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ASSESSMENT OF ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF NOVEL TRANSITION METAL DERIVED COMPOUNDS

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Abstract

Microorganisms are present everywhere in the biosphere and part of the human normal flora where it helps in the maintenance of the homeostasis. Along with these useful microorganisms some of these are harmful to the body and pathogenic in nature. These pathogenic microorganisms causes diseases in humans and other animals. Diseases caused by these pathogenic microorganisms are treated with a variety of antimicrobial agent both from natural and synthetic sources. Antimicrobial agents from synthetic sources are in common practice and prescribed by a clinician for the treatment of infectious diseases. However, today a majority of microorganism have shown resistance to these synthetic antimicrobial agents and it is time to discover new synthetic drugs to overcome the resistance issue; otherwise, it could become a global threat. Among these synthetic antimicrobial agents reported, studies on transitional metal compounds have shown activities against a large number of microorganisms.

Method: *In the current study transitional metals derivatives were studied for the evaluation of antimicrobial potential and anticancer activity which shows that the tested transitional metal compounds has both antibacterial and antifungal activities against some strains of bacteria and fungi*

Results: *The result showed that the transitional metal compounds L2 and L3 exhibited sensitivities against Proteus Mirabilis while L3 exhibit sensitivities to Acinetobacter baumannii at highest dilution, however antimicrobial activities of the other derivatives against E. Coli showed no significant zone of inhibition. Among the 5 synthetic transitional metal compounds, L2 and L3 were found sensitive at concentrations 800 µg/ml. Transitional metal compound L3 showed a significant antifungal activity against Aspergillus flavus while no prominent antifungal activities were noted for the other derivatives. Other two strain of bacteria Candida albicans and Candida krusei were found resistant. Furthermore anticancer activity was carried out by using HepG2 and Vero cell lines respectively however nonsignificant cytotoxic activity of all five transitional metal compounds against the HepG2 and Vero cell lines. The cytotoxic effect were consider mild to moderate on both cell lines.*

Conclusion: *Overall, the tested transitional metal compounds exhibited selective*

antimicrobial activity but limited anticancer potential. Further structural modifications may enhance their biological efficacy.

INTRODUCTION

Microorganisms are tiny living entities that form an active part of the biosphere and are invisible to the naked eye, requiring a microscope for their observation.. Based on structural morphology, growth characteristics and nutrient selection these microorganisms are classified into different classes including bacteria, protozoa, viruses and fungi (Lim et al., 2020). The microbes are not only present in the surrounding environment but also the active part as the normal flora of the human body where they perform some important functions for the host body (Hacquard *et al.,* 2015). Gastrointestinal normal floras help the hosts in digestion by secreting important digestive enzy mes and also help in the synthesis of an important vitamin like vitamin B-12 and vitamin-K (Eckburg*et al.,* 2005).

A medically important resistant mechanism among gram-negative bacterial species is the production of beta-lactamase enzymes against the broad-spectrum antibiotics (Livermore, 2002). Semisynthetic derivatives are made by reacting or adding functional groups to natural antimicrobials (Marshall and Arenas, 2003; Pettit et al., 2009) and are then evaluated for stability and antimicrobial activity. Their efficacy is enhanced by attaching additional groups to the core structure (Flick et al., 2017; Haug et al., 2008).

Transition metals, with incomplete d sub-shells and variable oxidation states e.g., MN (II), Fe (II), Fe (III), interact with negatively charged molecules and modulate redox systems. This has driven the development of metallo drugs for cancer (cisplatin, carboplatin, oxaliplatin) and other diseases including diabetes, ulcers, rheumatoid arthritis, inflammatory, and cardiovascular disorders (Bagchi et al., 2015). Recent studies indicate metal complexes also possess antitumor, antiviral, and antidiabetic activities, as well as diagnostic applications, with roles in cytotoxicity and anti-inflammatory effects (Sodhi et al., 2019). The study investigated five compounds, labeled L1 to L5. L1 is ethyl 2-(3-benzyl-5-phenyl-3H-1,2,3-triazol-4-yl) acetate, while L2 is 1,5-dibenzyl-1H-1,2,3-triazole. L3 corresponds to 5-benzyl-4-phenyl-4H-1,2,3-triazole, and L4 is ethyl 2-(5-phenyl-5H-1,2,3-triazol-4-yl) acetate. Finally, L5 represents the 4-OH derivative of H4.Cancer cell lines are essential in vitro models for research and therapeutic development, providing an unlimited supply of biological material (Masters, J.R.W., 2000). Cell culture refers to the maintenance of cells outside the organism. It can be primary, derived directly from tissues, or secondary, involving immortalized cells that divide indefinitely (Helgason & Miller., 2005).

Table 1. Details of cell lines

Cell Line	Morphology	Origin	Specie	Ploidy
HepG2	Epithelial	Liver	Human	Aneuploid
Vero	Epithelial	Kidney	Monkey	Aneuploid

METHODOLOGY

The synthetic metal compound (Triazole) were provide by our research collaborator to investigate the antimicrobial and anti-cancer activities.

Sample Preparation and Testing

Total of 05 samples named as (L1, L2, L3, L4, and L5) were tested. The following tests were performed to study the effect of Transitional metal compounds in selected bacterial and fungal species as well as on selected cell line:

Culture Media

Nutrient Agar: Bacterial media can be divided into three types: simple, synthetic, and complicated, and their nutritional composition varies. The chemical compositions of simple media are known and promote only the growth of non-fastidious bacteria. In complicated media, such as Tryptic Soy Broth, the specific chemical makeup is unknown. Solid nutrient agar medium, Stuart's semi-solid media, and nutrient broth liquid media are examples of bacterial media of various consistencies (Basu et al., 2015). **Procedure:** 1000 mL distilled water was poured into a beaker and heated. 28 grams of nutrient agar powder was added and mixed with a magnet until fully dissolved. The media was autoclaved at 121°C, 15 psi for 20 minutes. After cooling, 20 mL of media was poured into each petri plate and stored at 2–8°C.

Sabouraud Dextrose Agar: Fungi require media containing a strong carbohydrate source and a nitrogen source to thrive at pH 5–6 and temperature 15–37°C. Natural and synthetic fungal culture media are commonly used. Natural Medias are made of organic materials such as seeds, leaves, cornmeal, wheat germ, and oatmeal. Synthetic media contain known components (Basu et al., 2015). **Procedure:** 1000 mL distilled water was heated, and 65g Sabouraud dextrose agar powder was added and dissolved using a magnet. The media was autoclaved at 121°C, 15 psi for 20 minutes, cooled, and 20 mL was poured into each petri plate and stored at 2–8°C.

Culturing of Bacteria

The biosafety cabinet was sterilized with 70% ethanol for 30 minutes after turning it on. Colonies of *Staphylococcus aureus* were picked with swabs and streaked aseptically on nutrient agar plates. Plates were incubated at 37°C for 24 hours. The same inoculation procedure was used for other bacterial species. For sub culturing colonies of fresh *Staphylococcus aureus* culture were picked with swabs and streaked aseptically on nutrient agar plates. Plates were incubated at 37°C for 24 hours. The same procedure was used for other bacteria.

Culturing of Fungi

The biosafety cabinet was sterilized with 70% ethanol for 30 minutes after turning it on. Colonies of *Candida albicans* were picked with swabs and streaked aseptically on Sabouraud dextrose agar plates. Plates were incubated at 37°C for 24 hours. The same inoculation procedure was used for other fungal species. For subculturing, colonies of fresh *Candida albicans* culture were picked with swabs and streaked aseptically on sabouraud dextrose agar plates. Plates were incubated at 37°C for 24 hours. The same procedure was used for other fungal species.

Sample Preparation

Five different powdered samples of different colors and numbers were weighed on an analytical balance and dissolved in an appropriate amount of DMSO.

