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Antibacterial Activity of Polyoxometalates (POMS) Against Urinary Tract Infection (UTI) Causing Bacteria

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Abstract: Present study deals with the antimicrobial and antioxidant activities of polyoxometalates (Silicovanadate and Iron substituted Phosphotungstate) and their synergistic effect with beta lactam antibiotics against urinary tract infection causing bacteria. Three beta lactam antibiotics resistant strains such as *E. coli, P. aeruginosa* and *K. pneumoniae* are more prominent to cause urinary tract infections. Serial dilutions were prepared with concentration ranging from 5 to 15 µg/ml.MIC of both POMs was 5µg/ml concentration against all three strains and measured by scaling. The antioxidant activity of POMs was measured by spectrophotometer for different concentrations and compared with ascorbic acid used as standard. Antibacterial as well as antioxidant activity of silicovanadate was observed more than that of ironosubstituted phosphotundstate and increased as we increased the concentrations of both compounds.

Keywords Bacteria, Antibiotic Resistance, Poms, Silicovanadate, Ironosubstituted Phosphotungstate.

1. INTODUCTION

To find solutions of problems is human nature. To prevent from diseases and find remedies of causes and cure of diseases is a significant step that microbiologists intend to apply against different microorganisms of our environment that are the main cause to spread diseases in human and other animals. Scientists use different antimicrobial and antibacterial agents that can degrade them. But with the passage of time these microbes can develop resistance against these agents by mutating their genome. So there should be alternative ways to get rid from these microbes and diseases caused by microbial life. Antimicrobial drug resistance is a wide-reaching problem in both developing and developed countries (Tenover and Hughes, 1996). Urinary Tract Infections (UTIs) are one of the most widespread extra-intestinal bacterial infections.

Urinary tract infection (UTI) is a common infection that is nosocomial in nature caused by bacteria, encompassing of a wide range of clinical state of affairs and features which has been caused by microbial and bacterial life that usually invade cells and tissue lining tract of urinary system that lengthens from renal cortex to urethral meatus. In male population, UTI had been originated to be widespread and prominent in the age of 31-40 and 21-30 in female.

POMs are anionic cluster of early transition metals such as tungsten (W), niobium (Nb), Vanadium (V), antimony (Sb), molybdenum (Mo) and oxygen that are designed by the self-assembly procedures (Rhule *et al*., 1998).

The POMs display different characteristics and possess variability of physical features such as sizes, significant solubility in polar solvents like water, structures, electrochemical activities and an amalgamation of metals. This feature makes them a multipurpose and resourceful class of inorganic clusters (Yamase, 2005).

The present study has been designed to investigate and explore the antibacterial potential of Plyoxometalates against UTI causing bacteria that have become resistant towards beta lactam antibiotics. It is low cost, easy and a simple strategically comprehension study that would has appositive impact in reach and possess substantial and noteworthy inferences. By keeping all significant features, potential activities, simple procedures to perform antibacterial assays and effective perspectives of POMs under consideration we decide to do comparative study regarding antibacterial activity against pathogenic bacteria *E. coli, P. aeruginosa* and *K. pneumonia*. It has been studied for the first time a novel POM Iron substituted Phosphotungstate for its antibacterial activity and compares it with silicovanadate which may be exploited making new medical discoveries with synergistic antibacterial capability.

2. MATERIALS AND METHODS

The glassware and media to be used was autoclaved at 121ºC at 15 psi for 15 minutes. Pure culture of *Escherichia coli, Pseudomonas aeruginosa and K. pneumonia* were collected from Post Graduate Medical Institute Lahore (PGMI). The synthetic compounds

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were used to check its antibacterial activity against above mentioned UTI causing bacteria. The synthetic compounds were collected by the courtesy of COMSATS.

Antibacterial activity

Synthetic compounds Silicovanadate and Ironosubstituted Phosphotungstate were dissolved in solvents water and DMSO respectively. Inoculum was prepared by taking 1ml of the nutrient broth (autoclaved) in a test tube and inoculated it by picking a colony with the help of inoculating loop. We kept it in incubator for overnight at 37 degree centigrade temperature. To check sensitivity test took few cells of freshly prepared culture and were spreader on Nutrient Agar plates. Sterilize filter paper disks of Whatmann filter were used for disk diffusion method. These blank discs saturated with 5µg/ml, 10mg/ml and 15µg/ml of compound were diffused. Standard disc of kanamycin (10µg/disc) and a blank disc (saturated with solvent) were used as positive and negative control, respectively. This test was performed for *E. coli* and also repeated in the same manner for *P. aeruginosa* and *K. Pneumoniae*. After disks were diffused all the plates were kept in inverted position in incubator at 37 degree centigrade temperature for 18-24 hours. After incubation zones of inhibition were measured by transparent scale in mm **(Fig 1, and 2)**

The data was analyzed by univariate Analysis of variance on SPSS, (version 13.0) for the analysis of variance and to detect the significance difference between the drug susceptibility of UTI causing strain.

