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Cytotoxic and microbial studies of mixed metal complexes

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Breast cancer and its treatment have become a prominent and challenging problem today. The increasing multidrug resistance to microbial pathogens is the root cause of breast cancer. Women suffering from cancer showed high levels of *E. coli* and *S. aureus*. In the last few decades, there has been a considerable need in the medical field for the discovery of new compounds endowed with antimicrobial activity, despite the fact that several antibiotics and chemotherapy drugs are currently accessible. Substantial research was conducted, particularly on transition complexes as metal-based drugs in pharmacological applications to provide therapeutic options. The synthesis, characterization, antibacterial activity, and cytotoxic activity of copper complexes with specific ligands of amino acids such as tyrosine and arginine are discussed in this work.

Keywords: Amino acids, Anti-microbial, Breast cancer, Copper, Cytotoxicity

Among several cancers Breast Cancer is the cause for death among women worldwide[1-19]. To prevent women from being attacked with Breast cancer, drugs such as Cisplatin, Carboplatin are used for the treatment of breast cancer. Breast cancer and its treatment has become prominent and challenging problemtoday. The increasing multi drug resistance to microbial pathogens is the root cause of breast cancer. Women suffering from cancer showed high levels of *E. Coli* (gram –ve) and *S. aureus* (gram +ve). Most of the metal-based complexes have adverse effects which are dose related. Delivery of cancer drug is also a problem as it causes toxicity when absorbed in healthy tissues. Hence targeted delivery by various target ligands like amino acids or improved technology of drug delivery system enhanced by Nanotechnology can be the solutions.

Mixed-ligand complexes are biologically active than the ligand itself than its binary complexes (Malik *et al*., 1977). Inorganic complexes particularly metallic ions and chelates are essential cofactors in a variety of enzymes and proteins. Extensive research was done on metal-based drugs especially on transition metal complexes[-20-43]. The development of metal-based drugs is a promising one and it has started its pharmacological applications to offer therapeutic opportunities (Rafique *et al*., 2010). As copper compounds have anticancer role, copper organic complexes are capable of inducing cancer cell death. Out of 20 amino acids 11 non- essential amino acids play an important role in tumour assimilation. As decreased levels of L-Arginine were found in tumour bearing patients, supplementation may be a preventive for the inhibition of breast cancer. First, we synthesized and investigated copper complexes with amino acids like arginine and tyrosine. Secondly studying there in-vitro antimicrobial and anticancer activity and these two aspects have been examined in the present work.

Materials and Methods

Chemicals

All chemicals' reagents and solvents are procured from renowned companies and were of analytical grade used as received without further purification.

Instruments

IR spectra are obtained with a Shimadzu IR Prestige 21 FT-IR spectrophotometer. Electronic spectra are recorded on LABINDIA UV3000+ UV/Visible spectrophotometer. LC-MS spectra are recorded on AGILANT QQQ (ESI-MS). Mass spectrometer. TG-DTA spectra are obtained using SDT Q600 V20.9 BUILD 20.

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Synthesis of metal complex 1&2

Complex 1: An aqueous solution of Copper perchlorate is added to sodium hydroxide solution of Tyrosine and 4-Nitro pyrazole at 60°C under stirring conditions at 60°C for 30 min. Blue precipitate formed is washed with methanol and dried. Yield is about 0.35 g.

Results and Discussion

Characterization of the complex

IR Spectrum of Complex (1)

In complex1 stretching vibration of ν(N-H) correspond to peak at 3162 cm^{-1} and a band is found with medium intensity at 1416cm^{-1} due to the symmetric carbonyl stretching ν_{sym} (COO^{-}) with slightly marked shoulder at 1479 cm^{-1}. Due to the presence of crystallisation water ν(OH) stretching vibrations appears in the complex corresponding to 3422 to 3580 cm^{-1} (Fig. 1).

LC-MS Spectrum of Complex (1)

In complex1 peak at 413 (*m/z*) is due to copper bound to one tyrosine, one 4-nitro pyrazole and three water molecules [Cu(Tyr) (4-NP)3H_2O] correspond to peak at Similarly peaks at 391 (*m/z*) correspond to removal of one water molecule from the above complex [Cu(Tyr)(4-NP)2H_2O] and peak at 358 (*m/z*) correspond to complete removal of water molecules from the complex [Cu(Tyr)(4-NP)] (Fig. 2).