Table 2. Serial Dilution of Samples Blue [L1]

Tube. No.	Amount of Sample	DMSO Volume	Dilution Formula	Final
1	4mg (Powder stock sample)	5 mL	4 mg / 5 mL × 1000	800 µg/mL
2	2.5 ml from Tube No. 1 to Tube No. 2 (2 mg)	2.5 mL	2 mg / 5 mL × 1000	400 µg/mL
3	2.5 ml from Tube No. 2 to Tube No. 3 (1 mg)	2.5 mL	1 mg / 5 mL × 1000	200 µg/mL
4	2.5 ml from Tube No. 2 to Tube No. 4 (1 mg)	2.5 mL	0.5 mg / 5 mL × 1000	100 µg/mL

5	2.5 ml from Tube No. 2 to Tube No. 5 (1 mg)	2.5 mL	0.25 mg / 5 mL × 1000	50 µg/mL
6	2.5 ml from Tube No. 2 to Tube No. 6 (1 mg)	2.5 mL	0.125 mg / 5 mL × 1000	25 µg/mL
7	2.5 ml from Tube No. 2 to Tube No. 7 (1 mg)	2.5 mL	0.062 mg / 5 mL × 1000	12.5 µg/mL
8	2.5 ml from Tube No. 2 to Tube No. 8 (1 mg)	2.5 mL	0.031 mg / 5 mL × 1000	6.5 µg/mL

The same procedure of dilution were used for other four samples i.e. L2, L3 L4 and L5.

Soaking of Disk in Diluted Samples Containers: Disk of filter papers were placed in each 5 samples container and in each 8 diluted sample containing container and mixed appropriately.

Antibacterial Susceptibility Testing By Disk Diffusion Method

After formation of uniform bacterial lawn, the disc of the tested sample was placed on the agar and incubated at proper temperature. **Inoculation Procedures:** Biosafety cabinet was sterilized with 70% ethanol and left for 30 minutes. Placed the nutrient agar plates, swabs, diluted disks samples pin/needle and staphylococcus aureus culture plate inside the biosafety cabinets. From the labelled *Staphylococcus aureus* culture picked colonies with the help of disposable swabs and streaked on nutrient agar plates ascetically and labelled properly. **Placement of Diluted Sample Disks** By the help of Pin/Needle picked single disk one by one from each diluted L1 samples and placed on inoculated *Staphylococcus aureus* culture plates. Two cultured plates were used for single dilution to minimized error. The same procedures were used for other 4 species of bacteria. The plates were incubated at 37oC for 24 hours in incubator. The above practices were repeated for other four samples.

Antifungal Susceptibility Testing By Disk Diffusion Method

Clinical microbiology research labs frequently use AFST as a way to help in the selection of the best antifungal drug. It determines the drug concentrations necessary to inhibit a species to a particular degree, and hence offers an in vitro measure of susceptibility and resistance (Berkowet. *al.*, 2020) **Inoculation Procedure:** Turned On the biosafety cabinet and sterilized with 70% ethanol and left for 30 minutes. Placed the Sabouraud dextrose agar plates, swabs, Diluted samples disks, pin/Needle and *Candida albicans* culture plate inside the biosafety cabinets. From the labelled *Candida albicans* culture picked colonies with the help of disposable swabs and streaked on Sabouraud dextrose agar plates ascetically and labelled properly. **Placement of Diluted Sample Disks:** By the help of Pin/Needle picked single disk one by one from each diluted L1 samples and placed on inoculated *Candida albicans* culture plates. Two cultured plates were used for single dilution to minimized error. The same procedures were used for other 4 species of fungi. The plates were incubated at 37oC for 24 hours in incubator. The above practices were repeated for other four samples.

Preparation of 10x Minimal Essential Medium (MEM)

Harry Eagle invented Minimum Essential Medium (MEM), which is one of the most extensively used synthetic cell growth media. MEM is essentially a modified Basal Medium Eagle media with enhanced necessary nutrient contents. MEM has been used in the culture of a variety of monolayer-grown cells (Martins *et al.*, 2018). **Rehydration of Powder Medium:** Transferred the whole content of the medium bottle into the conical flask, intended to use for re-hydration. Added sterile triple distilled water mentioned on Media bottle. Magnet bar was placed into the flask. Sealed the flask and placed

over the magnetic stirrer at a gentle speed between 100- 200 rpm. All media contents were dissolved and the media solution became transparent. The flask was transferred into biosafety cabinet. **Filtration:** Sterile filtered assembly connected with suction pump. Media from flask poured into funnel of filter assembly and switch ON the suction pump. Filtrate was collected in sterile disposable 500 ml bottle. Mark the media as 10x MEM and stored in refrigerator at 2–8 °C.

Preparation of 10% Medium for Cell Culture

10% Growth medium is used for the revival and culturing of cell line. Fetal bovine serum is the main growth promoting regent. **Procedure:** Added 50 mL of 10X MEM to the sterile bottle at a concentration of 10%.Added 5 mL penicillin-streptomycin solution (PSS) 5 mL Amphotericin B, 5 mL, Non-essential amino acid, 3 mL L-Glutamine, 25 mL Fetal bovine serum and 407 mL, triple distilled water was added and make total 500 mL media. Added 7.5 % sodium, bicarbonate drop by drop until yellow color changes to reddish orange. The media was shaken properly and stored at 2–8 °C.

Table 3. Amounts of Ingredients required for growth mediums

S. No.	Ingredients	Amount for Growth Medium
1	10× Minimal Essential Medium (MEM)	10 mL
2	Penicillin–Streptomycin Solution	1.0 mL
3	Amphotericin-B	1.0 mL
4	Non-Essential Amino Acids	1.0 mL
5	L-Glutamine	0.6 mL
6	Fetal Bovine Serum (FBS)	10 mL
7	Triple Distilled Water (T.D.W)	Make up the volume to 100 mL
8	Sodium Bicarbonate	1–2 mL

Cell Line Revival

Cell lines is an important in vitro tools for a variety of investigations in life science, including virology, environmental toxicity, cytobiology, oncology, drug screening and development, gene expression studies, genetics, and genomics. Fish cells have an advantage over mammalian or avian cells in that they require less maintenance, multiply over a wide temperature range, and have a variable culture programme (*Yashwanth, et all 2020*). **Procedure:** The Biohazard safety cabinet was turned ON and leave for 30 minutes. The water bath was adjusted at 37° C. Inside the biosafety cabinet poured 10 ml 10% MEM into 25cm2 culture flask used for cell revival. By using safety gloves took out 1 ampoule of preserved Vero cell line from liquid Nitrogen cylinder and gently thaw in water bath. After complete thawing the ampoule were immediately disinfect and transferred into biohazard safety cabinet. Inside the cabinet poured the whole contents of ampoule into the culture flask containing 10 % MEM using 5mL pipette. The flask was Incubate at 37o C in incubator for 4 Hours. **Observation** After 4 hours the flask was observed under inverted microscope. It was observed that cell attached with the surface of flask. **Change Media** the Media from the flask was discarded carefully and new 10 mL media was poured into the flask. The flask was again incubated at 37o C in incubator for next 24 hour to complete monolayer.

Sub Culturing of Cell Line

A technique use for the enhancement of cell growth under specified media and proper temperature for certain time period (Ammerman *et al.,* 2008). **Procedure:** The cell culture growth medium, 1X Trypsin-EDTA, and PBS were pre-warmed at 37°C. After disinfection, all materials were placed in the Biohazard Safety Cabinet. The culture medium was decanted, and the cell monolayer was washed twice with PBS. Then, 2.5–3 mL of 1X Trypsin-EDTA was added and

spread evenly. The flask was left in the cabinet for 60 seconds, the trypsin decanted, and then incubated at 37°C for 10 minutes. Detached cells appeared as a milky suspension and detachment was confirmed under an inverted microscope. 5mL of growth medium were added, and the cell suspension was pipetted to obtain single cells. 1mL of this suspension was transferred into five 25 cm^2 flasks containing 10% medium. Each flask was labeled with the cell name, passage number, and date, then incubated at 37°C. The medium was replaced after 24 hours, and after another 24 hours of incubation, a complete cell monolayer was observed.

Culturing of Cell Line on Microtiter Plates

Procedure: The growth medium from five culture flasks was discarded into a beaker. The cell monolayer was washed with PBS, trypsinized, and re-suspended in 3.5 mL of 10% MEM. Cells were counted using an improved neubauer Chamber, and the concentration was adjusted to 5000 cells/50 µL. The suspension was stored at 2–8°C. **Preparation of Micro titration Plates:** Twenty milliliters of 10% MEM were placed in a sterile reservoir, 200 µL of medium was added to each well using a multichannel pipette. Then, 50 µL of cell suspension was added to each well except the last eight. Plates were sealed and incubated at 37°C for 24 hours. After observing the cell monolayer under an inverted microscope, the medium was decanted and replaced with 100 µL of fresh medium per well. **Test Procedure:** Microtiter plates were labeled according to sample numbers in triplicate. Using a micropipette, 20 µL of Sample L1 (800 µg/mL) was added to the first three wells, followed by 20 µL of 400 µg/mL dilution to the next three wells. The same steps were repeated for other dilutions of L1 and for samples L2, L3, L4, and L5, as shown in Table. Plates were incubated at 37°C.

