STUDIES ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF MIXTURES OF ZEOLITE DERIVATIVES

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Abstract

The present study is carried out with an aim to evaluate the antibacterial and antifungal activities of mixtures of various zeolite derivatives viz. Propolis-Zeolite, Silver sulfadiazine - zeolite and silver - zeolite. The antimicrobial activity of mixtures varying proportions were tested against two gram +ve bacteria: Staphylococcus aurens, Streptococcus pyogenes, and two gram –ve bacteria, Escherichia coli, Pseudomonas aeruginosa (Common pathogenic) bacteria and six fungal strains Penicillium, Rhizopus, Mucor fungal strain, Aspergillus niger, Aspergillus flavus and Candida albicans. All the activities were determined by using agar disk diffusion method. The zone of inhibition of growth of microbes were compared with the standard drug. The results show the remarkable inhibition of growth of bacteria in comparison to individual zeolite derivatives, hence present mixture of derivatives may be potentially used to develop new pharmaceutical products.

Keywords: Zeolite derivatives, Sulfadiazine, Silver and copper cations, antimicrobial activity, agar disk diffusion, zone of inhibition.

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Introduction: -

Zeolites play a pivotal role in the development of substrates with high exchanging efficiency, more surface area, hydrophilicity and ease of tunable chemical behavior [1-5]. Zeolites have tetrahedral skeleton of SiO_4 and AlO_4 [4]. Zeolites are also used in biomedical field [5-9]. It was reported that imidazole – Zeolite derivatives can be are used as an anticancer drug [8-11]. Several scientists found that zeolites can be used as a platform to deliver antibacterial agents [8,9,13-15]. One of excellent example is silver doped imidazole – zeolite composites which have superior antibacterial activity [13].

Various zeolite derivatives are used as pharmaceutical agents like Silver-sulfadiazine zeolite, Propolies-zeolite, transition metal-zeolites [16-18]. The present study is carried out explore the antimicrobial activity of mixtures of various zeolite derivatives. The present paper deals with the evaluation of antimicrobial (i.e., antibacterial and antifungal) activity of mixtures of propolis-zeolite, silver-sulfadiazine – zeolite and copper – zeolite by agar-disk-diffusion method. Different proportion of zeolite derivatives are used in this study. Common bacteria and fungi have been taken for the experiment.

Material and methods: -

The zeolite materials Propolis silver - sulfadiazine are purchased from local - market and Sigma - Aldrich. All other chemicals used were of pure grade.

A] Propolis – Zeolite (PZ):

Propolis – zoelite nanocomposite was prepared by reported method [16]. Propolis solution (21 g / 300 ml water) was mixed with zeolite nano composition (40 g) and stirred on magnetic stirrer for 1 day. The propolis embedded zeolite nano composite was centrifuged. The resultant solution was lypophilsed for 3 days to yield propolis – zeolite nanocomposite.

B] Silver – zeolite (SZ):

Silver – zeolite was prepared by literature method [17]. 100g of zeolite 4A dried powder stirred with 100 ml distilled water. 200 ml 0.5 N HNO_3 was added to slurry with maintaining pH 5 – 7. Then it was contacted by 0.1 M $AgNO_3$ with liquid: Solid rating 5:1 (by weight). Then stirred at room

temperature for 6 h. Solid was filtered, dried at 100°C and crushed in to ball mill.

C] Silver – sulfadiazine – zeolite (ASZ):

Silver – sulfadiazine – zeolite was prepared by method reported [18].

Silver – sulfadiazine first was dissolved in 30% (w/v) NH_4OH solution followed by mixing zeolite powder (dried at 100% for 7 day) with stirring. The solvent was evaporated at 70°C under reduced pressure. The resultant powder was dried at 100°C and used for further study.

1) Following mixtures of above zeolite derivatives were prepared in ball mill up to 100 mesh size.

Table 1 : Mixtures of zeolite derivatives

Mixture No.	Mixture of zeolite derivatives (wt. in g)		
	PZ	ASZ	SZ
1.	5	50	45
2.	10	50	40
3.	20	50	30
4.	30	50	20
5.	40	50	10
6.	45	50	5

Antimicrobial activity measurement

The antimicrobial and antifungal activities- The following pathogenic strains were used for testing antibacterial and antifungal activities of mixtures of zeolite derivatives.

Bacteria strains

S. No.	Bacteria strain	Gram
1.	Staphylococcus aereus	Gram +ve
2.	Streptococcus pyrogenaes	Gram +ve
3.	Escherichia coli	Gram - ve
4.	Pseudomonas aeruginosa	Gram - ve

Fungal strains:

1. Aspergillus niger,
2. Aspergillus flavus,
3. Penicillium expansum,
4. Candida albicans,
5. Rhizopus nigricans,
6. Botrydepladia thiobromine.