Electronic Spectrum of Complex (1)

The UV-VIS spectrum of the metal complex 1 with the wavelength range 200-800 nm is recorded using DMF (Dimethyl formamide) as solvent. Band at 294 nm is due to Tyrosine and at 373 nm due to 4-NitroPyrazole (Fig. 3).

TG-DTA spectrum of Complex (1)

In the complex thermal decomposition of the complex [Cu(Tyr) (4NP)3(H_2O)] takes place in two steps.In the first step, there is loss of water molecule in between 50-150°C with loss of mass 6.7% (cal. 6.8%) which is shown in DTA peak at 100°C. In the second step decomposition of Tyrosine and 4-Nitro pyrazole is found at the temperature 250-300°C with mass loss of 84.3% (cal.87.9%). In comparison to theoretical mass loss the overall loss in mass is 91%which is equal to calculated mass (Fig. 4A & B).

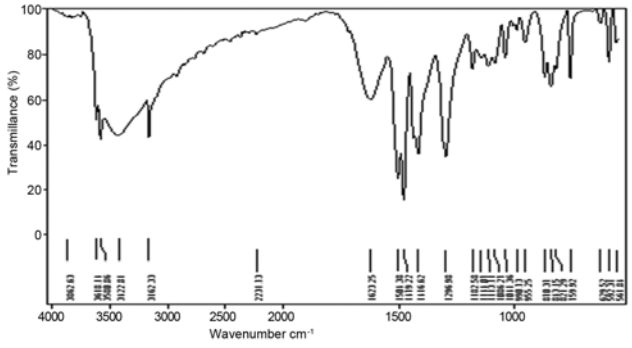

Fig 1 — IR Spectrum of Complex (1)

Antimicrobial Screening of Complex (1)

The Antimicrobial activity of the complex1 is tested for *invitro* antimicrobial properties by disc diffusion method against *E. coli*, *S. aureus* which is listed in (Table 1).

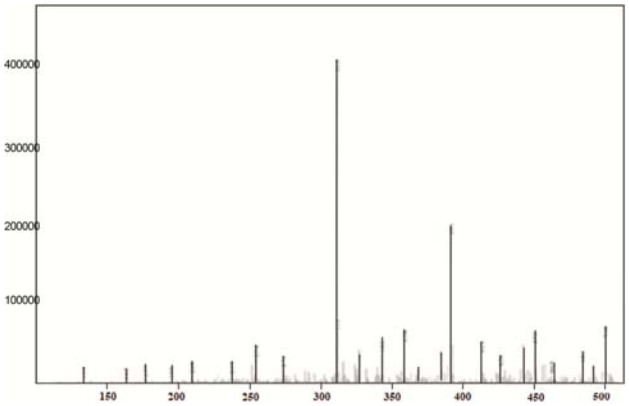

Fig 2 — LC-MS spectrum of Complex (1)

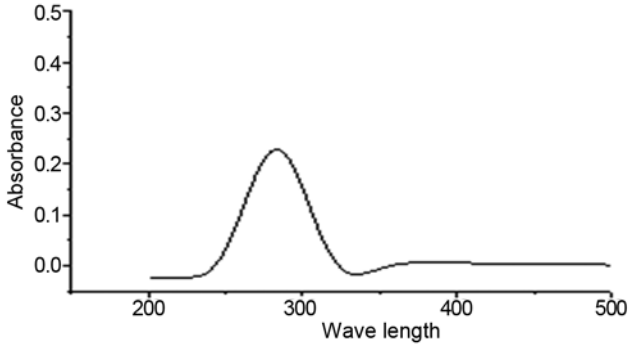

Fig 3 — Electronic Spectrum of Complex (1)

Table 1 — Inhibition zones for complex 1 in comparsion with Standard drug

Bacteria	Inhibition zone(mm)	Streptomycin
S. aureus	3	1.7
E. Coli	2	1.8

Dose response of Complex 1 on MCF-7 Cell line

Conc. (μg/mL)	%Cell Survival	%Cell Inhibition
1	95.8	4.2
10	48.28	51.72
100	14.46	85.54
500	14.21	85.79

Incubation time: 24 h

Complex 1 is tested for *in vitro* antimicrobial properties against the gram-positive bacteria *S. aureus* and gram-negative bacteria *E. Coli*. The diameter of inhibition zones is 3 and 2 mm, respectively, (Fig. 5).