Table 4. Pattern of ELISA on HepG2 cell line

Dilutions			Dilutions			
L1 Sample	L1 800 µg/mL	L1 400 µg/mL	L1 200ug/ml	L5 800 µg/mL	L5 400 µg/mL	L5 200 ug/ml
	L1 800 µg/mL	L1 400 µg/mL	L1 200ug/ml	L5 800 µg/mL	L5 400 µg/mL	L5 200ug/ml
	L1 800 µg/mL	L1 400 µg/mL	L1 200ug/ml	L5 800 µg/mL	L5 400 µg/mL	L5 200ug/ml
L2 Sample	L2 800 µg/mL	L2 400 µg/mL	L2 200ug/ml	Cell Control	Cell Control	Cell Control
	L2 800 µg/mL	L2 400 µg/mL	L2 200ug/ml	Cell Control	Cell Control	Cell Control
	L2 800 µg/mL	L2 400 µg/mL	L2 200ug/ml	Cell Control	Cell Control	Cell Control
L3 Sample	L3 800 µg/mL	L3 400 µg/mL	L3 200ug/ml	Blank	Blank	Blank
	L3 800 µg/mL	L3 400 µg/mL	L3 200ug/ml	Blank	Blank	Blank
	L3 800 µg/mL	L3 400 µg/mL	L3 200ug/ml	Blank	Blank	Blank
L4 Sample	L4 800 µg/mL	L4 400 µg/mL	L4 200ug/ml			
	L4 800 µg/mL	L4 400 µg/mL	L4 200ug/ml			
	L4 800 µg/mL	L4 400 µg/mL	L4 200ug/ml			

L5 Sample | Cell Control | Blank

MTT Assay

Added 20 µL of MTT solution (5 mg/mL in PBS) into each well except the blank and incubated at 37° C for 4 hours. After 4 hours of incubation all the contents from plates were replaced by 200 µL of DMSO and 20 µL of Glycine buffer and incubate the plate at 37° C in incubator for 30 minutes.

ELISA Test

Microtiter plate was placed in ELISA plate reader and set the wavelength at 570 nm. The absorbance of samples was taken and cell survival was determined by given formula.

Percentage of Cell Survival= D/E × 100
C= absorbance of control group
T= absorbance of test group
B= absorbance of blank
D= T-B
E= C-B

1. RESULTS & STATISTICS

Five different synthetic transitional metal compounds were tested for antibacterial, antifungal and anticancer activities.

3.1 Antimicrobial Activity

The antimicrobial activities of synthetic transitional metal compounds were tested against pathogenic strains of bacteria and fungi as mentioned in methodology section.

3.2 Antibacterial Activity of Transitional Metal Compounds

No synthetic transitional metal compounds showed sensitivities against the pathogenic strains of bacteria including *Staphylococcus* aureus, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *E. coli* while the transitional metal compounds exhibited sensitivities against the two species *Proteus mirabilis* and *Acinetobacter baumannii*.

Table 5. Antibacterial activity of synthetic transitional metal compoundsL1, L2, L3, L4 and L5 against Proteus mirabilis.

Dilution in µg/ml	**800µg/ml**	**400µg/ml**
Bacteria	*Proteus mirabilis*	*Proteus mirabilis*
Positive Control	24 mm Zone of inhibition	20 mm zone of inhibition
Negative control	0 mm zone	0 mm zone
L1	0 mm zone	0 mm zone
L2	14mm zone	05 mm zone
L3	15 mm zone	07 mm zone
L4	0 mm zone	0 mm zone
L5	0 mm zone	0 mm zone
Dilution in µg/ml	800µg/ml	400µg/ml
Bacteria	*Proteus mirabilis*	*Proteus mirabilis*
Positive Control	100%	100%
Negative control	0 mm zone	0 mm zone
L1	0	0
L2	70%	25%
L3	75%	355%
L4	0%	0%
L5	0%	0%
Mean	5.8000	2.4000
Std. Deviation	7.94984	3.36155
Std. Error Mean	3.55528	1.50033

P Value	0.156	0.003

Proteus species were found good sensitive (14mm, 15mm) to L2 and L3 at highest dilution 800 µg/mL and 400 µg/mL while no activities of L1, L4 and L5 were observed against proteus species at dilution 800 µg/mL as shown. By applying t-test statistical analysis showed significant value p=0.156 at dilution 800µg/ml and non-significant value p=0.003 at dilution 400µg/m. It is concluded that the sample results at dilution 800µg/m is effective while the result at dilution 400µg/ml were not effective against *Proteus mirabilis*.

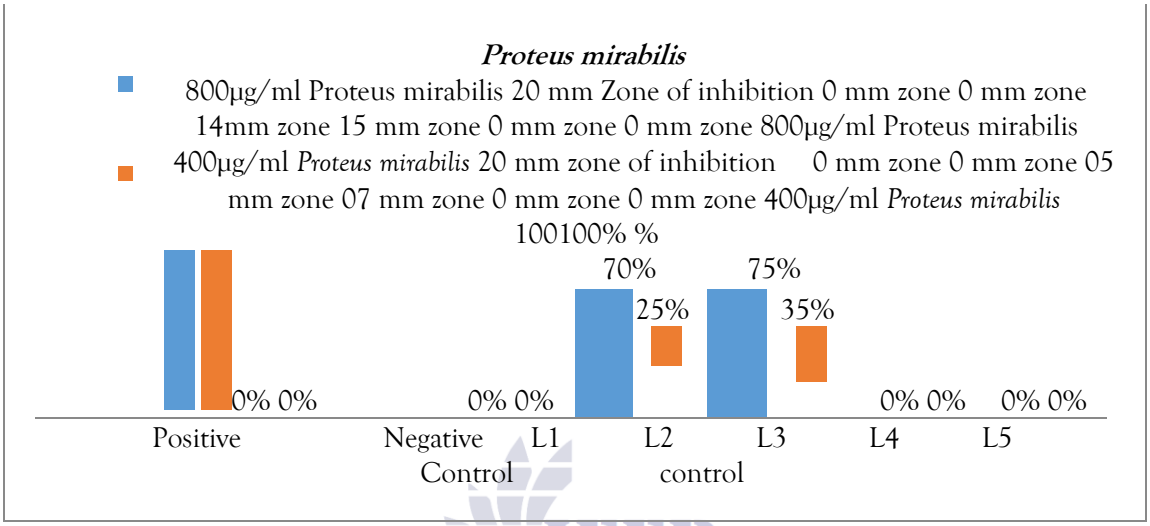

Figure 3.1 Antibacterial activity of synthetic transitional metal compounds against Proteus mirabilis.

In figure 3.1 the percentage measurement of zone of inhibition of L2 and L3 at 800 µg/ml dilution were 70% and 75% and on 400µg/ml dilution were 25% and 35% systematically. No zone of inhibition in sample L1, L4 and L5 found as indicated in table and figure respectively.

