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ANTI-BIOFILM ACTIVITY OF MIXED 'TRANSITION METAL (Mn, Fe, Co, Ni, Cu AND Zn) – CALCIUM TARTARATE' COMPLEXES

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Keywords:

Mixed metal complexes, Alkaline earth transition metal complexes, Biological activity, Anti-biofilm activity

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ABSTRACT: A series of six new, mixed transition metal and alkaline earth metal complexes of the general formulation $[MM'(C_4H_4O_6)_2.xH_2O]$ (where M= Mn, Fe, Co, Ni, Cu and Zn, M′ = Ca) are synthesized by using bidentate tartarate ligand and are characterized by different analytical techniques such as elemental analysis, TGA, FTIR, XRD, SEM, magnetic susceptibility study, UV-visible spectroscopy etc. All synthesized mixed metal complexes (sample A1 to A6) were then tested for *in vitro* anti-biofilm activity against some fresh bacterial cultures namely of *Pseudomonas aeruginosa* ATCC-27853, *E.coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923, *Klebsiella pneumoniae (Lab culture), Proteus vulgaris* (Lab culture). The Minimum Bactericidal Concentration (MBC) of these complexes was found slightly more than Minimum Inhibitory Concentration (MIC). It is found that the Biofilm Inhibition Concentration (BIC) levels of all the complexes are at higher side as compared to MIC but lower to MBC. The bio assays of all the complexes show a greater biofilm inhibition effect, than the individual tartarate ligand which indicates after the coordination the anti-biofilm activity of complexes is enhanced.

INTRODUCTION: Microbial biofilm is a community of bacteria, embedded in a self-producing matrix, forming on living and nonliving solid surfaces [1]. This formation of biofilm on biotic and abiotic surfaces is due to the ability of biofilm associated cells to adhere irreversibly on a wide variety of surfaces, including living tissues and in dwelling medical devices as catheters, valves, prosthesis and so forth [2].

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These biofilm making of microorganisms is considered an important virulence factor of bacteria that causes persistent chronic and recurrent infections; they are highly resistant to antibiotics and host immune defenses [3].

An estimated 75% bacterial infections involve biofilms which are protected by an extracellular matrix [4]. Biofilm formation can be increased due to several reasons like restricted diffusion of antibiotics into biofilm matrix, expression of multidrug efflux pumps, decreased permeability, and the action of antibiotic-modifying enzymes [5]. This increased biofilm formation and its resistance to conventional treatment enhances need to synthesize new drugs in the form of metal complexes.

In the recent years different herbal extracts are discovered for inhibition of biofilm of different microorganisms such as methanolic plant extract against Nosocomial microorganisms [6], *Artocarpus lakoocha* (Moraceae) extract against some oral pathogens [7], some bio inspired Ag-Au nanocomposites are discovered for biofilm inhibition [8]. Some transition metal complexes with Cefotaxime derivative [9], thiazole schiff bases [10] showing anti-biofilm activity are reported. Also antibacterial and antifungal activity of mixed metal tartarates were reported in the past [11 - 14], but mixed transition metal complexes with bidentate tartarate ligand showing anti-biofilm activity against microorganisms are reported very less.

In the present work we have synthesized six new, mixed transition metal and alkaline earth metal complexes, of the general formulation $[MM'(C_4H_4O_6)_2.xH_2O]$ (where M = Mn, Fe, Co, Ni, Cu and Zn, M/ = Ca) by using bidentate tartarate ligand and tested their *in vitro* anti-biofilm activity against bacterial cultures namely of *Pseudomonas aeruginosa* ATCC-27853, *E.coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923, *Klebsiella pneumoniae* (Lab culture), *Proteus vulgaris* (Lab culture).

Experimental: All the complexes (samples A1 to A6) were prepared by a simple co-precipitation method by using A.R grade salts of calcium and transition metals **(Table 1)**. All complexes are characterized by different analytical techniques such as elemental analysis, TGA, FTIR, XRD, SEM, magnetic susceptibility study, UV-visible spectroscopy etc. The complexes have been screened for their microbial activity and the work is reported earlier [15].

TABLE 1: COMPOSITION OF COMPLEXES SYNTHESIZED (SAMPLES A1 TO A6)

Complex	Symbol	Mol. Wt	Amount of $CaCl_2.2H_2O$ (gm)	Amount of metal salt (gm)	Tartarate Solution added	% Yield of complex
$MnCa(C_4H_4O_6)_25H_2O$	A1	481	4.584	6.174	15%	70
$FeCa(C_4H_4O_6)_210H_2O$	A2	571.85	3.859	4.254	15%	63
$CoCa(C_4H_4O_6)_25H_2O$	A3	485	4.546	7.358	15%	75
$NiCa(C_4H_4O_6)_28H_2O$	A4	538.69	4.093	6.618	15%	69
$CuCa(C_4H_4O_6)_22H_2O$	A5	435.5	5.063	5.871	15%	72.5
$ZnCa(C_4H_4O_6)_25H_2O$	A6	491.4	4.487	4.159	15%	77

MATERIALS AND METHODS: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Biofilm formation and Anti-biofilm (Biofilm Inhibition Concentration-BIC) studies were conducted using synthesized transition metal-Ca tartarate complexes (Samples A1 to A6).

