REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org

Synthesis and in vitro biological evaluation of metal complexes containing Pyridine

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Abstract:
In the present study, metal complexes containing pyridine have been synthesized and characterized by physicochemical and spectral means. Broth microdilution (96 well microtiter plate) method was used to evaluate antimicrobial activity of the synthesized compounds against Gram positive bacteria (*B. subtilis*), Gram negative bacteria (*E. coli*) and fungal species (*A.terrus* & *A.niger*) using cefadroxil (antibacterial) and fluconazole (antifungal) as standard drugs. Among the synthesized compounds, chalcone ligand **C1** showed good antifungal activity. against *A.terrus* & *A.niger* (MIC =3.12µg/ml.) while complex **KP1** showed significant activity against *E. coli* (MIC=1.56µg/ml) , *A. niger* (MIC=3.12µg./ml) and *A. terrus* (MIC=1.56µg/ml). Complex **KP7** showed significant activity against *B.subtilis* and *E.coli*.(MIC = 3.12 µg/ml.). Antioxidant activity of the synthesized compounds was determined using D.P.P.H. assay method. Complex **KP2** showed excellent antioxidant activity (48.46). The order of antioxidant activity **s**howed the increase in antioxidant activity **KP2>KP3> KP6>KP5>KP4>>KP7>C2>KP1>KP8>C1**

1. Introduction

Recurrent reporting of bacterial resistance poses a great threat to the efficacy of presently available treatment for the infectious diseases. Resistance is the consequence of reckless use of antibiotics. Multidrug-resistant organisms have originated due to which conventional treatment failed to provide its action. Thus, there is a need for the development of new antimicrobial agents (Bourichi *et al.,* 2018).

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. ROS, including free radicals and other reactive molecules, can cause damage to cellular components such as lipids, proteins, and DNA. This oxidative damage is associated with various diseases and aging processes. Antioxidant chemical compounds play a crucial role in counteracting oxidative stress by neutralizing and scavenging ROS. These antioxidant compounds work through various mechanisms, including scavenging free radicals, regenerating other antioxidants, chelating metal ions, and modulating cellular signaling pathways (Sies *et al*., 2015).

Pyridine is one of the crucial six membered heterocyclic ring containing nitrogen as a heteroatom. Pyridine found to be present in light oil fraction of coal tar as well as bone oil. It has a structure resemblance with benzene but has a nitrogen atom in place of one of the methine groups (=CH) as presented in **Fig 1**. The pyridine nucleus is found in a variety of physiologically and pharmaceutically important chemicals including vitamins (nicotinic acid, vitamin B6), food, flavourings, adhesives, pesticides and herbicides (Marinescu *et al.,* 2022).

There are many marketed drugs that contain pyridine ring system for treating the human illnesses. Benzehexol (antiparkinson), Clopidogrel (antiplatelet), Netupitant (antiemetic), Esomeprazole (proton pump inhibitors) are some of the drugs available in the market (Khan *et al*., 2021; Nagashree *et al.,* 2015). Moreover, many other chemical entities are there consisting of pyridine ring as a pharmacophore which have antiviral, antioxidant, antidiabetic, anticancer, antiamoebic, antimalarial as well as anti-inflammatory activities (Nagashree *et al.,* 2015). All these activities are exhibited by the single ring demonstrating the significance of this heterocyclic molecule. As a result, researchers have expressed an interest in developing pyridine derivatives to further analyse their therapeutic potential.

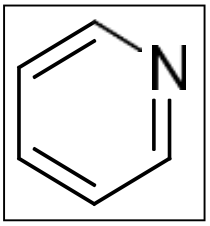


Fig 1: Structure of Pyridine

Chalcone is a very common bioactive scaffold that is found as a natural pigment in plants. Chalcones are formed as an

intermediate in the biosynthesis of flavonoids. Structurally, It is an α, β-unsaturated carbonyl system which contains 2 aryl groups connected at 1st and 3rd position (Zhuang *et al.,* 2017). They have a diverse set of biological activities like anti-inflammatory, antidiabetic, anticancer, antimycobacterial, anti-Alzheimer, anti-leishmania, antimicrobial (Johnson *et al.,* 2022). Chalcones can be prepared in the laboratory mainly by Claisen-Schmidt condensation. When appropriate benzaldehyde and acetophenone are cross- condensed with a base acting as a catalyst, Claisen-Schmidt condensation occurs. Chalcones having one or more hetero atoms (mainly O, N, S, P) can serve as a donor atom in a ligand molecule due to the presence of lone pair of electrons (Rammohan *et al.,* 2020).

Scaffolds of chalcone can be converted into other compounds like metal complexes that can ligate or function as ligands directly. Many of the transition as well as non-transition metal complexes based on chalcones have been identified so far. Metal complexes formed from chalcones have sparked the curiosity of medicinal chemists due to their diverse applications such as anti-cancer, antibacterial, anti-malarial, anti-inflammatory, anti-viral, anti-protozoal etc. (Johnson *et al.,* 2019). Many of the marketed formulations comprising of pyridine and its metal complexes are Isoniazid (antitubercular), copper pyrithione (antimicrobial), pyridoxine (Vit.B6.), Pirbuterol (bronchodilator) pioglitazone (antidiabetic), Benzhexol (anti-Parkinson), Disopyramide (anti-arrhythmic), Pantoprazole (anti-ulcer), Indinavir (anti-viral) as presented in **Fig 2.**