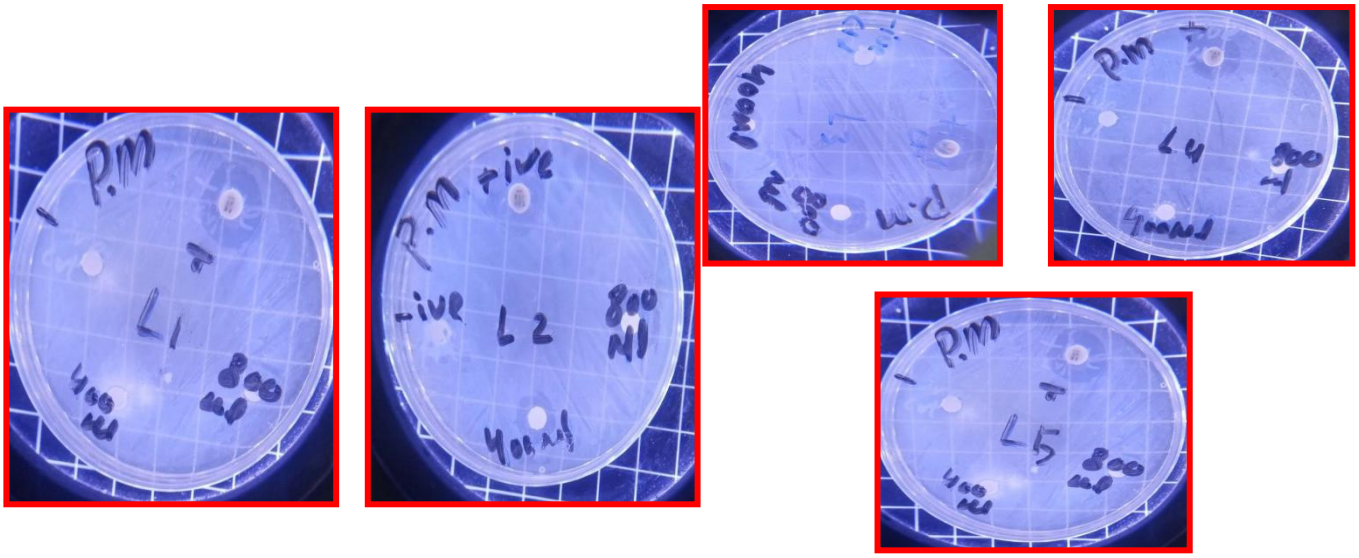

Figure 3.2 showed sensitivities pattern of Proteus mirabilis species to L1, L2, L3, L4 and L5 synthetic transitional metal compounds. Proteus specie shown sensitivity to L2 and L3 Transitional metal compounds while L1, L4 and L5 were found resistant.

Table 6. Antibacterial activity of synthetic transitional metal compounds L1, L2, L3, L4 and L5 against E. coli species.

Dilution in µg/ml	**800µg/ml**	**400µg/ml**
Bacteria	*E.coli*	*E.coli*
Positive Control	20 mm Zone of inhibition	20 mm zone of inhibition
Negative control	0 mm zone	0 mm zone
L1	0 mm zone	0 mm zone
L2	0 mm zone	0 mm zone
L3	0 mm zone	0 mm zone
L4	0 mm zone	0 mm zone
L5	0 mm zone	0 mm zone
Dilution in µg/ml	800µg/ml	400µg/ml
Bacteria	*E.coli*	*E.coli*
Positive Control	100%	100%
Negative control	0%	0%
L1	0%	0%
L2	0%	0%
L3	0%	0%
L4	0%	0%
L5	0%	0%
Mean	0.0000	0.0000
Std. Deviation	0.0000	0.0000
Std. Error Mean	0.0000	0.0000
P Value	0.0000	0.0000

E.coli species was found resistant to all test samples L1, L2, L3, L4 and L5 at both dilution 800µg/ml and 400µg/ml as shown in table and figure respectively. By applying t-test statistical

analysis showed non-significant value p=0.0000 at dilution 800 µg/mL and 400µg/ml. It is concluded that the sample results at dilution 800 µg/mL and 400µg/ml were not effective against *E coli*.

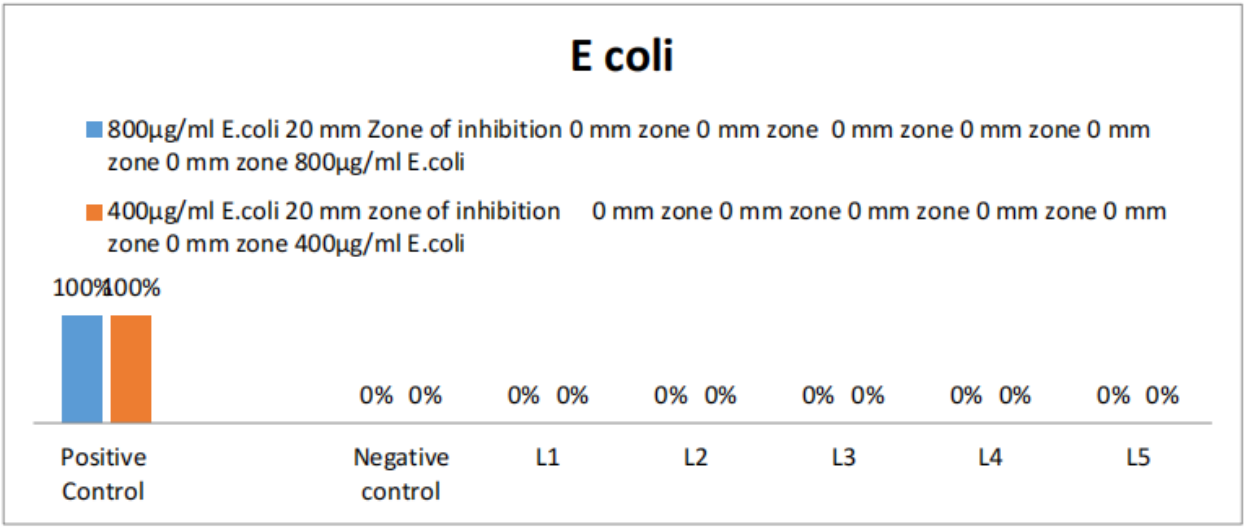

Figure 3.3 Showed antibacterial activity of synthetic transitional metal compounds L1, L2, L3, L4 and L5.

Synthetic Transitional metal compounds did not show any sensitivity at highest to lowest dilution 800 µg/mL and 400 µg/mL as indicated Figure 3.3.

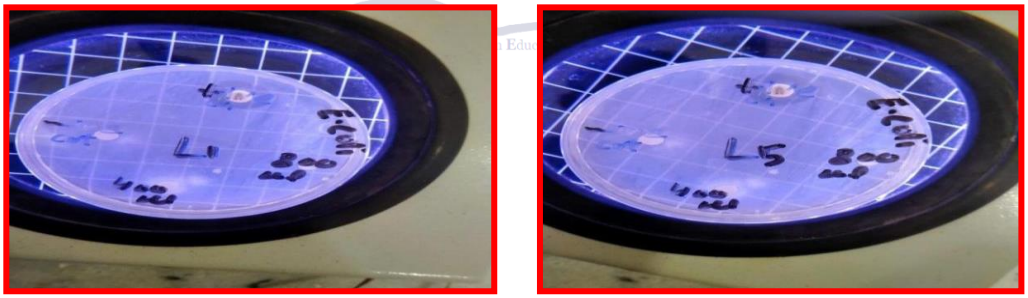

Figure 3.4 Antibacterial activity of synthetic transitional metal compounds L1, L2, L3, L4 and L5 against Acinetobacter species.

Table 7. Antibacterial activity of synthetic transitional metal compoundsL1, L2, L3, L4 and L5.

Dilution in µg/ml	**800µg/ml**	**400µg/ml**
Bacteria	*Acinetobacter baumannii*	*Acinetobacter baumannii*
Positive Control	20 mm Zone of inhibition	20 mm zone of inhibition
Negative control	0 mm zone	0 mm zone
L1	0 mm zone	0 mm zone
L2	0 mm zone	0 mm zone
L3	12 mm zone	05 mm zone
L4	0 mm zone	0 mm zone

L5	0 mm zone	0 mm zone
Dilution in µg/ml	**800µg/ml**	**400µg/ml**
Bacteria	*Acinetobacter baumannii*	*Acinetobacter baumannii*
Positive Control	100%	100%
Negative control	0%	0%
L1	0%	0%
L2	0%	0%
L3	60%	25%
L4	0%	0%
L5	0%	0%
Mean	2.4000	1.0000
Std. Deviation	5.36656	2.23607
Std. Error Mean	2.40000	1.0000
P Value	0.016	0.000

Acinetobacter baumannii was found sensitive (12mm zone of inhibition) to L3 highest dilution 800 µg/mL and (5mm zone of inhibition) at dilution 400 µg/mL while no activities of L1, L2, L4 and L5 were observed against *Acinetobacter baumanni* at dilution 800 µg/mL as shown in table

And figure respectively. By applying t-test statistical analysis showed significant value p=0.016 at dilution 800 µg/mL and non-significant value p=0.000 at dilution 400 µg/mL. It is concluded that the sample results at dilution 800 µg/mL is effective while the result at dilution 400 µg/mL were not effective against *Acinetobacter baumannii.*

