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Bactericidal Activity of Di-m-Toluidine Phosphate Having C-N-P Linkage

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***Abstract*: Antibiotic resistance is rising to perilously high levels in all parts of the world. Due to the increasing development of drug resistance to antibacterial agents, demand for searching novel antibacterial agents is being screened. Di-m-toluidine phosphate has been synthesized by the reaction of m-toluidine, with phosphorylating agent POCl3 by known method and characterized by elemental and IR spectral analysis. Synthesized di phosphate ester has been screened for bactericidal activity against isolated bacteria. The isolation of bacteria and their characterization by different methods has been carried out. Different concentrations of phosphate esters in DMSO were applied to examine their antibacterial activity against coccus gram positive and gram negative bacteria by paper disc diffusion method. The study reveals that phosphate ester is highly effective against coccus gram negative bacteria as compared to gram positive bacteria.**

***Keywords*: Di-m-toluidine phosphate, Paper disc diffusion, Phosphorylating agent.**

1. Introduction

Esters of phosphoric acid have an important role in the studies of biological and biochemical processes1. As a result of improved aqueous solubility, the development of phosphate ester prodrug is an interesting approach to increase intestinal absorption of poorly water soluble drugs. Clindamycin is an antibacterial agent used orally in treating gram positive infections, anaerobic infections, methicillin-resistant staphlococcal and streptococcal infections. Clindamycin 2-phosphate, unlike clindamycin, is highly water soluble and does not produce pain upon injection [2].

A wide range of phosphate esters are used as insecticides [3], pesticides [4], herbicides [5], fungicides [6] & bactericides [2]. Dimethyl pentachlorophenyl phosphate, O-aryl phosphorodichloridothioate shows the biological activity as bactericidal, fungicidal and insecticidal [3]. Some phosphate esters such as triphenyl phosphate, trichesil phosphate are used to prepare a self-sustaining antibacterial food packaging film having transparency, antifogging and antibacterial property.

New antibiotics phoslactomycins A, B, C, D, E and F, which contain alpha, beta-unsaturated delta-lactone, phosphate ester, conjugated diene and cyclohexane ring moieties show strong activity against various fungi, particularly phytopathogenic fungi (Botrytis cinerea and Alternaria kikuchiana) [7]. 8′-phospho derivatives of amicoumacins A and B shows the antibacterial activity against Staphylococcus aureus bacteria8. Difficidin and oxydifficidin, two novel macrocyclic polyene lactone phosphate esters were showed a broad spectrum of activity against aerobic and anaerobic bacteria [9].

These days, the treatment of bacterial infections is being complicated increasingly by the ability of bacteria to develop resistance against antimicrobial agents. Due to this resistance to the traditional antibiotics, there is a significant need to search for new types of antibacterial agents [10], [11]. In same context, the present investigation belongs to synthesis of new phosphate ester with C-N-P linkage and study of their bactericidal activity against bacterial strains.

2. Experimental

The synthesis of di-m-toluidine phosphate was carried out according to the method described by Rudert [12], which involves the reaction of m-toluidine and phosphorus oxychloride in 2:1 mol ratio in benzene to give crude di-ester as a solid. Solid crude di-ester obtained was dissolved in ammonia and recrystallized by requisite volume of hydrochloric acid, to get pure sample. The compound was characterized by elemental and IR spectral analysis. All the chemicals used were of AR grade.

For the study of antibacterial activity, bacteria (A & B) were isolated from soil and rotten banana fruit. These samples were collected in sterile bottle and processed for isolation of bacteria. Bacterial samples were inoculated into proper growth medium (Nutrient Agar Medium) through serial dilution and isolated bacteria were re-cultured as pure culture through streak plate method in Nutrient Agar Medium. Characterization of selected bacteria was done by Gram staining, acid fast staining methods and microscopic study [13], [14]. Microscopic characters of selected bacteria are summarized in Table 1. Paper discs of Di-m-toluidine phosphate were prepared in 40% DMSO at three concentration levels of 500 µg/ml, 1000 µg/ml, 1500µg/ml. The

antibacterial activity was tested by paper disc diffusion method 13, 15. The Di-m-toluidine phosphate and the reference drug norfloxacin were screened under identical conditions and zones of inhibition were measured in mm.