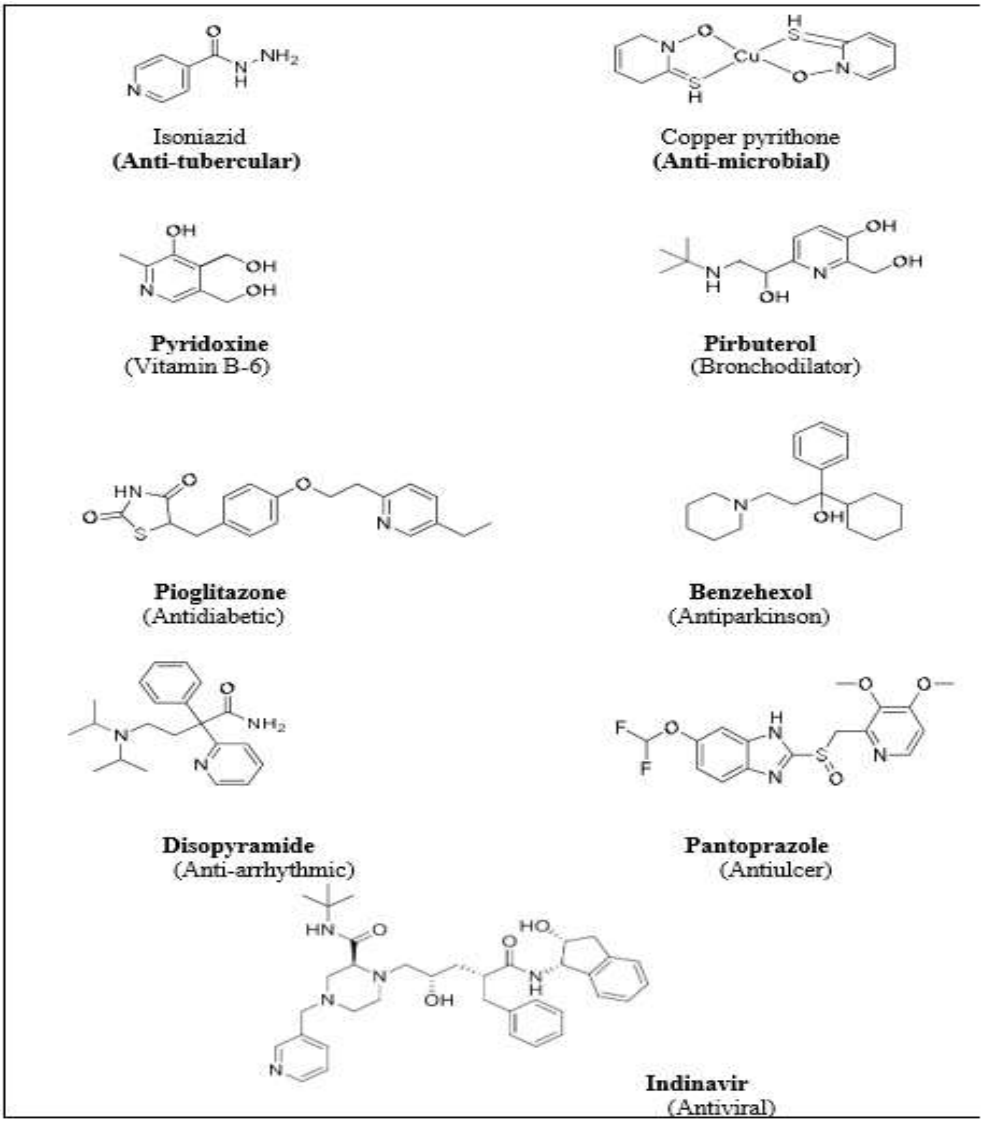


Fig. 2: Marketed formulations containing pyridine moiety

1. Experimental

1.1. Materials and Method

The starting materials for the synthesis were obtained from different sources, mainly Loba Chemie Pvt. Ltd, C.D.H. Fine Chemicals Pvt. Ltd. and Hi-Media Laboratories Pvt. Ltd. The progress of the chemical reactions was monitored using thin-layer chromatography (TLC). Silica gel G was used to prepare TLC plates, whichserved as the stationary phase. For the synthesized derivatives, mobile phases of Cyclohexane:Ethyl acetate and Benzene:Ethylacetoacetate were used. The

melting points of the synthesized derivatives were determined using a Sonar melting point apparatus (Sunbim, India). For the analysis of the compound's structures and protons, 1H-Nuclear Magnetic Resonance (NMR) spectroscopy was performed using a Bruker Avance III 600 NMR spectrometer operating at 600 MHz. The appropriate deuterated solvents, such as DMSO, were used. The infrared (IR) spectra of the compounds were evaluated using a Bruker 12060280 spectrophotometer with OPUS 7.2.139.1294 software. The spectra were recorded in the range of 4000-400 cm-1. The absorbance measurement of the 96-well microtitre plate were taken using a Thermo Scientific Multiscan FC ELISA plate reader. The data was analyzed using Skan-It software version 7.0.2 RE. The antioxidant activity of the synthesized derivatives was determined using the DPPH assay method. In the NMR data of the compounds, the multiplicity of signals was indicated by abbreviations such as singlet (s), doublet (d), triplet (t), and multiplet (m). These designations represent the number of protons present in the compounds.

1.2. Synthetic procedure for synthesis of pyridine derivatives:Step a: Synthesis of Chalcone Ligands(C1-C2)

Aldehyde (0.02 mol) was first dissolved in 10ml. ethanol. Subsequently, a 10% NaOH solution (approximately 100 ml) was added to the mixture resulting in clear solution. The solution was cooled to 0ºC by using ice bath. Over a span of 30 minutes, dropwise addition of 2-acetyl pyridine (0.02 mol) took place. The resulting solution was kept at 0ºC for one hour and then the same solution was allowed to stir atroom temperature. With continuous stirring, solid substance started to separate out, which was further stirred for approximately one hour. The solid product was then filtered and recrystallized from ethanol resulting in the formation of chalcone powder (Lamara *et al.,* 2021.

Step b: Synthesis of Metal complexes (KP_1-KP_8)

Chalcone Ligands (0.02 mol) synthesized in the first step was dissolved in 15ml of methanol. Then, pH was adjusted to 7-8 by using ammonia solution in order to provide deprotonation of chalcone. A solution of metal acetate (Co(II), Ni(II), Cu(II) or Zn(II)) (0.01 mol) in 20ml methanol was then added dropwise to solution of chalcone ligand. The resulting mixture was stirred under reflux for 4-6 hr. Solid complex was collected by filtration and washed sequentially with deionised water andice cold Et-OH (Sulpizio *et al.,* 2018).

1.3. In vitro antimicrobial evaluation

An antimicrobial agent refers to a substance that impedes the growth or eliminates microorganisms, including bacteria, fungi, and protozoans. These substances can either halt the growth of microorganisms (microbistatic) or resultin their death (microbicidal).

Method for antimicrobial evaluation.

Pure cultures were employed to check the *in vitro* antimicrobial activity ofthe synthesized compounds. (Cappuccino *et al*., 1999). An antimicrobial assay is critical for evaluating the effectiveness of different chemicals against bacteria. These assays provide valuable insights about antimicrobial drugs to suppress or kill microorganisms such as bacteria, fungus, and protozoans.

The discovery of new antimicrobial drugs and the assessment of their effectiveness both dependent on these assays. Antimicrobial evaluation helps in identifying potential candidates for further investigation, guiding drug formulationand dosage determination, and assessing the development of antimicrobial resistance. Additionally, *in vitro* assays serve as preliminary screening tools in research and development, providing a cost-effective and time-efficient means of evaluating numerous compounds.