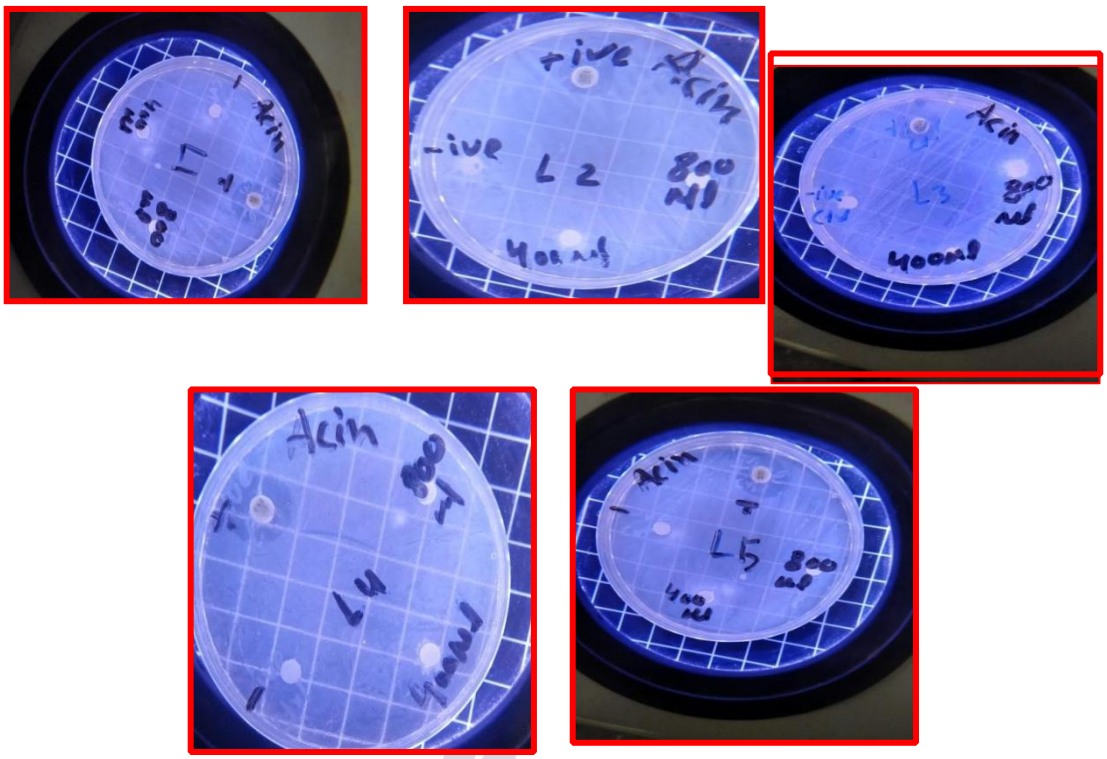

Figure 3.5 Antibacterial activity of synthetic transitional metal compoundsL1, L2, L3, L4 and L5 against Acinetobacter species.

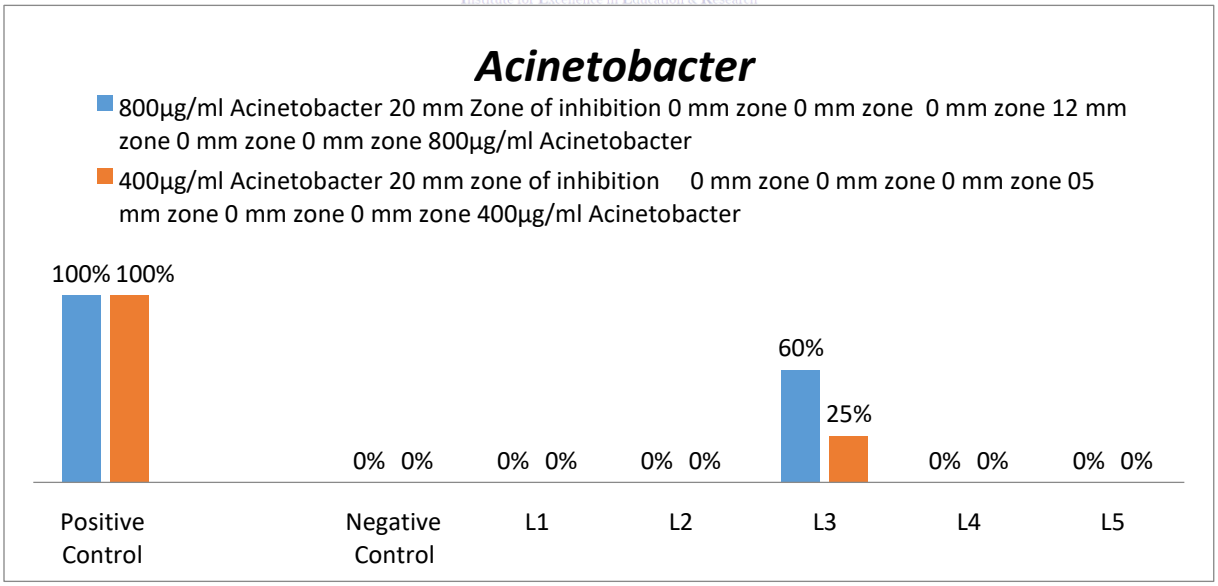

Figure 3.6 Antibacterial activity of synthetic transitional metal compounds L1, L2, L3, L4 and L5 against Acinetobacter species.

In Figure 3.6 the percentage measurement of zone of inhibition of sample L3 at 800 µg/mL dilution were 60% and on 400 µg/mL dilution were 25%. No zone of inhibition in sample L1, L2, L4 and L5 found as indicated in table and figure respectively.

ANTIFUNGAL ACTIVITY OF SYNTHETIC TRANSITIONAL METAL COMPOUNDS

Among the Three pathogenic species of fungi, only one pathogenic species *Aspergillus flavus* exhibited sensitivity against the synthetic transitional metal compounds while the rest all species, *Candida albicans* and *Candida krusei* showed resistant to the Transitional metal compounds.

Table 8. Antifungal activity of synthetic transitional metal compounds L1, L2, L3, L4 and L5 against Aspergillus flavus species.

Dilution in µg/ml	**800µg/ml**	**400µg/ml**
Fungi	***Aspergillus flavus***	***Aspergillus flavus***
Positive Control	23 mm Zone of inhibition	23mm zone of inhibition
Negative control	0 mm zone	0 mm zone
L1	0 mm zone	0 mm zone
L2	0 mm zone	0 mm zone
L3	16 mm zone	09mm zone
L4	0 mm zone	0 mm zone
L5	0 mm zone	0 mm zone
Dilution in µg/ml	**800µg/ml**	**400µg/ml**
Fungi	***Aspergillus flavus***	***Aspergillus flavus***
Positive Control	100%	100%
Negative control	0%	0%
L1	0%	0%
L2	0%	0%
L3	69.5%	39%
L4	0%	0%
L5	0%	0%
Mean	3.2000	1.8000
Std. Deviation	7.15542	4.02492
Std. Error Mean	3.20000	1.80000
P Value	0.051	0.005

Aspergillus flavus was showed good sensitivity (16mm zone of inhibition) to sample L3 at highest dilution 800 µg/mL and (9mm zone of inhibition) at 400 µg/mL while no activities of L1, L2, L4 and L5 were observed against *Aspergillus flavus* at dilution 800 µg/mL and 400 µg/mL as shown in table and figure respectively. Data analysis of t test showed significant value p=0.051 at dilution 800 µg/mL and non-significant value p=0.005 at dilution 400 µg/mL. It is concluded that the sample results at dilution 800 µg/mL is effective while the result at dilution 400 µg/mL were not effective against *Aspergillus flavus*.

