing of the paper.

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