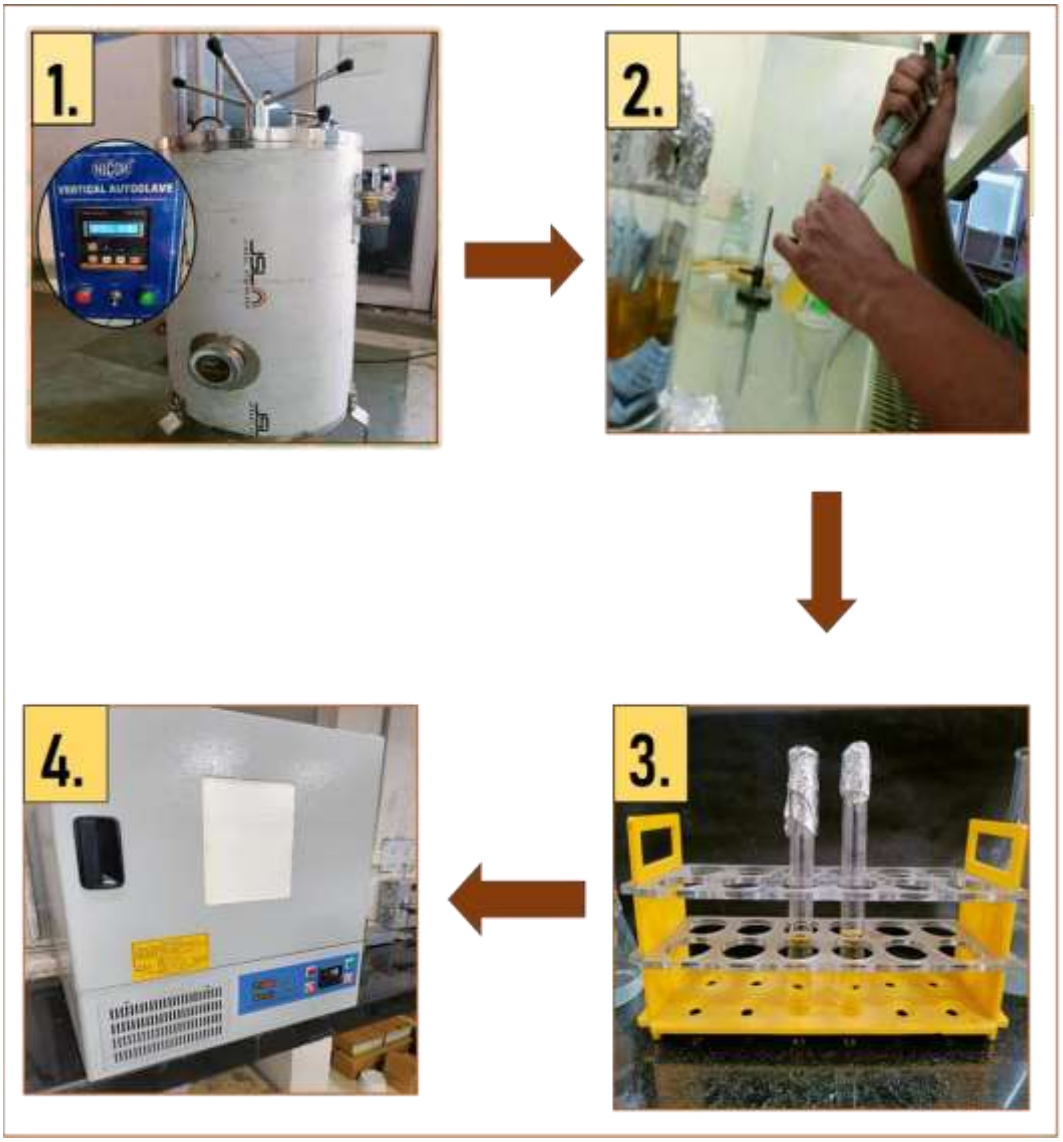
By determining the MIC, MBC/MFC, and inhibition zones, microbiological assays provide valuable information on the potential of compounds to inhibit microbial growth or cause microbial death. With their widespread application in research, pharmaceutical development, and clinical microbiology, evaluation of new synthesized compounds continues to contribute significantly to our understanding of antimicrobial agents and their impact on combating microbial infections (Andrews *et al*., 2001). Two different methods were utilized to
determine the susceptibility of microorganisms to these compounds in a laboratorysetting.

- Diffusion through agar media.
- Tube Dilution method (Broth micro-dilution method)

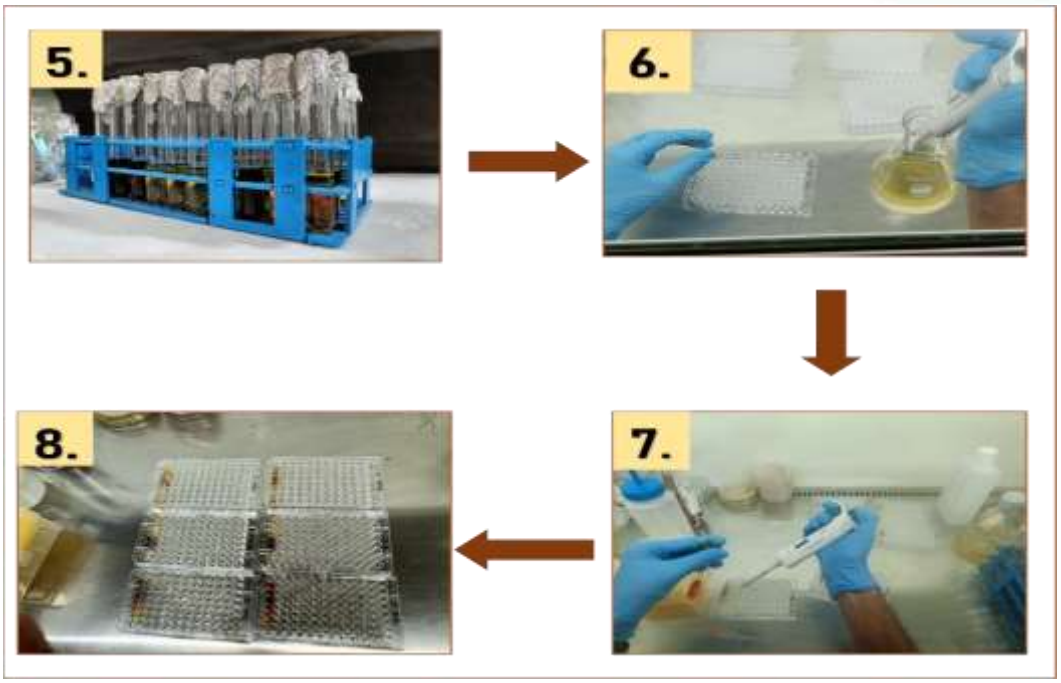
Broth Micro-dilution method

1. The nutrient broth media and Sabouraud dextrose broth were prepared as per (Indian Pharmacopoeia 2018).
2. The prepared media was sterilized by autoclaving for 30 minutes at 121°C.
3. Bacterial strains (*E. coli* and *B. subtilis*) and Fungal strains (*A.niger* and*A.terrus*) were revived by sub-culturing them in media and incubate them in a vortex shaker incubator at 37°C for 24 hours and 25ºC for 7 days respectively.
4. A stock solution (1000 µg/ml) of the synthesized compounds and standard drugs (cefadroxil and fluconazole) was prepared using dimethyl sulfoxide(DMSO) as the solvent.
5. The wells of a 96-well microplate were filled with 50 µL of the media, leavingthe last two wells as positive and negative controls for the entire procedure. The positive control contained 100 µl of the microbial inoculum, while the

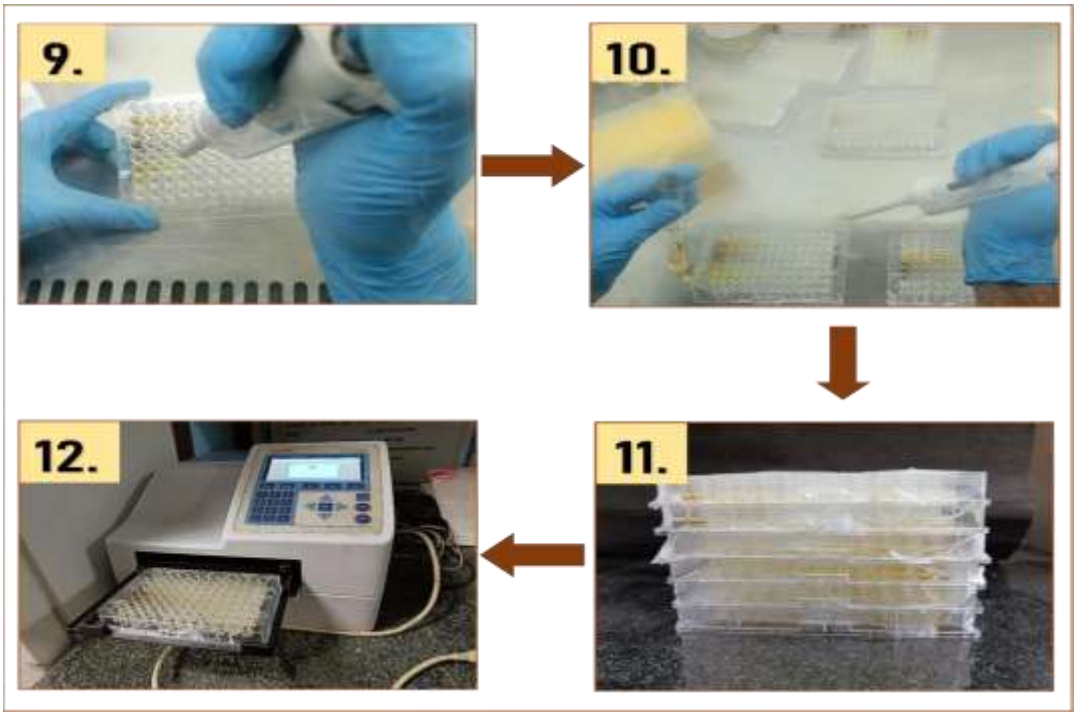
negative control contained both media and inoculum in equal quantities. (50 µL: 50 µL).
6. 100 µl of the prepared sample was pipetted into the first well of each row.
7. Serial dilution was performed by transferring 50 µL of the sample from the first well of each row to the second well, and subsequently from the second well to the third well, resulting in various concentrations of the solution (i.e., 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.56 µg/ml, 0.78 µg/ml, 0.38 µg/ml).
8. The revived microbial strains were added to each well, excluding the last well of each row, which serves as the positive control.
9. All 96-well plates were covered with para-film to ensure a sterile environment.
10. All the plates were incubated at 37°C for 24 hours and 25ºC for 7 days respectively to allow for proper microbial growth and interaction with the samples.
11. The absorbance of each well was measured using an ELISA plate reader at a wavelength of 620 nm to assess the microbial response to the samples.
12. The M.I.C. value of compounds was determined from the absorbance where MIC values for a drug were expressed as lowest concentration that inhibits the microbial growth (Tang *et al*., 2021).