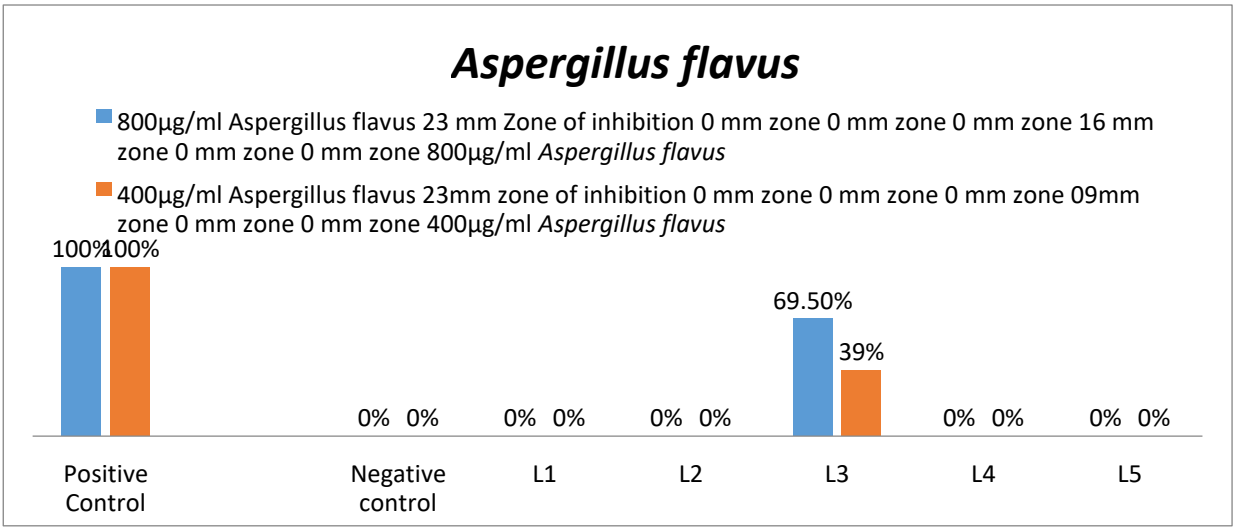

Figure 3.7 Antifungal activity of synthetic transitional metal compounds L1, L2, L3, L4 and L5 against Aspergillus flavus species in percentage form.

In figure 3.7 the percentage measurement of zone of inhibition of L3 at 800 µg/mL dilution were 69.50% and on 400 µg/mL dilution were 39% respectively. No zone of inhibition were showed by sample L1, L2, L4 and L5 as indicated in table and figure respectively.

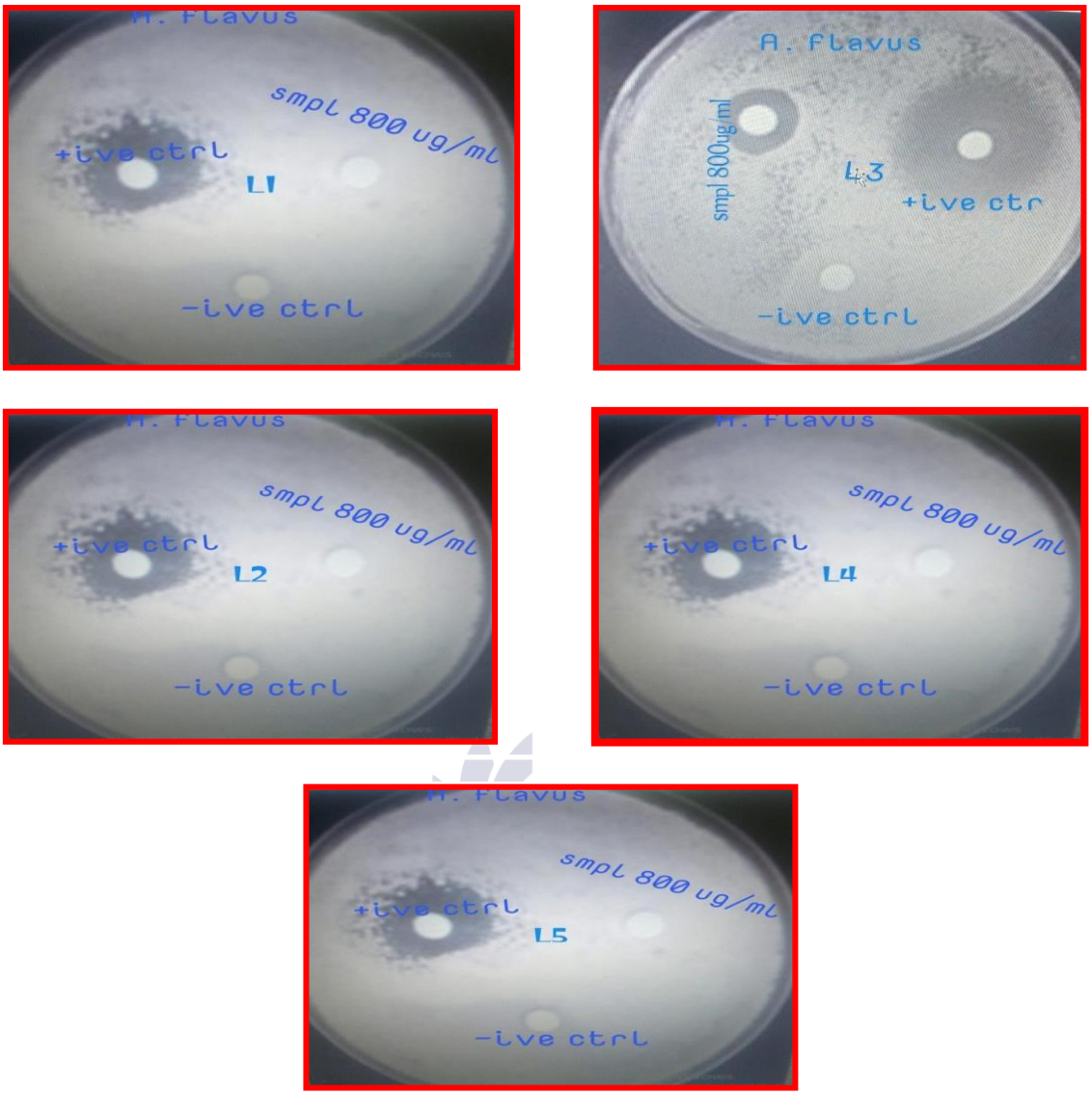

Figure 3.8 Antifungal activity of synthetic transitional metal compoundsL1, L2, L3, L4 and L5 against Aspergillus flavus species.

Among the 5 synthetic Transitional metal compounds, only L3 were found sensitive. L3 was the high sensitive compound at highest concentrations 800 µg/ml dilution while the rest dilutions showed resistant against the tested species of fungi as indicated in table and figure. L1, L2, L4 and L5 were found resistant to *Aspergillus flavus*.

Cell Line Revival

After 24 hours of incubation at 37°C intact uniform sheet of cells on the surface of cell culture plate was observed under inverted microscope.

Sub Culturing of Cell Line

After 24 hours of incubation at 37°C intact uniform monolayer sheet of Vero and Hep-G_2 cells on the surface of cell culture plates were observed under inverted microscope.

Culturing of Cell Line on Micro titration Plates
After 24 hours of incubation at 37°C intact uniform monolayer sheet of Vero and Hep-G_2 cells on the surface of microtiter plates were observed under inverted microscope.

MTT Assay
After 30 minutes of incubation at 37°C the wells of plate's changed the color and observed through naked eye. The plate was further analyzed by ELISA technique.

Cytotoxic activity of synthetic transitional metal compound by ELISA Assay
The cytotoxic effect of five different synthetic transitional metal compounds on Hep-G_2 and Vero cell lines was determined by ELISA at 570 nm wavelength. The absorbances of samples were taken in triplicate and mean of that absorbance were obtained. After complete calculation Percentage of cell survival were obtained.