Materials:

1. Sterile Nutrient Broth tubes containing 5 mL medium.
2. Dilutions of synthesized transition metal -Ca tartarate complexes (samples A1 to A6) in sterile Nutrient media- 10, 15, 20, 25 --- 200 µgs / mL of medium. (Solid and broth media).
3. Nutrient agar plates.
4. Soft nutrient agar plates. (with 0.5 % agar concentration)
5. Test culture bacteria (Known Biofilm Formers) used: *Pseudomonas aeruginosa* ATCC-27853, *E.coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923, *Klebsiella pneumoniae* (Lab culture), *Proteus vulgaris* (Lab culture).

Method:

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) Studies: Test bacterial fresh cultures of *Pseudomonas aeruginosa* ATCC-27853, *E.coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923, *Klebsiella pneumoniae* (Lab culture), *Proteus vulgaris* (Lab culture) were inoculated in loop full amounts in sterile nutrient broth tubes containing concentrations of tartarate complexes (samples A1 to A6) and incubated at 37 ^{0}C for 48 h. The lowest concentration of tartarate complexes (samples A1 to A6) showing no turbidity in the medium is taken as MIC while lowest concentration of tartarate complexes (samples A1 to A6) showing no growth on solid medium plates is taken as MBC.

Biofilm and Anti-biofilm studies: Test bacterial fresh cultures of *Pseudomonas aeruginosa* ATCC-

27853, *E.coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923, *Klebsiella pneumoniae* (Lab culture), *Proteus vulgaris* (Lab culture) were point inoculated at centre of soft nutrient agar plates and incubated at 37 ^{0}C for 24 h. The swarming (spreading) growth on medium surface is taken as biofilm activity of test bacteria.

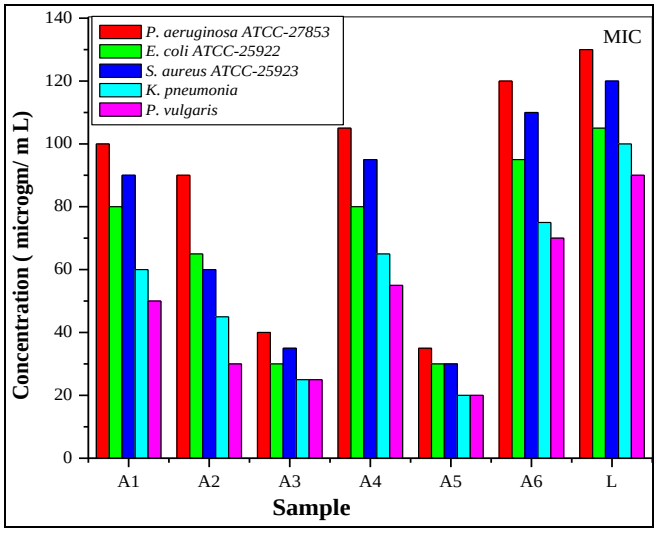
To study anti-biofilm activity of tartarate complexes (samples A1 to A6), the test bacterial fresh cultures of *Pseudomonas aeruginosa* ATCC-27853, *E.coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923, *Klebsiella pneumoniae* (Lab culture), *Proteus vulgaris* (Lab culture) were point inoculated at the centre of soft nutrient agar plates with different concentrations of tartarate complexes and plates were incubated at 37 ^{0}C for 24 - 48 h. Inhibition of swarming growth and formation of compact growth at point of inoculation is taken as anti-biofilm activity of tartarate complexes.