Cytotoxic studies

Complex 1 is subjected to the preliminary anti proliferative activity test for their cytotoxicity against the cancer cell line MCF-7 (breast, ER positive) by MTT assay method. Complex displayed their IC_{50} (µg/mL) values of cytotoxic activities in (Table 1 and Fig. 6).

Synthesis of metal complex (2)

A methanol solution of copper nitrate solution is added toaqueous solution of Arginine and sodium azide under stirring for 30 min at room temperature. Solution is allowed for slow evaporation and blue crystals are formed in 8 days which are washed with methanol.

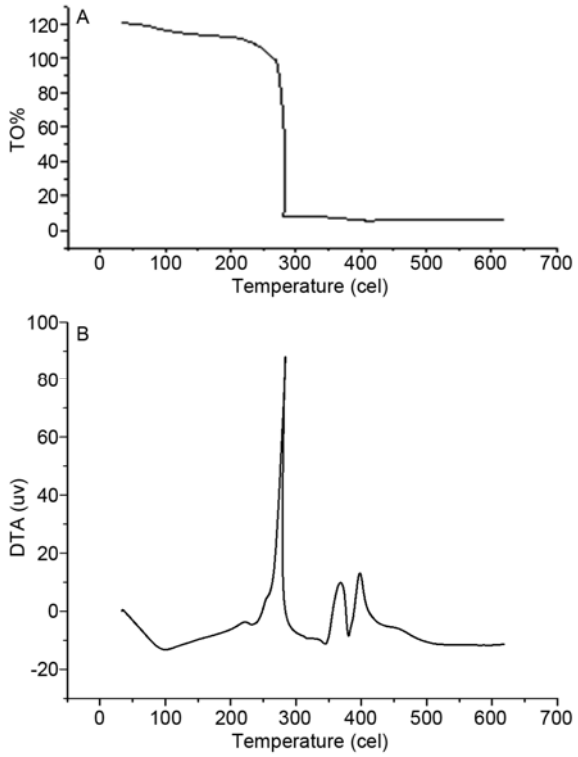

Fig 4 — (A) TG spectrum of Complex (1); and (B) — DTA spectrum of complex (1)

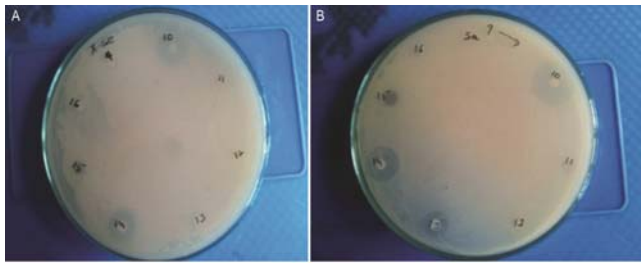

Fig 5 — Inhibition zones for complex against (A) *S. aureus*; and (B) *E. Coli*

Results and Discussion

IR Spectrum of Complex (2)

The spectrum exhibited a band at 3385 cm^{-1} due to the stretching vibration of υ(N-H) of the amine group. Due to the stretching vibrations of both symmetric and a symmetric vibrations bands are observed in between the region 1680-1350 cm^{-1}. The absence of N_3 ligand in the complex is shown due to the absence of strong peak at 2085 cm^{-1} in IR spectrum (Fig. 7).

LC-MS spectrum of Complex (2)

In complex 2 peak at 237 (*m/z*) and 410 (*m/z*) is due to copper bound to one mole of Arginine [Cu (Arg)] and two moles of Arginine [Cu $(Arg)_2$] (Fig. 8).