Table 1: Antibacterial Activity of Silicovanadate against Urinary Tract Infection Causing (Uti) Bacteria

	P. aeruginosa			*E. coli*			*K. pneumoniae*		
Dilutions	**5µg/ml**	**10µg/ml**	**15µg/ml**	**5µg/ml**	**10µg/ml**	**15µg/ml**	**5µg/ml**	**10µg/ml**	**15µg/ml**
Zone of inhibition by action of compound(mm)	**4**	**7**	**12**	**6**	**7**	**13**	**7**	**10**	**12**
	3	**8**	**13**	**7**	**8**	**11**	**6**	**11**	**13**
	5	**6**	**11**	**5**	**9**	**12**	**8**	**9**	**14**
Mean ± SD	**4±0.81**	**7±0.79**	**12±0.8**	**6±0.78**	**8±0.8**	**12±0.8**	**7±0.8**	**10±0.7**	**13±0.78**
Zone of inhibition by synergistic effect	**5**	**12**	**15**	**8**	**14**	**17**	**9**	**15**	**18**
	6	**11**	**14**	**7**	**13**	**16**	**8**	**16**	**17**
	4	**13**	**16**	**9**	**15**	**18**	**10**	**14**	**19**
Mean ± SD	**5±0.84**	**12±0.8**	**15±0.7**	**8±0.87**	**14±0.7**	**17±0.8**	**9±0.8**	**15±0.7**	**18±0.8**

Table 2: Brine Shrimp Micro well Cytotoxicity results

Compounds Names	A	B	N	G	M=[A-B-N/G-N] 100
Ironosubstitutedphosphotungstate	**8**	**0**	**1**	**18**	**38.88%**
Silicovanadate	**8**	**0**	**1**	**21**	**35%**

Antioxidant activity of POMs

The antioxidant activity of compounds was measured by spectrophotometer for different concentrations (5µg/ml, 10µg/ml and 15µg/ml). The DPPH capability of the sample was testified as the % of DPPH scavenging activity. Higher concentration of DPPH has been related to higher antioxidant activity. The antioxidant activity present in the figure reveals that antioxidant activity was increased as we increased the concentrations of both compounds **(Fig 3).**

Brine Shrimp Micro well Cytotoxicity Assay

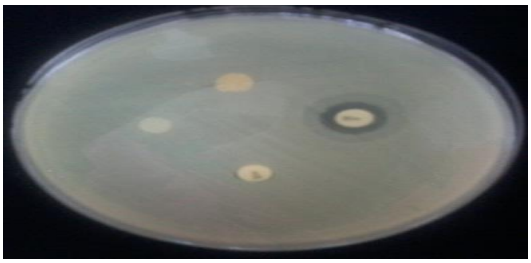
Cytotoxicity of Polyoxometalates was calculated by using living organisms (brine shrimp larvae) (*Artimia salina*) in the laboratories. This special cytotoxicity assay was anticipated by Sleet and Brendel, 1983. It is actually associated with the potential ability of POMs to kill laboratory cultured brine shrimp (Pelka *et al*., 2000) 400 ml of artificial seawater was taken and dried eggs of *Artemia salina* (0.5 g) were added to the sea water. Proper aeration was maintained by bubbling air and kept it for duration of 24 to 48 hours in the laboratory. The suspension containing larvae was kept for 1 hour undisturbed, and the left behind eggs settled down. It was intension to collect the active larvae for the present study. For this purpose aluminum foil was used to cover one side of the funnel and the other side was brightened by using a lamp. The phototropic larvae began to move at the irradiated side. By the help of a pipette 30 to 40 larvae were taken and have been transferred to special plate called as micro titer plate having deep wells (wells diameter 1.8 cm, depth 2 cm). 0.2 ml of sea water was added to each well. The dead larvae were counted and showed as value N. A solution of 10µg of the compound (POMs) in l ml of DMSO was added by keeping the plate at room temperature in the dark. Number of dead organisms had been counted and named as A after 24 hours. These dead larvae were counted under microscope. Added 0.5 ml of methanol in the mixture of surviving larvae and the remaining larvae were killed hence the G value could be determined that showed total number of organisms. DMSO was used as a solvent. The mortality rate M was deliberated by using the formula M= [A-B-N/G-N] 100.