Table 1
Microscopic characters of selected bacteria

Bacteria	Source	Shape	Gram staining	Acid fast stuffy
A	Soil	Spherical	positive	negative
B	Rotten fruit	Spherical	negative	negative

3. Results and Discussion

Di-m-toluidine phosphate was tested for antimicrobial activity against selected bacteria with standard antibiotic norfloxacin. Antibacterial activity of norfloxacin and Di-m-toluidine phosphate against gram positive and gram negative bacteria were tested at different concentrations and the zones of inhibition obtained were measured. Results are summarized in Table 2, which show that the zone of inhibition increases with increase in concentration of compound. The zones of inhibition of di-m-toluidine phosphate at 500 µg/ml, 1000 µg/ml and 1500 µg/ml concentrations were 13 mm, 19 mm and 25 mm respectively as compared with 19 mm, 24 mm and 28 mm of norfloxacin against gram positive bacterial strains shown in fig. 1.

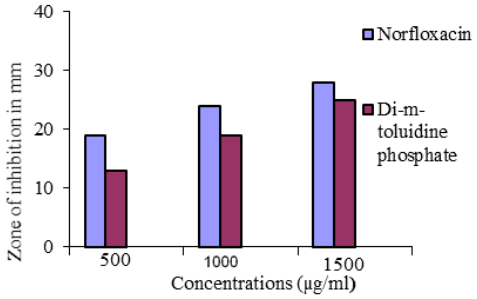

Fig. 1. Representing bactericidal activity of Di-m-toluidine phosphate against gram positive bacteria

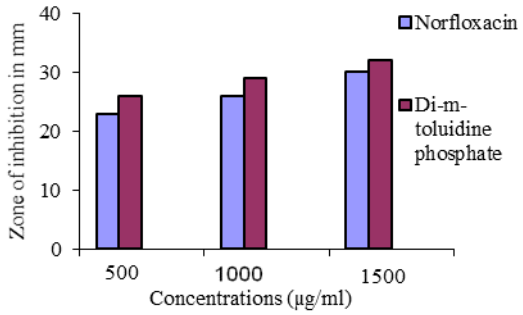

Fig. 2. Representing bactericidal activity of Di-m-toluidine phosphate against gram negative bacteria

Sensitivity of standard antibiotic norfloxacin is highest as compared to the di-m-toluidine phosphate against gram positive bacteria, while in case of gram negative bacteria the di-m-toluidine phosphate is highly sensitive. The zones of inhibition of di-m-toluidine phosphate at 500 µg/ml, 1000 µg/ml and 1500 µg/ml concentrations were 26 mm, 29 mm and 32 mm respectively as compared with 23 mm, 26 mm and 30 mm of norfloxacin against gram negative bacterial strains shown in fig. 2. Similar observations have also been reported on the Synthesis and antimicrobial activity of alkyl -2-{[3-(3'-chloro-4-nitrophenyl)-2-oxo-3,4-dihydro-2H-1,3,2λ5-benzoxazaphosphinin-2-yl] alkanoates, and some oxazaphosphinine oxides against gram positive and gram negative bacteria using paper disc diffusion method by C. S. Reddy et. al. [16]. Thus the di-m-toluidine phosphate shows maximum sensitivity against coccus gram negative bacteria in comparison with standard norfloxacin.

4. Conclusion

Di-m-toluidine phosphate has been successfully synthesized by method described earlier. It is found to be effective to inhibit the growth of selected bacterial strains. The findings of the present study reveal that di-m-toluidine phosphate is found to possess significant bactericidal activity against coccus gram negative bacteria as compared to gram positive bacteria.

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Table 2
Antibacterial activity Di-m-toluidine phosphate against gram positive and gram negative bacteria at various concentrations

Compounds	Gram positive bacteria (Zone of inhibition in mm)			Gram negative bacteria (Zone of inhibition in mm)		
	(500 µg/ml)	(1000 µg/ml)	(1500 µg/ml)	(500 µg/ml)	(1000 µg/ml)	(1500 µg/ml)
Norfloxacin	19	24	28	23	26	30
Di-m-toluidine phosphate	13	19	25	26	29	32

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