Step 1: Preparation & sterilization of broth media.
Step 2: Revival of bacteria.
Step 3 & 4: Incubation of test tubes containing nutrient broth media & inactivebacteria.



Step 5: Preparation of sample using DMSO as the solvent.
Step 6: Preparation of microplate wells using sterilized broth media and positive andnegative controls..
Step 7: Pouring of sample into 96-well plate.
Step 8: Plate containing sample and sterilized broth media.



Step 9: Serial Dilution process to create concentration gradient.
Step 10: Inoculation of revived Bacterial Strains into Microplate Wells (ExcludingPositive Control).
Step 11: Covering of 96-well plate with para-film for sterility protection for theprocess of incubation.
Step 12: Measurement of absorbance using ELISA plate reader after incubationperiod.

Antioxidant study

The ability of any substance to scavenge the stable 1,1-diphenyl-2-picryl hydrazyl(DPPH) free radical is used to measure it's antioxidant capability. The evaluation of antioxidant activity using this method involves measuring the reduction in absorbanceof DPPH at 517 nm, resulting in a color change from purple to yellow. This decrease in absorbance occurs

because of the formation of a stable DPPH molecule when it reacts with an antioxidant, either through hydrogen donation or electron transfer. The DPPH radical contains a free electron that contributes to its absorbance peak at 517 nm, which gives it a purple color. The antioxidant compound interacts with the free electron of the DPPH radical by either donating an electron or releasing a hydrogen atom, leading to the formation of a stable DPPH-H molecule. The change in color from purple to yellow corresponds to a decrease in the molar absorptivity of the DPPH radical, as the odd electron pairs up with the antioxidant compound. The extentof color change is proportional to the number of electrons captured, indicating the stoichiometry of the reaction. The DPPH model was employed to evaluate the anti- radical activity of the compounds (Lone *et al*., 2013).

Preparation of Stock Solution:
10 mg of each synthesized compound and the standard drug, ascorbic acid, were accurately weighed. These weighed compounds were then transferred into separate 10ml volumetric flasks. To achieve a concentration of 1000 µg/ml for each stocksolution, the volume was adjusted to 10 ml using methanol.

Preparation of Dilutions:
From the previously prepared stock solutions of the sample and standard drug, 0.25 ml, 0.50 ml, 0.75 ml, and 1 ml were carefully pipetted out into separate 10 ml volumetric flasks. To achieve the desired dilutions, methanol was added to each flask to bring the final volume up to 10 ml. As a result, different dilutions of the sample andstandard drug were obtained, with concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml, respectively.

Preparation of DPPH Solution:
A solution of DPPH with a concentration of 0.1 mM was prepared by dissolving 3.9432 mg of DPPH in 100 ml of methanol.

Screening of Antioxidant Activity: For each concentration (25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml), 1 ml of the sample or standard drug solution was taken separately. Similarly, as a control, 1 ml of methanol was taken. To each sample or control solution, 1 ml of the DPPH (2,2-diphenyl-1-picrylhydrazyl) solution wasadded. All solution mixtures, including the samples, standard drug, and control, were kept in a dark environment for 30 minutes to allow for reaction. After 30 minutes, the absorbance of each solution mixture was measured at a wavelength of 517 nm using a spectrophotometer (Singh *et al*., 2015). The absorbance was noted and % inhibition was calculated using the fromula given below-
% inhibition **= [(Acontrol – Asample)/ Acontrol x100]**
Where $A_{control}$ = Absorbance of controlA_{sample} = Absorbance of sample.

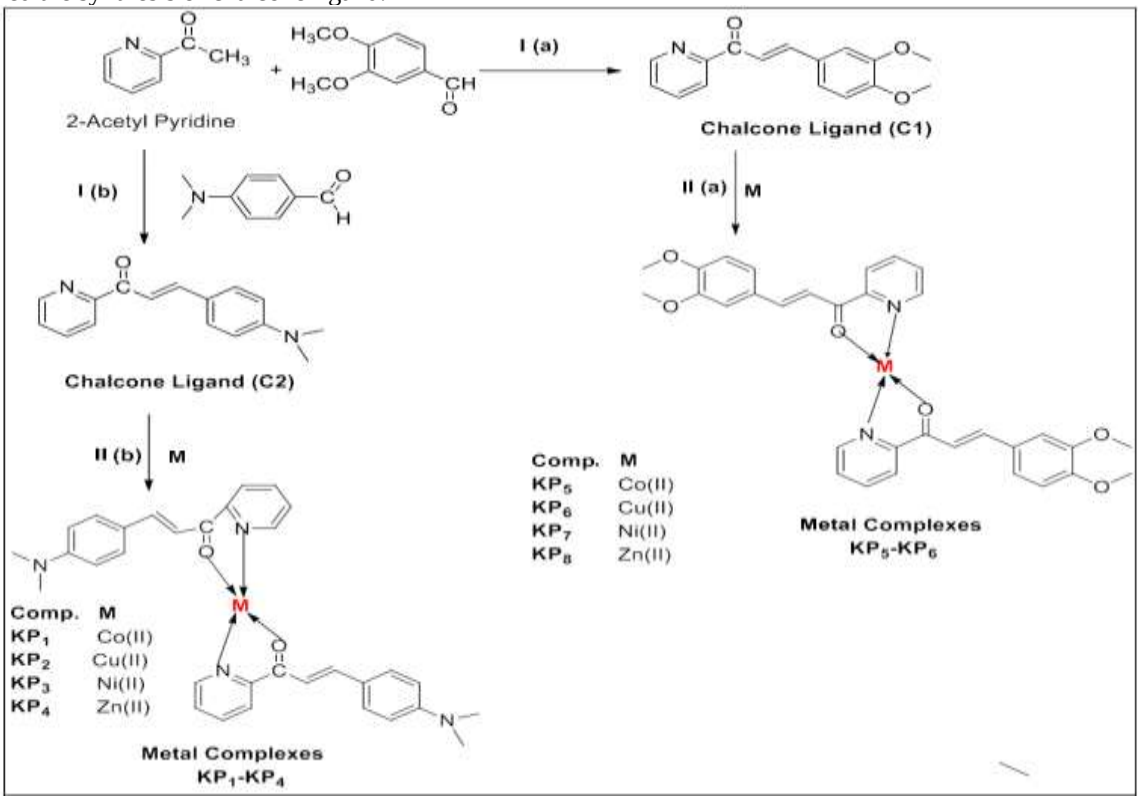
Fig 3: Picture representing the solution mixtures contaning D.P.P.H. andsynthesized compound

