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ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY STUDY OF POLY SUBSTITUTED BENZENE DERIVATIVES

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ABSTRACT

Antimicrobial and antifungal activity of some poly substituted benzene derivatives against gram-positive bacteria i.e. *Psydomamonas aeruginosa ATCC 29212, Bacillus subtilis RSKK 244, Bacillus megaterium (clinical isolate)*, gram-negative bacteria *Micrococcus Luteus NRRLB* and as fungus *Candida albicans ATCC 90028* were studied.
Poly substituted benzene derivatives were 2,4,5- trichloroflourbenzene; 2,4,5-trichlorobromo-benzene; 2,4,5-trichloro iodobenzene; 1,2,3,4-tetrachlorobenzene; 1,2,4,5-tetrachlorobenzene.
As a result, the substituent which attached to the benzene ring, affected the antimicrobial and antifungal activity. Even substituent is same but position of substituent over the benzene ring is different activity were affected differently.

KEYWORDS: Poly substitutued benzene derivatives, antimicrobial activity, antifungal activity, Minimum inhibition concentration (MIC), Disc diffusion method.

INTRODUCTION

Many haloarens are important environmental pollutants. In the last three decades the environmental impact of halogenated chemicals has become increasingly apparent[1]. Polychlorinated benzenes (PcBzs) are used as solvents and starting materials or intermediates in the synthesis of many other substances e. g. phenols, dyestuffs in chemical industry and as pesticides and fungicides in agriculture.. Hexachloro benzene (HcBz) has many uses in industry e. g. as a plasticizer for PVC as a fungicide in agriculture [2].
Hexabromobenzene and its metabolites are present in water, fish, birds, sediments and human tissues. Among polybromobenzenes hexabromobenzene has been used most widely. The products that result from the hexabromobenzene debromination (penta-, tetra-, tribromobenzenes) are formed by means of environmental degradation or through metabollisms of various organisms [3-4]. They are more volatile and water soluble than the parent compound. Dibromobenzenes found in natural environments mainly originates

from their use as fumigants, additives to cleaning agents, or as intermediates in the production of pharmaceutical preparations [5].

In mammals, polybrominated biphenyls cause loss of weight, chloracne, edema, hepatic hypertrophy, porphyria,estrogenic activity and immunosupression[6]. An interesting relationship was found in phenobarbital (PB)- and polychlorinated biphenyls (PCB)-pretreated mice between increased rates of covalent binding of bromobenzene to liver microsomes and a decrease in in vivo bromobenzene-induced lung injury[7].

The monohydroxylation of halobenzenes by phenobarbital-induced rat liver microsomes was studied. The p-halophenol was found to be the major metabolite from all four halobenzenes; o-halophenol formation decreased as the halogen atom size increased[8]. For hepatocarcinogenicity, As to the methods of determination, some scientist focus set the on the bioassay to assess the concentration of 1,2,4,5-tetrachlorobenzene and 1,4-dichlorobenzene using a medium-term liver[9-10].

In this work , we report on the synthesis of polysubstituted benzene derivatives and on the biological activities of these compounds against gram-positive bacteria i.e. *Psydomamonas aeruginosa ATCC 29212, Bacillus subtilis RSKK 244, Bacillus megaterium (clinical isolate)*, gram-negative bacteria *Micrococcus Luteus NRRLB* and as fungus *Candida albicans ATCC 90028.*The chemical structures were given in Figure 1.

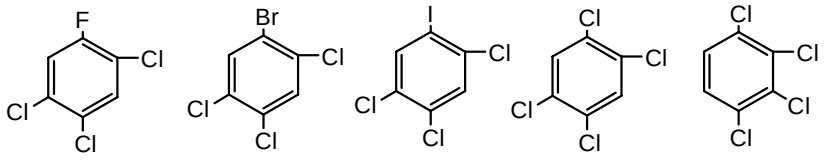

Figure 1. Chemical structures of tetra-substituted benzene derivatives

MATERIALS AND METHODS

Test microorganisms and medium

In this study, *Psydomamonas aeruginosa* ATCC 29212, *Bacillus subtilis* RSKK 244, *Bacillus megaterium* (clinical isolate), gram-positive bacteria *Micrococcus Luteus* NRRLB, and as fungus *Candida albicans* ATCC 90028 were used. Bacterial strains were cultured overnight at 37 °C in Mueller-Hinton broth and the yeast were cultured overnight at 30 °C in YEPDE Agar for antibacterial and antifungal activity tests. Test strains were suspended in Nutrient agar to give a final density of 5x 10^5 cfu/ ml.

Screening of Antimicrobial Activity

Minimum inhibitory concentrations (MICs) were determined by macrodilution broth method following the procedures recommended by the National Committee for Clinical Laboratory Standards[9]. MICs were defined as the lowest concentrations of the antimicrobial activity of compounds determined by the disc diffusion method. For testing antifungal activity of the compounds, *Micrococcus luteus* NRRLB were used[11].