Electronic spectrum of Complex (2)

The UV-Visible spectrum of the metal complex 2 is recorded in Dimethyl formamide as solvent. Complex displayed two bands at 283 nm and 384 nm due to d-d transitions of Cu(II) ion in the complex (Fig. 9).

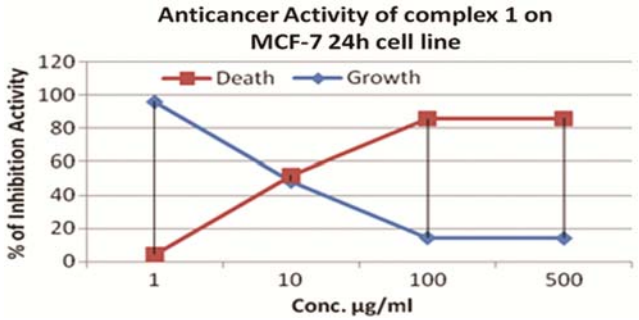

Fig. 6 — Complex displayed good cytotoxicity of IC_{50} 9.13 µg/mL

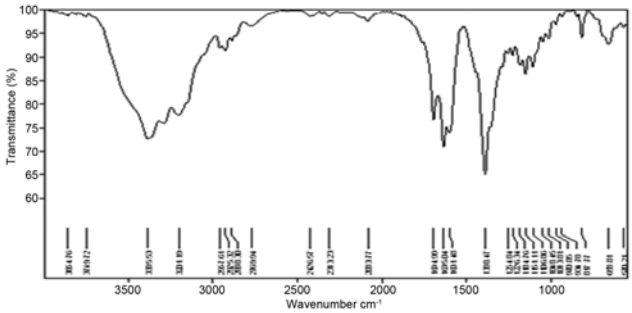

Fig 7 — IR spectrum of Complex (2)

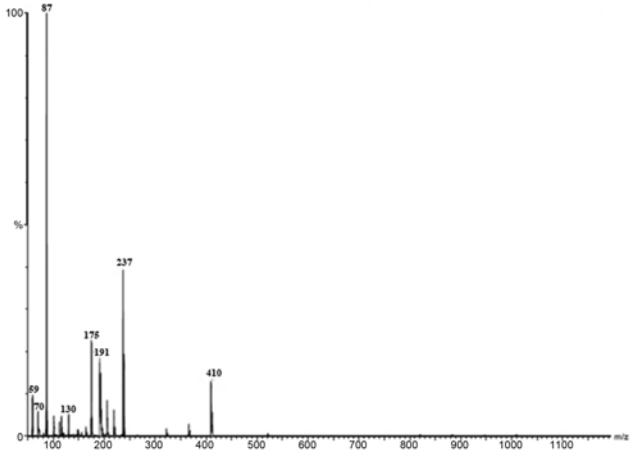

Fig 8 — LC-MS spectrum of Complex(2)

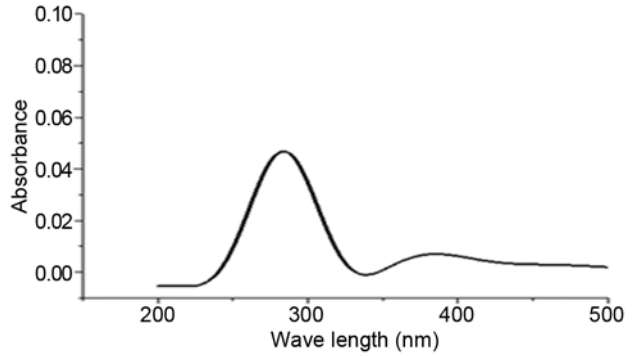

Fig 9 — Electronic spectrum of Complex (2)

TG-DTA spectrum Complex (2)

The decomposition of the complex [Cu $(Arg)_2$] on heating takes place in three steps. During the first step loss of uncoordinated water takes place in between 40-110°C with loss in mass of 8.7% (obs. 9.0%). This is in accordance with endothermic band observed in the DTA curve at 110°C. Peak is observed at 290°C in the temperature range 120-290°C which corresponds to the partial decomposition of arginine mass loss of 35% (obs. 33%). Finally, the peak observed at 460°C correspond to the decomposition of Arginine with mass loss of 43% (obs. 42.2%). The overall mass loss is 84.9% (obs. 84.1%) which is equal to theoretical value (Fig. 10A & B).