3. RESULTS AND DISCUSION

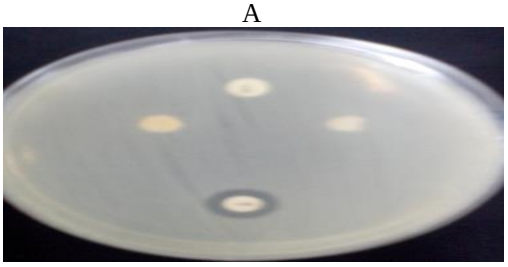
Three strains were collected as samples from Post Graduate Medical Institution Lahore (PGMI) .These bacteria were identified by morphological and biochemical tests. The synthesized compound showed antibacterial activity was subjected to MIC (Minimum inhibitory concentration) assay. When 5µg/ml, 10µg/ml and 15µg/ml concentrations of ironosubstituted phosphotungstate were used the zone of inhibition activities measured against *E. coli* were 2mm, 3mm and 4mm respectively and for synergistic effect these are 10mm, 11mm and 12mm. Against *P. aeruginosa* the inhibition zones were measured 1mm, 2mm and 3mm and for its synergistic effect the zones of inhibition were 4mm, 5mm and 6mm respectively. For all three concentrations antibacterial activity of this compound against *K. pneumoniae* were 4mm, 5mm and 6mm and its synergistic effect with beta-lactam antibiotic was measured were 10mm, 12mm and 13mm respectively (Table 1).

When 5µg/ml, 10µg/ml and 15µg/ml concentrations of silicovanadate were used the zones of inhibition measured against *E. coli* were 6mm, 8mm and 12mm respectively and by checking its synergistic effect with beta-lactam antibiotic the effect was enhanced and measured as 8mm, 14mm and 17mm. Against *P. aeruginosa* these zone of inhibition activities were 4mm, 7mm and 12mm and its synergistic effect were also evaluated as their zones of inhibition were 5mm, 12mm and 15mm respectively. Inhibition activities against *K. pneumoniae* as their zone of inhibition were 7mm, 10mm and 13mm (**Fig 1, 2).**

Antibacterial and antioxidant activity of ironosubstituted phosphotungstate was compared with silicovanadate results showed that values obtained for the silicovanadate were highly significant than ironosubstituted Phosphotungstate ($p<0.05$). *K. pneumonia* showed highest sensitivity against both compounds than *E. coli* and *P. aeruginosa.* The order of sensitivity is given *K. pneumonia* >*P. aeruginosa* >*E. coli* Antioxidant activity of silicovanadate was more than iron osubstitutedphosphotungstate and measured as 4.5±0.763, 15±1 and 25.5±0.72 and the readings taken for ironosubstituted Phosphotungstate were 1.2± 0.529, 13±1 and 20±1 for 5µg/ml, 10µg/ml and 15µg/ml concentrations respectively. All these readings were compared with ascorbic acid (55 ± 0.72) used as standard.



A



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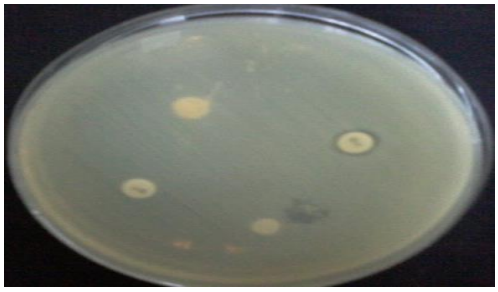
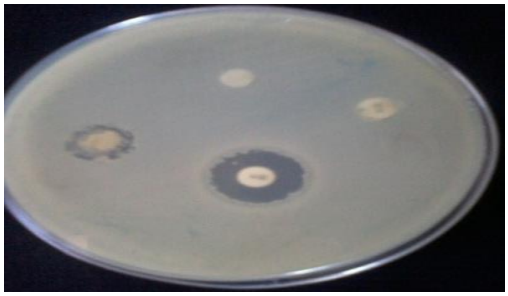
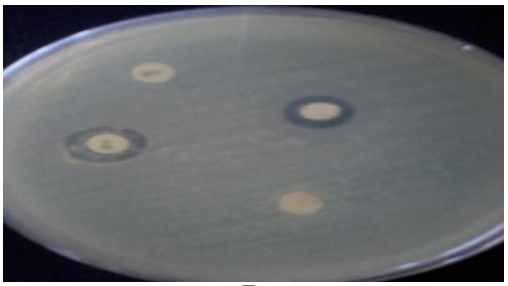


C

Fig 1: Maximum inhibitory activity of synergistic effect of Ironosusbstituted Phosphotungstate with beta-lactam antibiotic disc against (A=*K. pneumoniae*,B= *E. coli*,C=*P. aeruginosa*)



A



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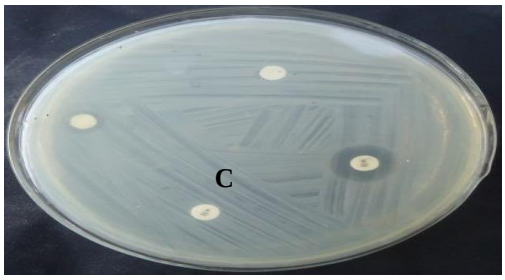