2. Results and discussion

2.1. Chemistry

The metal complexes containing pyridine were synthesized using **Scheme 1**. Initially, 2-acetyl pyridine was treated with substituted benzaldehydes (3,4-dimethoxy benzaldehyde and para dimethyl amino benzaldehyde) in presence of sodium hydroxide solution to form chalcone ligands (**C1-C2**) which on further treatment with metal acetates to form metal complexes (**KP1-KP8**). Synthesized compounds were characterized for their physicochemical properties (Table 1).

Chemical structures of the synthesized chalcone compounds (**C1-C2**) and it's metal complexes (**KP1-KP8**) were determined by spectral analysis (ATR, ^{1}H-NMR) (Table 2). The bidentate chalcone ligand have one benzene ring, one pyridine ring andunsaturated linkage, respectively. The IR (cm^{-1}) data of chalcone ligand showed the characteristic band at 1542.47(**C1**) and 1524.05 (**C2**) which represented the presenceof ν(C=C) unsaturated linkage. Nitrogen atom in the pyridine ring combine with metalions and reduce electron density and decrease the ν(C=N) absorption frequency i.e. indicated by the lowering of stretching band due to ν(C=N) shifted towards the frequency range between 1516-1650 cm^{-1} and characteristic bands at 1524-1542 (C=C), 3026-3027 (C-H), 1756-1781 (C=O) and 1027-1090 (C-O) which confirmed the synthesis of chalcone ligand.



Scheme 1: Synthesis of Chalcone ligands and metal complexes of pyridine

The IR data obtained for the metal complexes displayed characteristic bands inthe range of 700-600 cm^{-1}. These bands are indicative of the metal ions forming linkages with the nitrogen (N) and oxygen (O) atoms present in the formed complexes. The 1H-NMR spectra of both the chalcone ligand and its metal complexeswere recorded using chloroform (CHCl3) and DMSO-d6 solvents. These spectraconfirmed the binding of the chalcone ligand to the metal atoms. In the spectra, multiplet signals of aromatic protons in the chalcone ligand and its metal complexes were observed within the range of 6.71-7.72 δ ppm. Additionally, peaks appeared in the region of 7.18-8.86 δ ppm, which were assigned to the chemical shift of protons present in the pyridine ring. The observed up field shifting of the substituted aromatic ring indicated hydrogen peaks at 6.64-7.99 δ ppm, signifying its coordination with themetal complexes. Moreover, a singlet was observed at 7.23-8.48 δ ppm, indicating thepresence of a proton on the unsaturated position of the chalcone ligand. Interestingly, the proton on the unsaturated carbon of the metal complexes remained unchanged upon complexation. These NMR results further supported the coordination of the chalcone ligand with the metal atoms in the formed complexes.

Table 1: Physicochemical properties of the synthesized derivatives

Comp.	Mol. Formula	Mol. wt.	Colour	R*f*	Yield (%)	M. pt. (°C)
C1	$C_{16}H_{15}NO_3$	269.30	Mustard yellow	0.65[a]	60.99	92-94
C2	$C_{16}H_{16}N_2O$	252.31	Orange	0.58[a]	58.41	80-83
KP1	$C_{30}H_{27}N_3O_2Co$	520.64	Dark black	0.55[a]	42.77	208-210
KP2	$C_{30}H_{27}N_3O_2Cu$	524.26	Black	0.63[a]	39.88	220-222
KP3	$C_{30}H_{27}N_3O_2Ni$	519.40	Light green	0.57[b]	46.56	201-203
KP4	$C_{30}H_{27}N_3O_2Zn$	526.92	Light grey	0.54[b]	42.12	199-201
KP5	$C_{32}H_{30}CoN_2O_6$	597.51	Black	0.65[a]	48.30	207-209
KP6	$C_{32}H_{30}CuN_2O_6$	601.13	Black	0.72[a]	40.80	220-222
KP7	$C_{32}H_{30}NiN_2O_6$	597.27	Dark black	0.67[b]	41.19	213-215
KP8	$C_{32}H_{30}ZnN_2O_6$	603.96	Brown-black	0.58[b]	38.99	217-219

TLC Mobile phase: benzene: ethylacetoacetate (7:3)[a] ethyl acetate: n-hexane (2:3)[b]

2.2. Antimicrobial screening results

The antimicrobial activity of the synthesized compounds was evaluated usingthe broth microdilution method in a 96-well microtitre plate. The minimum inhibitoryconcentration (MIC) was determined in micrograms per milliliter (µg/ml). Standard drugs(cefadroxil, and fluconazole) were used for comparison (Table 4, Fig. 7, and 8). The synthesized compounds exhibited promising antimicrobial activity againstvarious strains, including Gram-positive bacteria (*B. subtilis*), Gram-negative bacteria(*E. coli*) and fungal strains (*A. niger* and *A. terrus*).

Among the synthesized derivatives, complex **KP1** demonstrated good antifungal activity, with a MIC of 1.56 µg/ml against *A. terrus* and *E. coli*. Complex **KP7** showed significant activity against E. coli and B. subtilis, with a MIC 5of 3.12 µg/ml. Compound **C2** and metal complex **KP2** displayed notable activity against B. subtilis, with a MIC of 3.12 µg/ml. Additionally, they exhibited activity against both fungal strains, with a MIC of 3.12 µg/ml. The observed increase in antimicrobial activity could be attributed to the presence of an additional alkene (C=C) linkage in the metal complexes. This linkage might be involved in the binding of the antimicrobial target, thereby enhancing the efficacy of the compounds against the tested strains.

Table 2: Spectral characteristic of synthesized derivatives:

Comp.	IR (ATR, cm^{-1})	^{1}H-NMR (DMSO), δ ppm
C1	[1542.47 (C=C str.), 3027.35 (C-H str.), 1688.86 (C=N str.) of aromatic ring],1027.29 (C-O str.), 1756.69 (C=O), 2900.78 (CH_3 str.)	6.94-7.22 (3H, m of Ar-H), 6.70-7.70(2H, s of CH=CH), 3.83 (6H, s of CH_3), 7.97-8.85 (4H, s, pyridine ring)
C2	[1524.05 (C=C str.), 3026.69 (C-H str.), 1693.75 (C=N str.) of aromatic ring],1090.99 (C-O str.), 1780.41 (C=O), 2884.90 (CH_3 str.), 1090.99 (C-N str.)	6.71-7.72 (3H, m of Ar-H), 6.70-7.70(2H, s of CH=CH), 3.06 (6H, s of CH_3), 7.90-8.85 (4H, s, pyridine ring)
KP1	[1542.47 (C=C str.), 3022.48 (C-H str.), 1696.93 (C=N str.) of aromatic ring],1766.43 (C=O), 2884.76 (CH_3 str.), 692.31 (M-N str.), 824.29 (M-O str.), 1165.62 (C-N str.)	
KP2	[1524.70 (C=C str.), 3027.32 (C-H str.), 1692.66 (C=N str.) of aromatic ring],1748.28 (C=O), 2885.41 (CH_3 str.), 692.19 (M-N str.), 815.22 (M-O str.), 1094.97(C-N str.)	
KP3	[1548.23 (C=C str.), 3061.11 (C-H str.), 1675.13 (C=N str.) of aromatic ring],1748.28 (C=O), 2906.42 (CH_3 str.), 677.91 (M-N str.), 815.16 (M-O str.), 1004.13(C-N str.)	
KP4	[1596.38 (C=C str.), 3190.23 (C-H str.), 1653.22 (C=N str.) of aromatic ring],1748.28 (C=O), 2854.40 (CH_3 str.), 670.90 (M-N str.), 815.01 (M-O str.), 1059.11(C-N str.)	
KP5	[1516.42 (C=C str.), 3020.14 (C-H str.), 1693.31 (C=N str.) of aromatic ring], 1087.79 (C-O str.), 1758.67 (C=O), 2884.40 (CH_3 str.), 813.42 (M-O str.), 698.27 (M-N str.)	6.94-7.22 (16H, m Ar-H), 5.67-6.79 (4H, s of CH=CH), 3.83 [(6H, s of $CH_3)_2$], 7.18-8.86 (8H, s of pyridine ring)
KP6	[1591.42 (C=C str.), 3086.92 (C-H str.), 1653.09 (C=N str.) of aromatic ring], 1006.77 (C-O str.), 1780.36 (C=O), 2892.45 (CH_3 str.), 818.36 (M-O str.), 694.53 (M-N str.)	6.94-7.22 (16H, m Ar-H), 5.64-6.82 (4H, s of CH=CH), 3.90 [(6H, s of $CH_3)_2$], 7.25-8.95 (8H, s of pyridine ring)
KP7	[1518.92 (C=C str.), 3023.58 (C-H str.), 1653.19 (C=N str.) of aromatic ring], 1088.05 (C-O str.), 1757.31 (C=O), 2884.98 (CH_3 str.), 954.09 (M-O str.), 695.03 (M-N str.)	6.88-7.15 (16H, m Ar-H), 5.62-6.34 (4H, s of CH=CH), 3.83 [(6H, s of $CH_3)_2$], 7.18-8.86 (8H, s of pyridine ring)
KP8	[1516.15 (C=C str.), 3016.02 (C-H str.), 1692.69 (C=N str.) of aromatic ring], 1087.47 (C-O str.), 1750.07 (C=O), 2885.57(CH_3 str.), 815.61 (M-O str.), 698.41 (M-N str.)	6.94-7.22 (6H, w Ar-H), 5.67-6.79 (4H,w of CH=CH), 3.83 [(6H, s of $CH_3)_2$], 7.18-8.86 (8H, w of pyridine ring)

Among the synthesized derivatives **KP7** showed significant antimicrobial activity which may be used as a prime complex to develop better antimicrobial agents.

Table 4: Antimicrobial screening results of the synthesized derivatives (MIC=µg/ml.)

Compounds	Bacterial strains		Fungal strains	
	Gram positive	Gram negative		
	B. subtilis	*E. coli*	*A. niger*	*A.terrus*
C_1	12.5	6.25	12.5	12.5
C_2	**3.12**	12.5	**3.12**	**3.12**
KP_1	25	**1.56**	**3.12**	**1.56**
KP_2	**3.12**	12.5	25	3.12
KP_3	6.25	3.12	6.25	12.5
KP_4	12.5	50	12.5	6.25
KP_5	12.5	12.5	25	25
KP_6	12.5	25	6.25	12.5
KP_7	**3.12**	**3.12**	12.5	25
KP_8	12.5	12.5	6.25	12.5
Standard	**3.12[a]**	**1.56[a]**	**3.12[b]**	**3.12[b]**

Std. drug =Cephadroxil[a], Fluconazole[b], **MIC**=Minimum Inhibitory Concentration

1.. Antioxidant activity

The inhibition percentage (%) of free radicals in the DPPH assay is calculated based on the ability of the tested compounds to scavenge the DPPH free radical. The assay measures the reduction of DPPH by hydrogen-donating species present in the compounds. Initially, the DPPH solution appears as a dark violet color, but when it reacts with compounds that possess antioxidant activity, the DPPH is reduced, resulting in a change of color from violet to yellow. The absorbance of the reaction mixture is measured at 517 nm using UV spectrophotometry. To determine the inhibition percentage, different dilutions of the synthesized compounds are prepared with variable concentrations, along with a standard drug (ascorbic acid) for comparison. Each dilution is mixed with the DPPH solution and allowed to react in a dark environment at room temperature for a specified period. After the reaction, the absorbance of the reaction mixture is measured at 517 nm. The inhibitory concentration of the compounds is inversely proportional to their free radical scavenging potential. In other words, a lower inhibitory concentration indicates a higher ability to scavenge free radicals (Mukherjee *et a*l., 2012). The inhibition percentage is then calculated by comparing the absorbance of the tested compounds with that of the blank reference, which consists of DPPH and methanol. By assessing the inhibition percentage, researchers can evaluate the antioxidant potential of the synthesized compounds and determine their effectiveness in scavenging free radicals, which is beneficial in combating oxidative stress and reducing the risk of chronic illnesses such as cardiovascular disease and cancer. Inhibition percentage(%) of free radical of DPPH is calculated by the below equation-% inhibition **= [(Acontrol – Asample)/ Acontrol x100]**

where $A_{control}$ = Absorbance of control A_{sample} = Absorbance of sample.

The calculation of percentage (%) inhibition is calculated from the above equation and 50% inhibitory concentration value of newly synthesized metal complexes was determined using the plotted graph between concentration of test/standard and % inhibition. Outocme of antioxidant evalaution revealed, metal complex KP_2 exhibit excellent antioxidant activity with IC_{50} value of 48.46%

Table 5 : Antioxidant acvity of newly synthesized metal complexes

Analogs	Percentage Inhibition				IC50
	(25)µg/ml.	(50) µg/ml.	(75)µg/ml.	(100)µg/ml.	
C1	15.62	28.56	42.34	57.28	87.81
C2	21.03	33.65	48.56	75.62	69.89
KP1	15.71	32.56	44.62	65.54	76.16
KP2	35.68	52.23	65.38	78.63	**48.46**
KP3	31.58	47.54	63.25	78.65	54.19
KP4	21.72	36.39	58.65	72.36	66.47
KP5	27.25	38.56	57.12	75.77	62.99
KP6	17.63	31.59	50.64	75.63	59.06
KP7	21.25	36.62	54.28	69.58	69.52
KP8	25.32	35.99	52.36	67.23	70.90
Ascorbic acid	37.61	56.62	67.81	85.62	**43.30**