Table 9. Mean absorbance of test samples and percentages of cell survival of HepG2 and Vero cell line

Sample No	Description	Absorbance 1	Absorbance 2	Absorbance 3	Mean	T-B = D	C-B = E	% Cell Survival (D/E ×100)
L1	Absorbance on Hep-G2 cell line (800 µg/mL)	0.35	0.37	0.37	0.36	0.36−0.05=0.31	0.52−0.05=0.47	**65.9%**
	Absorbance on Vero cell line (800 µg/mL)	0.30	0.34	0.32	0.32	0.32−0.05=0.27	0.52−0.05=0.47	**57.4%**
	Absorbance on Hep-G2 cell line (400 µg/mL)	0.49	0.48	0.50	0.49	0.49−0.05=0.44	0.52−0.05=0.47	**93.6%**
	Absorbance on Vero cell line (400 µg/mL)	0.48	0.45	0.47	0.46	0.46−0.05=0.41	0.52−0.05=0.47	**87.2%**
L2	Absorbance on Hep-G2 cell line (800 µg/mL)	0.38	0.40	0.39	0.39	0.39−0.05=0.34	0.52−0.05=0.47	**72.2%**
	Absorbance on Vero cell line (800 µg/mL)	0.34	0.37	0.36	0.35	0.35−0.05=0.30	0.52−0.05=0.47	**63.8%**

	Absorbance on Hep-G2 cell line (400 µg/mL)	0.50	0.46	0.48	0.48	0.48−0.05=0.43	0.52−0.05=0.47	**91.4%**
	Absorbance on Vero cell line (400 µg/mL)	0.45	0.47	0.48	0.46	0.46−0.05=0.41	0.52−0.05=0.47	**87.2%**
L3	Absorbance on Hep-G2 cell line (800 µg/mL)	0.30	0.28	0.29	0.29	0.29−0.05=0.24	0.52−0.05=0.47	**51.0%**
	Absorbance on Vero cell line (800 µg/mL)	0.42	0.46	0.43	0.43	0.43−0.05=0.38	0.52−0.05=0.47	**72.3%**
	Absorbance on Hep-G2 cell line (400 µg/mL)	0.47	0.43	0.45	0.45	0.45−0.05=0.40	0.52−0.05=0.47	**85.1%**
	Absorbance on Vero cell line (400 µg/mL)	0.50	0.51	0.49	0.50	0.50−0.05=0.45	0.52−0.05=0.47	**95.7%**
L4	Absorbance on Hep-G2 cell line (800 µg/mL)	0.38	0.39	0.40	0.39	0.39−0.05=0.34	0.52−0.05=0.47	**72.3%**
	Absorbance on Vero cell line (800 µg/mL)	0.34	0.35	0.34	0.34	0.34−0.05=0.29	0.52−0.05=0.47	**61.7%**
	Absorbance on Hep-G2 cell line (400 µg/mL)	0.49	0.48	0.50	0.49	0.49−0.05=0.44	0.52−0.05=0.47	**93.6%**

	Absorbance on Vero cell line (400 µg/mL)	0.48	0.47	0.49	0.48	0.48−0.05=0.43	0.52−0.05=0.47	**91.4%**
L5	Absorbance on Hep-G2 cell line (800 µg/mL)	0.36	0.38	0.39	0.37	0.37−0.05=0.32	0.52−0.05=0.47	**68.0%**
	Absorbance on Vero cell line (800 µg/mL)	0.40	0.39	0.41	0.40	0.40−0.05=0.35	0.52−0.05=0.47	**74.4%**
	Absorbance on Hep-G2 cell line (400 µg/mL)	0.49	0.48	0.49	0.48	0.48−0.05=0.43	0.52−0.05=0.47	**91.4%**
	Absorbance on Vero cell line (400 µg/mL)	0.46	0.48	0.47	0.47	0.47−0.05=0.42	0.52−0.05=0.47	**89.3%**
Cell Control	Absorbance of Hep-G2 cell line	0.49	0.54	0.53	0.52	0.52−0.05=0.47	0.52−0.05=0.47	**100%**
	Absorbance of Vero cell line	0.46	0.47	0.48	0.47	0.47−0.05=0.42	0.52−0.05=0.47	**100%**
Blank	Absorbance	0.04	0.07	0.06	**0.05**	–	–	–

The calculated percentages of Hep-G2 cell survival from L1 to L5 on 800 µg/mL dilution were 65.9, 72.2, 51.0, 72.3, and 68.0 while on dilution 400 µg/mL percentage of cell survival were 93.6, 91.4, 85.1, 93.6, and 91.4 respectively. The effect of L3 sample was found highest on Hep-G_2 Cell line at dilution 800 µg/mL while lowest effect of 72.3 percent was showed by L4. Other samples L1, L2 and L5 showed 65.9, 72.2, and 68.0 percent cell survival at dilution 800 µg/mL. On the other hand the effect of L1 on Vero cell was highest 57.4 percent at dilution 800 µg/mL and the lowest effect 74.4 percent showed by L5. The effect of others three samples L2, L3 and L4 on Vero cell line were 63.8, 72.3 and 61.3 respectively. The survival rate of Hep-G_2 cell line and Vero cell line on 400 µg/mL dilution were more than 85 percent.

Sample No.	HepG2 Cell Survival Percentage (%)		Vero Cell Survival Percentage (%)	
	800 µg/mL dilution	400 µg/mL dilution	800 µg/mL dilution	400 µg/mL dilution
L1	65.9	93.6	57.4	87.2
L2	72.2	91.4	63.8	87.2
L3	51.0	85.1	72.3	95.7
L4	72.3	93.6	61.3	91.4
L5	68.0	91.4	74.4	89.3
Cell line control	100	100	100	100
Blank	0.05	0.05	0.05	0.05

Table 10. Percentage of cell survival of Hep-G2 and Vero Cell line at dilution 800 µg/mL and 400 µg/mL of different synthetic transitional metal compound.

Table showed the effect of transitional metal compound on Hep-G_2 and Vero cell line and the percentage of cell survival. Sample L3 showed the highest activity on Hep-G2 cell line 51.0 percent and sample L1 showed the highest activity 57.4 percent against Vero cell line at dilution 800 µg/mL. The lowest activity was observed in case of sample L4 on Hep-G2 cell line at dilution 800 µg/mL while in case of Vero cell line the lowest activity 74.4% showed at dilution 800 µg/mL by sample L5. The remaining samples showed different range of activities to Hep-G2 and Vero cell line.

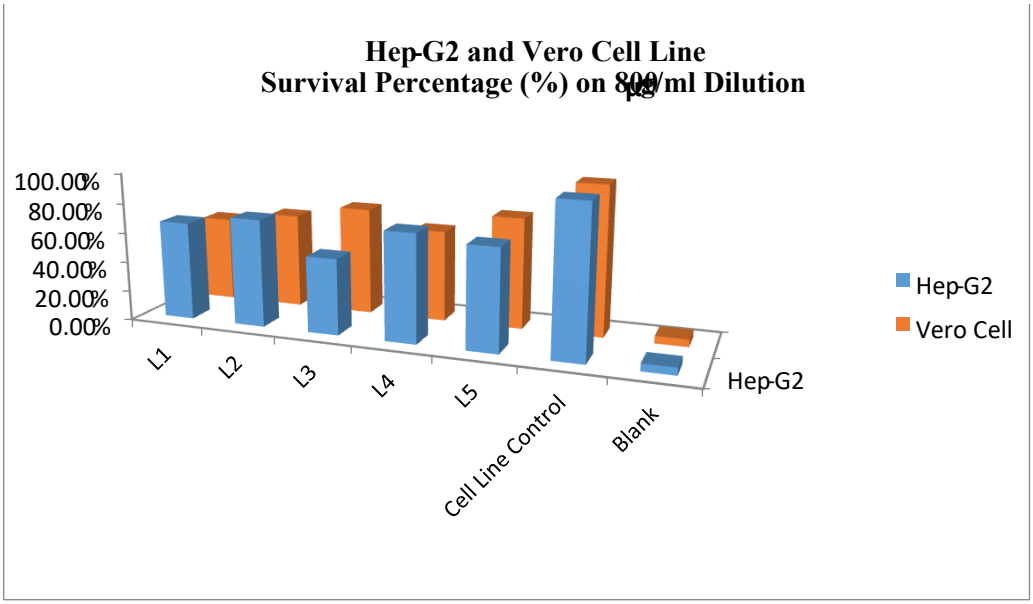

Figure 3.9 Effect of transitional metal compounds and percentage of cell survival of Hep-G_2 and Vero cell line on dilution 800 µg/mL

Showed the effect of synthetic transitional metal compound on Hep-G2 and Vero cell line at 800 µg/mL dilution .The highest percentage of Hep-G2 cell survival 91.4 on L2 and L5 were observed while the lowest percentage of cell survival of L3 sample was observed. In case of Vero cell line the highest cell survival 95.7 was observed by sample L3 and the lowest cell survival 87.2 for L1 and L2 respectively.