RESULTS AND DISCUSSION:

1. All the five test bacterial cultures *Pseudomonas aeruginosa* ATCC-27853, *E.coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923, *Klebsiella pneumoniae* (Lab culture), *Proteus vulgaris (Lab culture)* showed luxuriant biofilm activity on soft nutrient agar at 37 ^{0}C for 24 h. of incubation. (**Plate -1**)
2. The Minimum Inhibitory Concentration (MIC) of Transition metal - Ca tartarate complexes (samples A1 to A6) ranged from 20-40 µgs / mL, where sample A3 and A5 found the most active **(Table 2).** The Ligand bidentate tartarate was found less active against all test organisms **(Fig. 1).**

TABLE 2: MINIMUM INHIBITORY CONCENTRATION (µg / m l) OF SAMPLES A1 TO A6 AGAINST TEST BACTERIA

Sr. No	Complex	*Pseudomonas aeruginosa* ATCC-27853	*E.coli* ATCC-25922	*Staphylococcus aureus* ATCC-25923	*Klebsiella pneumoniae* (Lab culture)	*Proteus vulgaris* (Lab culture)
1	$MnCa(C_4H_4O_6)_2 5H_2O$	100	80	90	60	50
2	$FeCa(C_4H_4O_6)_2 10H_2O$	90	65	60	45	30
3	$CoCa(C_4H_4O_6)_2 5H_2O$	40	30	35	25	25
4	$NiCa(C_4H_4O_6)_2 8H_2O$	105	80	95	65	55
5	$CuCa(C_4H_4O_6)_2 2H_2O$	35	30	30	20	20
6	$ZnCa(C_4H_4O_6)_2 5H_2O$	120	95	110	75	70
7	Ligand (Tartarate)	130	105	120	100	90



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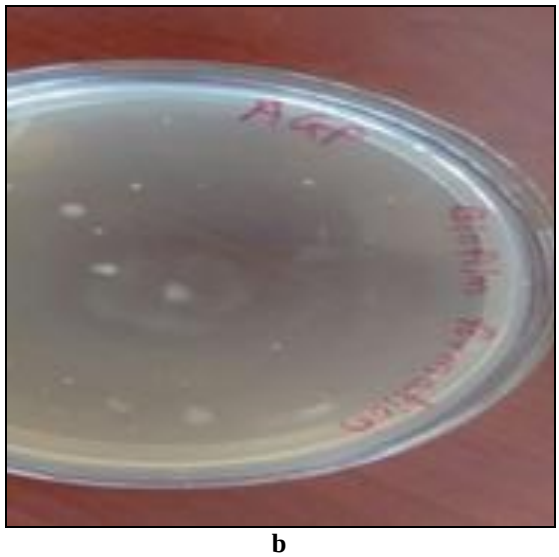

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FIG. 1: a. MIC OF TRANSITION METAL- Ca TARTARATE COMPLEXES (SAMPLES A1 TO A6). b. IMAGE OF BIOFILM FORMATION

3. The Minimum Bactericidal Concentration (MBC) of Transition metal - Ca tartarate complexes (samples A1 to A6) was found slightly more than MIC. It ranged from 25-50 µgs / mL **(Table 3)** (**Fig. 2).**

TABLE 3: MINIMUM BACTERICIDAL CONCENTRATION (µg/ml) OF MEDIUM OF SAMPLES A1 TO A6 AGAINST TEST BACTERIA

Sr. No	Complex	*Pseudomonas aeruginosa* ATCC-27853	*E.coli* ATCC-25922	*Staphylococcus aureus* ATCC-25923	*Klebsiella pneumoniae* (Lab culture)	*Proteus vulgaris* (Lab culture)
1	$MnCa(C_4H_4O_6)_25H_2O$	110	85	95	70	65
2	$FeCa(C_4H_4O_6)_210H_2O$	95	70	70	50	45
3	$CoCa(C_4H_4O_6)_25H_2O$	50	40	45	30	30
4	$NiCa(C_4H_4O_6)_28H_2O$	115	90	105	75	70
5	$CuCa(C_4H_4O_6)_22H_2O$	40	40	35	25	30
6	$ZnCa(C_4H_4O_6)_25H_2O$	130	100	120	85	85
7	Ligand (Tartarate)	140	120	130	120	105