Antimicrobial screening of $[Cu(Arg)_2]$

In vitro Antimicronial activity is screened for the complex by disc diffusion method against *E. coli*, *S. aureus* and listed in (Table 2).

In complex 2 the diameter of inhibition zone is of 3 mm (Fig. 7) against *E. Coli* and *S. aureus*.

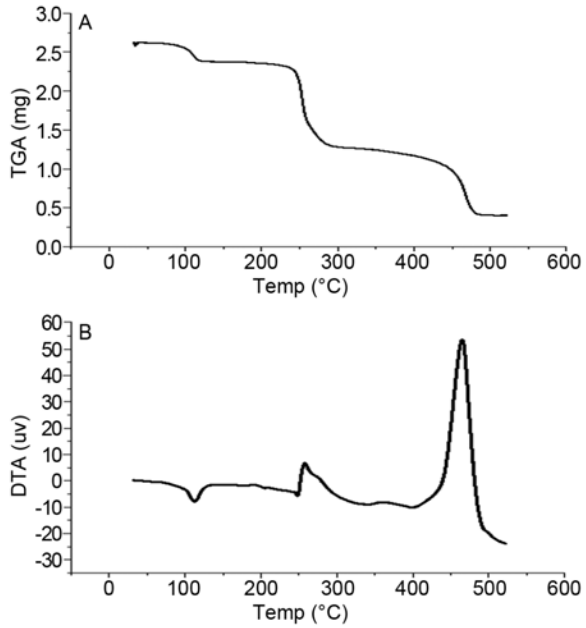

Fig 10 — (A) TG spectrum of Complex 2; and (B) DTA spectrum of complex (2)

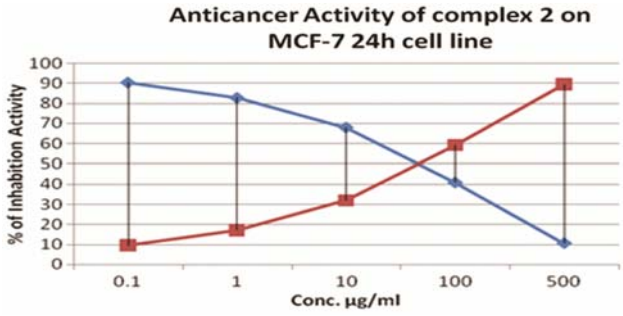

Fig 11 — Effect of complex 2 on MCF-7 Cell Viability for 24 h Incubation time

Table 2 — Inhibition zones for complex 2 in comparsion with Standard drug

Bacteria	Inhibition zone (mm)	Streptomycin
S. aureus	3	1.7
E. Coli	3	1.8

Dose response of complex 2 on MCF-7 Cell line

Conc (µg/mL)	%Cell Survival	%Cell inhibition
0.1	90.46	9.54
1	82.83	17.17
10	68.02	31.98
100	40.85	59.15
500	10.58	89.42

Incubation time: 24 h

Cytotoxic studies

The Complex was subjected to the cytotoxicity against human cancer cell line MCF-7 (breast, ER positive) by MTT assay method (Fig. 11). The IC_{50}

values against MCF-7 are listed in (Table 2). Complex (2) displayed cytotoxicity of IC_{50} 70.54 µg/mL.

Conclusion

Mixed ligand complexes are more biologically active than individual ligands. Chelation has been linked to the development and treatment of a variety of diseases, including cancer. Copper complex conjugates with amino acids like arginine and tyrosine have been shown to be highly effective against breast cancer. These complexes outperformed traditional drugs like streptomycin in terms of activity. Complexes were also shown to be cytotoxic against breast cancer cells at micro molar concentrations for 24 h, with IC_{50} values of 9.13 g/mL and 70.54 g/mL. As a result, these complexes may lead to the creation of novel metal-based medicines with antibacterial action as well as cytotoxicity. Unwanted side effects can be eliminated using improved medication delivery technologies such as nanotechnology.

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