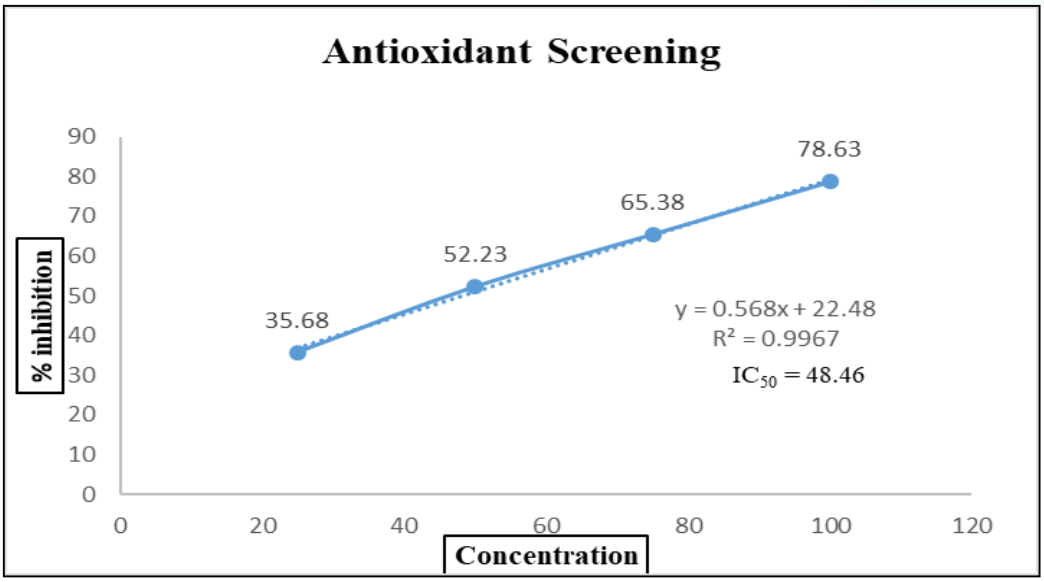


Fig. 4 : % inhibition value of most active antioxidant compound(KP2)