Nutrient agar (20 ml) was poured into each sterile Petri dish after injecting cultures (100 µl) of microorganisms and distributing medium in Petri dish homogeneously. Compounds were filtered with a pore size of 0.45µm for sterilization. All of the compounds were dissolved in DMSO of 5 mg/ml. Empty sterilized discs of 6 mm (Schleicher and Schuell, No. 2668, Germany) were impregnated with 50 µl of compounds. Discs were placed on agar plates, and the plates were incubated at 37 ^{o}C for 24 h for bacteria. The culture suspensions were prepared and adjusted by comparing against 0.3 Mc Farland turbidity standard tubes. Inhibition zones formed on the medium were evaluated in mm. The solvent control (DMSO) did not show any antimicrobial activity. Studies performed in duplicate and the inhibition zones were compared with those of reference discs. Reference discs used for control were as follows: Ketoconazole (it is used to prevent and treat skin and fungal infections), Ampicillin (it is used to treat bacterial infections), Tetracycline (It is commonly used to treat acne today), Penicillin (It is used in the treatment of bacterial infections) Chloroamphenicol (it is effective against a wide variety of microorganisms) and Gentamisin (It is used to treat many types of gram-bacterial infections)

RESULTS AND DISCUSSIONS

Poly substituted benzene derivatives were assayed in vitro for their ability to inhibit the growth of representative *Psydomamonas aeruginosa* ATCC 29212, *Bacillus subtilis* RSKK 244, *Bacillus megaterium* (clinical isolate), gram-positive bacteria *Micrococcus Luteus* NRRLB, and as fungus *Candida albicans* ATCC 90028. The susceptibilities of certain strains of bacteria and fungus to Poly substituted benzene derivatives cause the inhibition of a visible growth of the microorganism. The MIC of Ketoconazole, Ampicillin, Tetracycline, Penicillin, Chloramphenicol and Gentamisin was individually determined in parallel experiments in order to control the sensitivity of the test organisms. MIC values of the compounds are presented in Table 2. Disc diffusion experiment results for poly substituted benzene derivatives and antibiotics are presented in Table 1 and Table 3.

Table 1. Disc diffusion method results of poly substituted benzene derivatives (*Diameter of zone mm)

Compounds ↓	Bacteria→	B. subtilis	M. luteus	P. aeruginosa	B. megaterium	C. albicans
2,4,5- trichloroflourbenzene		11	13	9	10	10,5
2,4,5-trichlorobromobenzene		8	11	---	11	---
2,4,5-trichloro iodobenzene		11	12	8,5	13	---
1,2,3,4-tetrachlorobenzene		14	---	12	11	---
1,2,4,5-tetrachlorobenzene		10	17,5	10,5	10	---

Table 2. MIC results of tetra substituted benzene derivatives and standard reagents (µg/ml)

Compounds ↓	Bacteria →	B. subtilis	M. luteus	P. aeruginosa	B. megaterium	C. albicans
2,4,5- trichloroflourbenzene		800	400	800	800	200
2,4,5-trichlorobromobenzene		400	100	---	800	---
2,4,5-trichloro iodobenzene		100	100	100	800	---
1,2,3,4-tetrachlorobenzene		200	---	100	800	---
1,2,4,5-tetrachlorobenzene		600	200	400	800	---

Table 3. The results of antibiogram test against to antibiotics (zone diameter , mm)

Bacteria ↓	Antibiotic→	Penicilin	Tetracycline	Ampicillin	Gentamisin	Ketoconazole
Bacillus subtilis		--	--	15	--	--
P.aeruginosa		--	--	--	16	--
Micrococcus luteus		31	9	28	--	--
Bacillus megaterium		--	--	10	--	--
Candida albicans		--	--	--	--	16

CONCLUSIONS

All compounds showed activity against to test bacteria or fungi. Only 2,4,5-trichloroflourbenzene showed activity against to *Candida albicans.* Also, the other poly halobenzene derivates inhibit some bacteria growth.
All compounds show activity against *Bacillus subtilis RSKK 244* and *Bacillus megaterium*.

2,4,5- trichloroflourbenzene is the most active compound against the all our test bacteria and fungi. 2,4,5-trichlorobromobenzene, show activity of except *Pseudomamonas aeruginosa ATCC29212 and Candida albicans ATCC 90028*. 2,4,5-trichloroiodobenzene and 1,2,4,5-tetrachlorobenzen show activity of all the test bacteria and has no activity against to *Candida albicans*. 1,2,3,4-tetrachlorobenzene, show activity of bacteria except *Micrococcus Luteus NRRLB 4375* and *Candida albicans*. Their antibiogram tests showed better results than some known antibiotics.

According to results of the antibiogram tests, 2,4,5- trichloroflourbenzene has the most activity to all antibiotics against all test bacteria. So, this compound can be used for broad-spectrum antibiotics. It is also means that it acts against both Gram-positive and Gram-negative bacteria. 2,4,5-trichlorobromobenzene is active against to bacteria except *Pseudomamonas aeruginosa ATCC29212 and Candida albicans ATCC 90028*. So, antibiotics which in the antibiogram except gentamisine and Ketoconazole, efficient for the bacteria that were affected by 2,4,5-trichlorobromobenzene. 2,4,5-trichloroiodobenzene and 1,2,4,5-tetrachlorobenzen show activity to all the test bacteria. It means that antibiotics except Ketoconazole efficient for these bacteria. 1,2,3,4- tetrachlorobenzene is active against to *Bacillus subtilis RSKK 244, Bacillus megaterium* and *Pseudomamonas aeruginosa ATCC29212*. Ampicillin and gentamisine efficient for these bacteria.

ÖZET: Bu çalışmada bazı poli sübstitüe benzen türevlerinin antimikrobiyal ve antifungal etkinlikleri incelenmiştir.Kullanılan bileşiklerde aynı gurupları benzen halkasının farklı konumlarında bulunduran izomerlerin antimikrobiyal ve antifungal etkinlik degerlerinin farklı olduğu saptanmıştır.

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