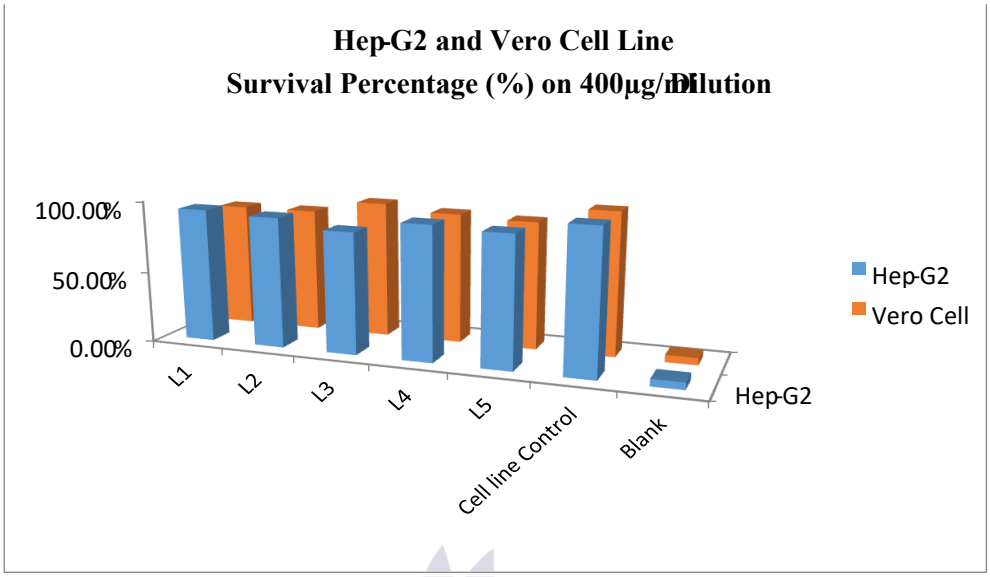

Figure 3.10 Cytotoxic activity of transitional metal compounds on Hep-G_2 and Vero cell line on dilution 400 µg/mL

Showed the comparative analysis of Hep-G_2 and Vero cell survival at dilution 400 µg/mL. Sample L3 showed the highest effect on HepG_2 cell line and the survival rate of cell line was found low 51.0 percent while the sample L4 showed less effect and the cell survival rate was higher 72.3 percent. On the other hand sample L1 showed highest effect on Vero cell line and the survival rate of cell was low 57.4 percent while the sample L5 show less effect and the survival rate of cell was high 74.4 percent. At dilution 400 µg/mL both Hep-G2 and Vero cell line showed less effect and the survival rate of cell was above 85 percent.

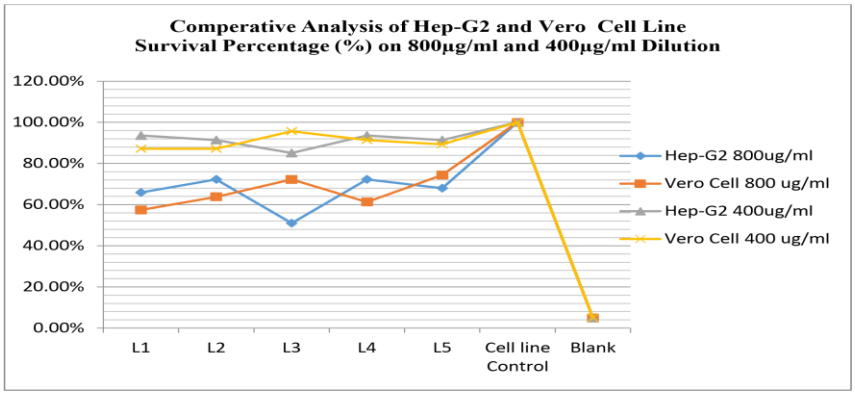

Figure 3.11 Comparative analysis of Hep-G2 and Vero cell line survival at dilution 800 µg/mL and 400 µg/mL

Indicated that cell viability of both cell line were more than 85% at dilution 400 µg/mL while the cell viability decrease up to 51.0 % in case of HepG2 cell line and 61.3 in case of Vero cell line at dilution 800 µg/mL. Survival of cell line control was much higher than other test sample. Blank showed no growth of both cell lines.

DISCUSSION

The morbidity rate of bacterial associated infections goes high and high all around the world and which alarmed and threatened the public health department (Ali *et al.*, 2020, Edlin *et al.*, 2013, Khoshnood *et al.*, 2017). This high morbidity rate is due to the emergence of resistance in bacterial strains against broad spectrum antibiotics (Gharbi *et al.*, 2019). There are many ways which help the bacterial specie to emerge high resistance against antibiotics such as self-medication, incompletion of antibiotic therapies, home medication etc. (Zafar *et al.*, 2008).

The current research study screened synthetic transitional metal compounds with different concentrations against the pathogenic species of both bacteria and fungi as well as HepG2 cells and Vero cells to check the cytotoxicity. Another previous study also screened the antimicrobial activity of transition metal complexes against pathogenic strains of fungi and bacteria (Prashanthi and Raj, 2010). Similarly, transition metal complexes of folic acid were investigated against the pathogenic species of bacteria (both gram-positive and gram-negative) and fungi (Abd ElWahed et al., 2008). Similarly, Mohamed et al., (2011) investigated the antimicrobial and cytotoxic activity of transition metal compounds against bacteria, fungi and cancer cells.

The current study considered bacterial pathogenic strains including *Acinetobacter baumannii, E. coli* and *Proteus merabilis.* A previous study supports our current study in case of tested pathogenic strains of bacteria. They also tested and investigated the antibacterial activity of synthetic metals against the *Acinetobacter baumannii, E. coli and Proteus merabilis* (Tahaa et al., 2020). Another previous study also screened the same bacterial pathogenic strains against the synthetic transition metal compounds (Chohan et al., 2004). In the current research study, the pathogenic strains of bacteria including *Proteus merabilis and Acinetobacter baumannii* showed high sensitivity against the tested synthetic transitional metal compounds while *E coli* were highly resistant. One of the previous study reported almost similar results to our study. They reported that some of the pathogenic bacterial species showed high sensitivity against the tested Transitional metal compounds (Carlson-Banning et al., 2013). Another previous study is also in line with our research study. They reported more of the bacterial species were sensitive to Transitional metal compounds while some were remained resistant (Chohan et al., 2007). Its means that the potency of Transitional metal compounds depends upon its nature and the concentration to be tested against the pathogenic strains of bacteria and fungi.

The present study screened synthetic metal compounds against the pathogenic strains of fungi including *Candida albicans, Candida Krusei and Aspergillus flavus*. Similar research was conducted by Chohan et al., (2004), they tested the same fungal species against the synthetic transition metal complexes. Another previous study also conducted similar practice against the mentioned fungal pathogenic strains in vitro. They also tested the fungal species including Aspergillus species as well as Candida species and the study compiled fruitful results in this regard (Chohan et al., 2007). In the current research study, the *Aspergillus flavus* showed maximum sensitivity to L3, when tested against the synthetic transitional metal compounds while L1, L2, L4 and L5 were showed no effect against *Aspergillus flavus.* Other fungal species *Candida albicans* and *Candida krusei* remained resistant to all five samples. One of the previous research study supported our results. They reported that the synthetic transitional metal compounds have high potency to control the growth of Candida species as well as Aspergillus species (Chen et al., 2011).

In the current study all five synthetic transitional metal compounds were tested for cytotoxic activity against the Hep-G2 and Vera cell lines. Similar research was conducted by one of the previous research study, they also inspected the cytotoxic activity of metal complexes against the tested cells (Tahaa et al., 2020).

In the current study, L3 Transitional metal compound were showed moderate cytotoxic activities against Hep-G2 cell line at highest dilution 800 µg/mL while the rest all samples were found mild cytotoxic activities which is consider non-significant. On the other hand the L1 sample were showed moderate activity against Vero cell line while the other four sample showed less activities which is non-significant. The study conducted by (Tahaa et al., 2020) to evaluate the cytotoxicity of transitional metal compound were accepted our results. But another previous study were much similar with our results and some cell lines of their study showed less cytotoxic effect (Rehman et al., 2005).

CONCLUSION

It is concluded from the present study that promising biological activities have been determined from synthetic Transitional metal compounds, including antibacterial, antifungal activities but did not show any anticancer activity. Some of these activities like antibacterial and antifungal activities can be used for identifying and synthesis of novel products in the future.

It is concluded that synthetic transitional metal compound did not show anticancer activity against the growth of tested parameter, only showed antibacterial and antifungal activity there is a possibility of using these synthetic transitional metal compounds for the antimicrobial agents and other pharmaceutical sectors.

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