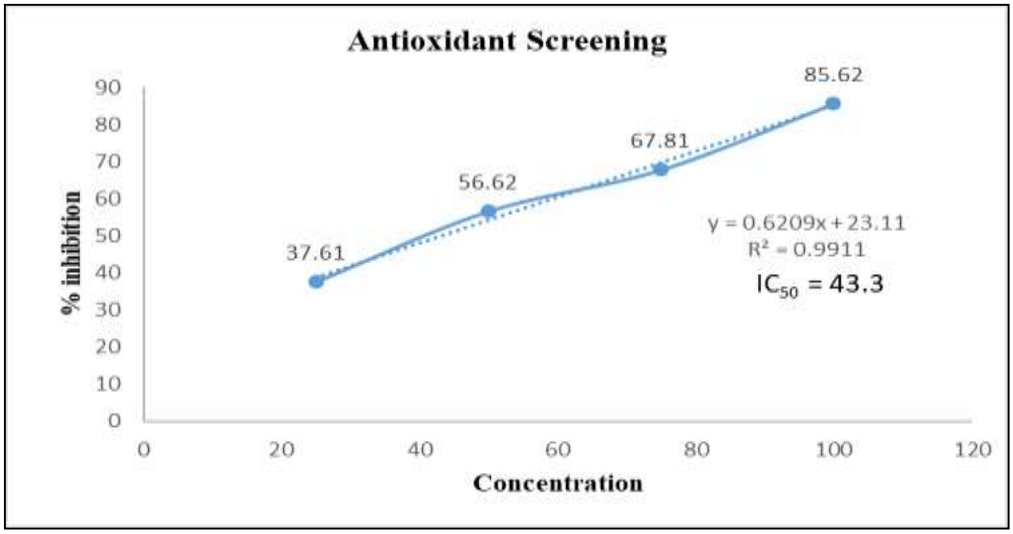


Fig. 5 : % inhibition value of standard compound i.e., Ascorbic acid

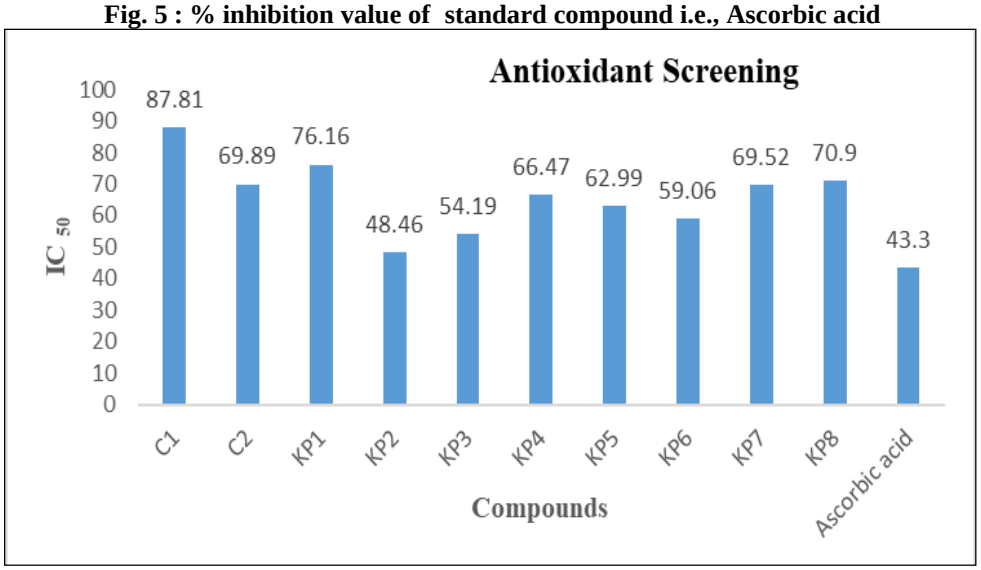


Fig. 6 : Graphical representation of IC50 of antioxidant compounds

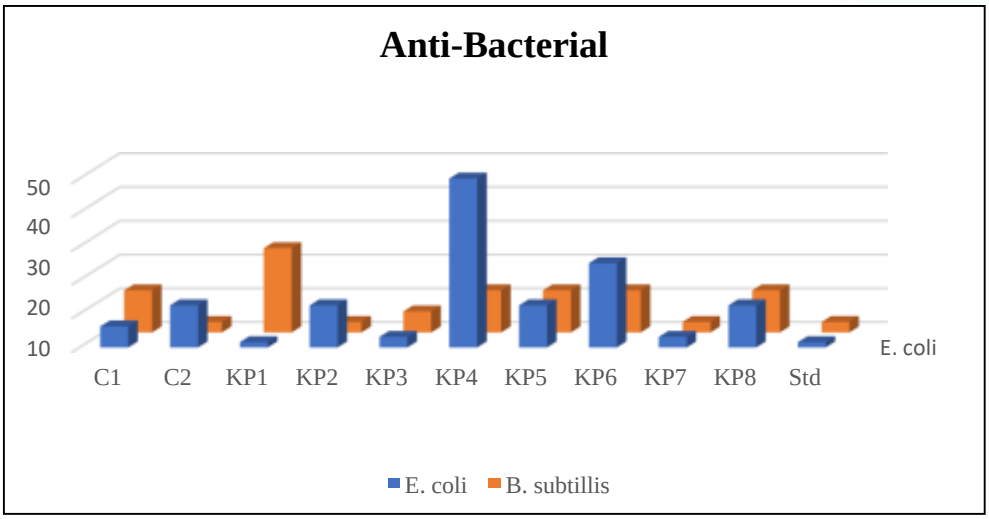


Fig.7 : Graphical representation of antibacterial activity

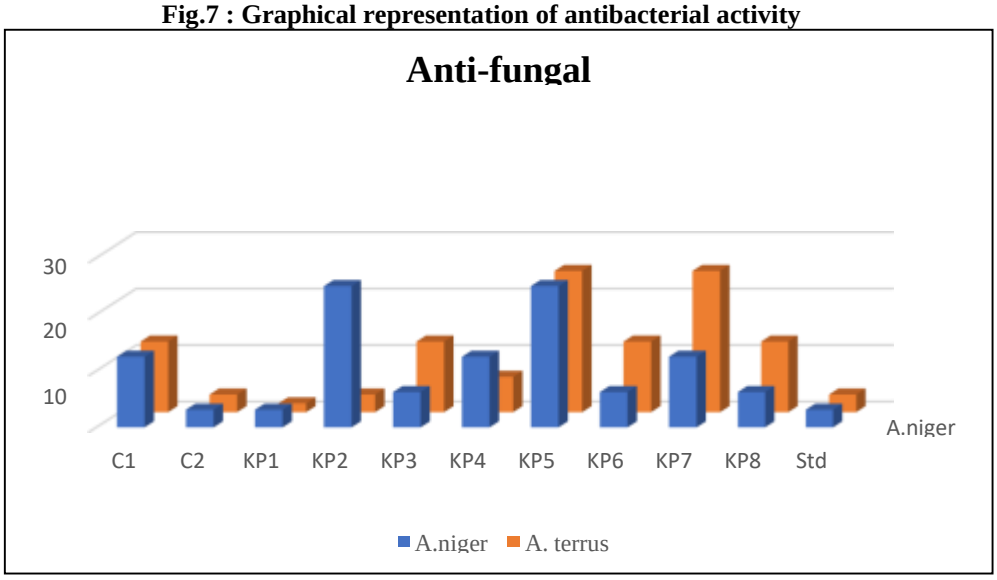


Fig. 8 : Graphical representation of antifungal activity

2.. Structure activity relationship (SAR) study

From the antimicrobial and antioxidant results, the following structure activityrelationship (Fig. 3) may be deduced:

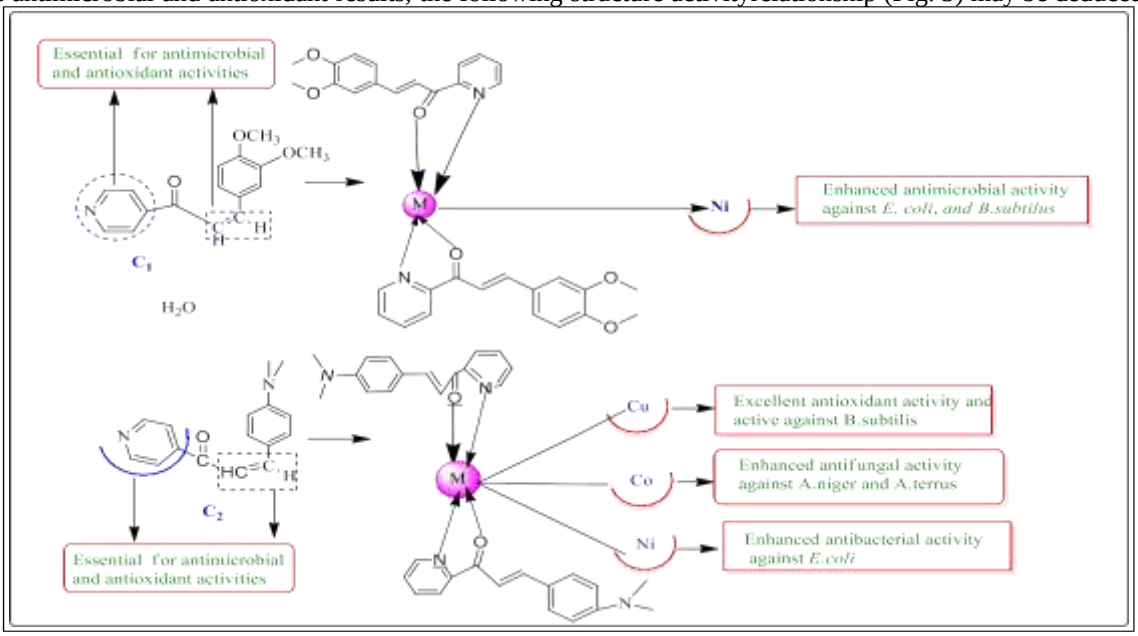
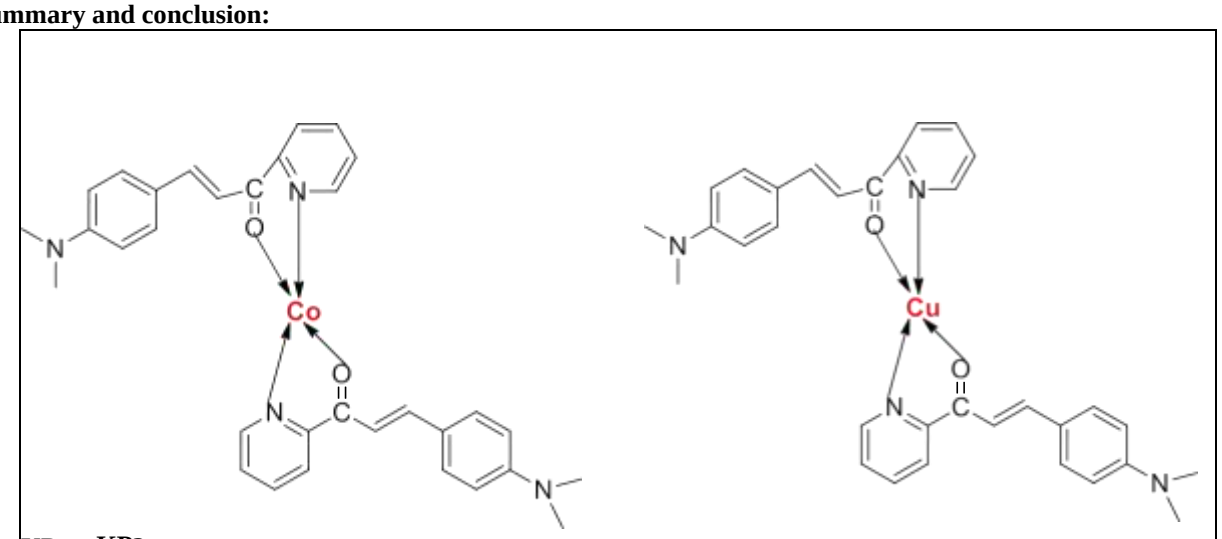


Fig. 9 : Structure activity relationship of pyridine complexes

a) Pyridine ring and alkene group are important for antimicrobial and antioxidantactivities respectively.
b) Presence of Ni(II) as transition metal improved antibacterial activity against *E.coli and B. subtilus.*
c) Presence of Cu(II) contributed to antimicrobial activity against *B. subtilis* andalso has excellent antioxidant activity.
d) Presence of Co(II) in metal complexes enhanced the antifungal potentialagainst *A. terrus* and *A. niger* .

Summary and conclusion:



KP1 **KP2**

(**MIC*ec,at*** = 1.56 µg/ml) IC50 = 76.16	(**MIC*ec*** = 3.12 µg/ml) **IC50 = 96.67 %**

Fig.10: Most active antimicrobial and antioxidant complexes

Metal complexes containing pyridine were synthesized and characterized by physicochemical and spectral means. The synthesized derivatives showed promising antibacterial and antifungal activities. The complex **KP1** exhibited promising antimicrobial activity. Antioxidant activity screening by D.P.P.H. assay method indicated that complex **KP2** had excellent antioxidant activity. It can be concluded that metal complexes**, KP1** and **KP2** (Fig. 10) may be used as lead molecules for the development of novel antimicrobial and antioxidant agents, respectively.

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