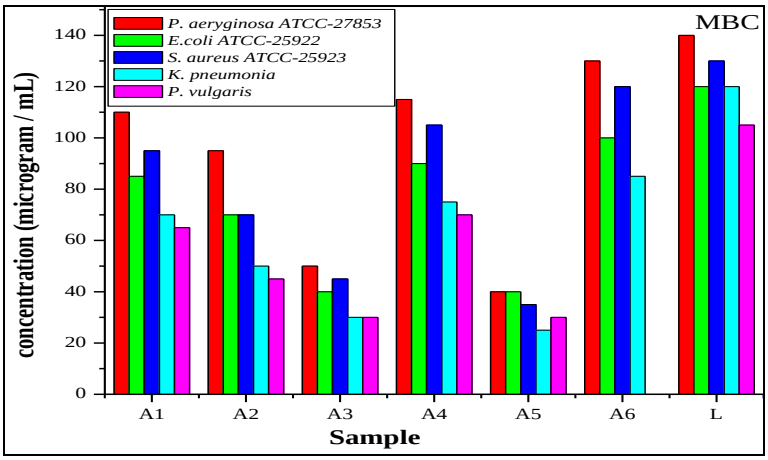
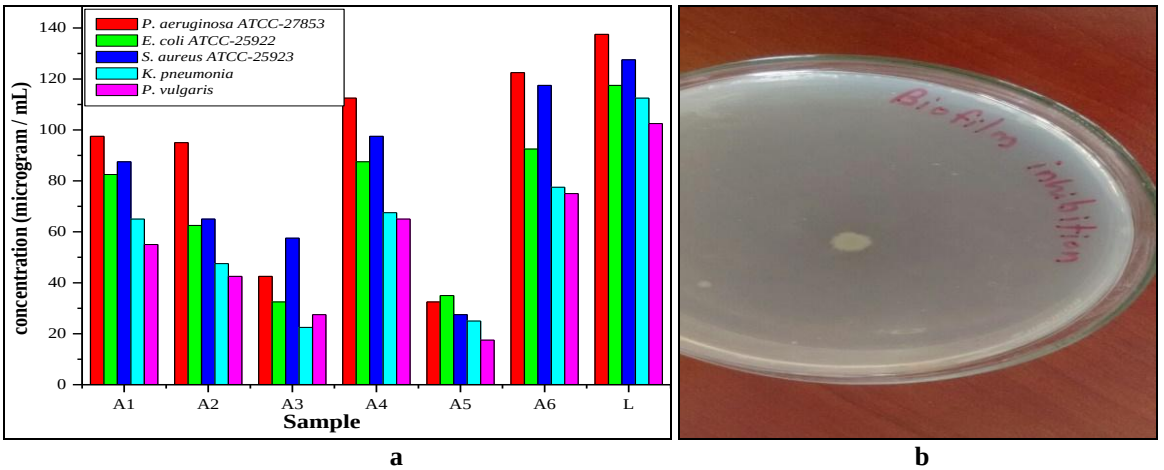


FIG. 2: MBC OF TRANSITION METAL- Ca TARTARATE COMPLEXES (SAMPLES A1 TO A6)

TABLE 4: ANTI-BIOFILM ACTIVITY OF SAMPLES A1 TO A6 (AT µg / ml CONCENTRATION OF COMPLEXES IN MEDIUM) AGAINST TEST BACTERIA

Sr. No	Complex	*Pseudomonas aeruginosa* ATCC-27853	*E.coli* ATCC-25922	*Staphylococcus aureus* ATCC-25923	*Klebsiella pneumoniae* (Lab culture)	*Proteus vulgaris* (Lab culture)
1	$MnCa(C_4H_4O_6)_25H_2O$	95-100	80-85	85-90	60-70	50-60
2	$FeCa(C_4H_4O_6)_210H_2O$	90-100	60-65	60-70	45-55	40-45
3	$CoCa(C_4H_4O_6)_25H_2O$	40-45	30-35	35-40	20-25	25-30
4	$NiCa(C_4H_4O_6)_28H_2O$	110-115	85-90	95-100	65-70	60-70
5	$CuCa(C_4H_4O_6)_22H_2O$	30-35	30-40	25-30	20-30	15-20
6	$ZnCa(C_4H_4O_6)_25H_2O$	120-125	90-95	115-120	75-80	70-80
7	Ligand (Tartarate)	135-140	115-120	125-130	110-115	100-105
8	Control	No inhibition of biofilm	No inhibition of biofim	No inhibition of biofilm	No inhibition of biofilm	No inhibition of biofilm



a **b**

FIG. 3: a. ANTI-BIOFILM ACTIVITY OF TRANSITION METAL- Ca TARTARATE COMPLEXES (SAMPLES A1 TO A6), b. IMAGE OF BIOFILM INHIBITION

4. All five bacterial test cultures showed luxuriant biofilm formation on soft nutrient agar medium at 37 ^{0}C in 24 h. of incubation. The samples A3 and A5 found more active in the inhibition of biofilm formation (BIC) ability of test bacteria. It ranged from 15-45 µgs / mL. The BIC levels are at higher side as compared to MIC but lower to MBC **(Table 4) (Fig. 3). (Plate 2).**

CONCLUSION: Microorganisms cause infections to man and animals, dwell in hospital instruments and surfaces, and cause fouling of surfaces of dockyard through biofilm formation and damage the ships. But transition metal- Ca Tartarate complexes, especially A3 and A5 inhibit biofilm activity of test bacterial cultures, indicating potential use of these complexes in reducing dwelling of bacteria in hospital instruments and at dockyard for preventing damage to the surface of ship by fouling and depending on animal and human toxicity can be used to control infections caused by biofilm forming pathogenic bacteria.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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