Fig 2: Maximum inhibitory activity of synergistic effect of s ilicovanadatewith beta-lactam antibiotic disc against (A=*K. pneumoniae*,B= *E. coli*,C=*P. aeruginosa*)

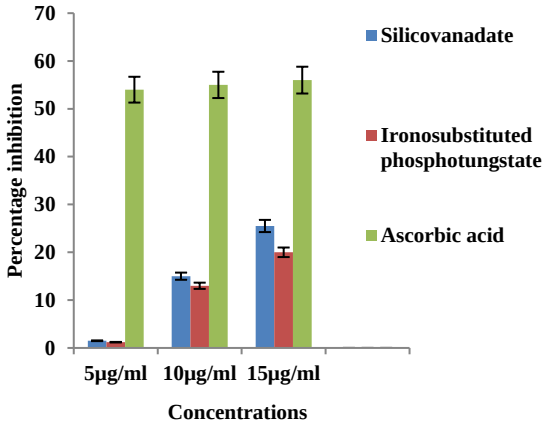

Fig 3: DPPH radical scavenging activity of POMs at different concentrations

In present study *K. pneumonia* depicted the highest sensitivity to both POM and was more adversely affected by the silicovanadate (acid) than ironosubstituted phophotungstate.In a similar study synthesized compound named ammonium encapsulated silicovanadate in synergism with beta-lactam antibiotic (sulfamethoxazole) were treated against Uropathogenic *E. coli* (UPEC) which is an etiological agent of urinary tract infection. There would be three different concentrations (5µg/ml, 10µg/ml and 15µg/ml). Their results revealed that the compound has enhanced the activityof β-lactam antibiotics by effecting at the genetic level of bacteria. It was reported that β-lactam antibiotics shows activity by effecting on the cell wall of bacteria (Matsuhashi *et al*.,1986).

In our study we also used ironosubstituted phosphotungstate in which there is a special constituent (tungstate) that is responsible for the enhancement of antibacterial activity. A similar study that supported our result was conducted to analyze its inhibitory effects against pathogenic strains. In that study it was reported that the effect of tungstate and phosphate together with beta-lactam antibiotics. It has been investigated that a factor is effectively responsible to increase the antibacterial activity of POMs against pathogens. The name of that factor was "Factor T" for Tungstate (Tajima *et al*., 1997).

Another supported study in which antibacterial activity of Quinolone derivatives were applied against three test organisms *E. coli*, *P. aeruginosa* and *K. pneumoniae* and other antibiotics resistant strains (*streptococcus* and *staphylococcus aureus*). It was proposed that the compound contained constituents that are effective for antibacterial activity and low cytotoxicity and is a more potential drug candidate (Chen *et al*., 2001).

Antioxidants have scavenging power and they use it for the controlling of some diseases in body of organisms (Koleva *et al*., 2002). Antioxidant activity of silicovanadate measured was greater than that of Ironosubstituted Phosphotungstate readings taken were 4.5 ± 0.763, 15 ± 1 and 25.5 ± 0.72 and the readings taken for ironosubstituted Phosphotungstate are 1.2 ± 0.529, 13 ± 1 and 20 ± 1 for 5µg/ml, 10µg/ml and 15µg/ml concentrations respectively as shown in table 3. All these readings were compared with ascorbic acid (55 ± 0.72) used as standard.

In a similar study which supported our results where the absorbance of the complex (Mo (VI)–PR–CTA) at 612.0 nm was analyzed against a blank reagent as the reference to evaluate the effect of components of complex such as PR (pyrogallol red) and CTAC (*N*-cetyl-*N*-*N*-*N*-trimethyl ammonium ions) (Gharehbaghi and Farzaneh, 2011).The absorption was amplified by increasing the PR concentration. At a constant concentration of 0.05% (w/v) CTAC and 100 ng mL^{-1} of Mo, and the PR concentration would be in the specific range of 3.0×10^{-6}–1.2×10^{-3} mol L^{-1}. We got maximum absorbance at a concentration of 6.0×10^{-4} mol L^{-1} of the ligand and contrary to the present study that after that particular concentration the absorbance remained about constant in recent study the antioxidant activity of POMs (silicovanadate and ironosubstituted Phosphotungstate) was increased as we increased the concentration of compounds.

Based on our results, it can be concluded that chemical compounds contain great amount of some important constituents that have antibacterial and antioxidant activity. Statistical results exhibit that both POMs showed a significant inhibitory activity against test strains and the antioxidant agents that can check us from the free radicles that are extremely destructive and reactive oxygen species that are also being formed through metabolism that run in our body. It was concluded that *K. pneumonia* was more sensitive than that of *E. coli* and *P. aeruginosa* in present study. The results showed that the values obtained for silicovanadate were highly significant ($p<0.05$).

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