The antibacterial activity was investigated using agar disk diffusion method [19 - 22]. The activity of zeolite derivative mixture was studied against above mentioned bacteria strains (1 to 6). 50 mg of two sample of each mixture and reference drug were slurred in DMSO solvent. Bacteria form a 24 h culture contain about 100-105 CFU/ml were spread on nutrient agar (Prepared form 1% Tryptone, 1% Agar, 0.5% NaCl 0.5% yeast extract in 1 litre water at pH 7) which was autoclaved at 120°C for half an hour. Then the disks were loaded with the 50 mg sample slurred in DMSO. Ciprofloxacin (1 mg/ml) was used as a reference drug. The disks were kept in an incubator at 37°C for 2 days and examined for inhibition zone of various zeolite derivative mixtures. The experiment was performed in duplicate. The inhibition zone was measured by caliper to receive mean value in mm.

All the mixture (1 to 6) were screened separately for their antifungal strain. The antifungal activity was performed by ager cup method as follows.

A potato – dextrose – agar (PDA) (40:10:15) thick syrup was prepared and sterilized by autoclaving at 120°C under pressure for half an hour. The sterilized syrup (20ml) was then solidified kept in an incubator to about 100 – 105 CFU/mg Fungal strain (listed above) was spread out uniformly on solid petri dish by sterilized folded glass road, left for 10 minutes till culture is properly absorbed on the surface of PDA. Small wells of 4mm size were cut into the dish with the help of sterile metallic bores. The various mixtures of 50mg in zeolite derivatives were loaded into wells fluconazole (1mg/ml) was used as reference. The petri dishes were kept for incubation at 30°C for 5 days. Then inhibition zone was measured.

Table-2 : Antibacterial activity of mixture of Zeolite derivatives

Zeolite derivative mixture	**Zone of inhibition of growth of bacteria (mm) of 100-105 CFU/ml culture**			
	Gram +ve bacteria		Gram +ve bacteria	
	Staphylococcus aereus	Streptococcus pyrogenaes	Escherichia coli	Pseudomonas aeruginosa
1.	19	20	21	19
2.	17	18	20	18
3.	17	17	20	18
4.	19	21	22	23
5.	19	19	20	18
6.	18	17	16	19
Ciprofloxacin	25	24	24	25

Table-3: Antibacterial activity of mixture of Zeolite derivatives

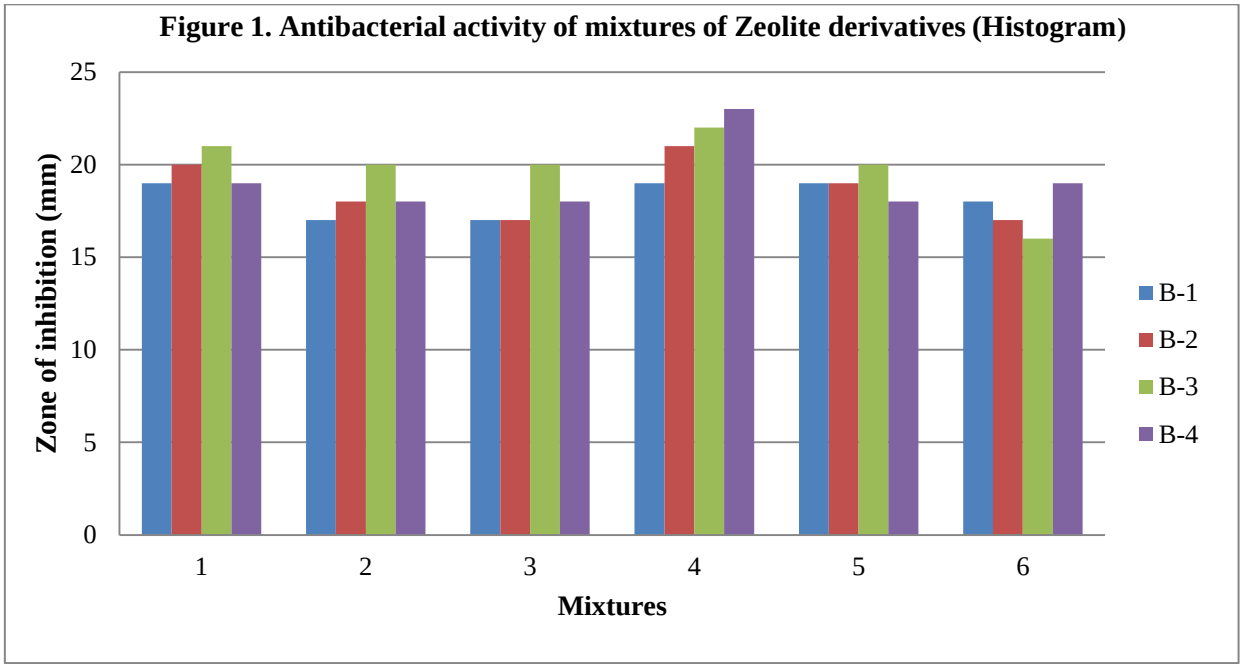
Zeolite derivative mixture	**Zone of inhibition of growth of fungi (%) of 1000 ppm**					
	Aspergillus niger	Aspergillus flavus	Pennicillium expansum	Candida albicans	Rhizopus nigrieuns	Botrydepladia thiobromine
1.	82	80	80	78	75	80
2.	75	77	78	80	75	75
3.	68	68	70	73	75	70
4.	85	85	82	80	80	85
5.	76	75	73	70	71	70
6.	67	70	65	67	65	70
Fluconazole	90	93	95	90	94	95

Result and discussion: –

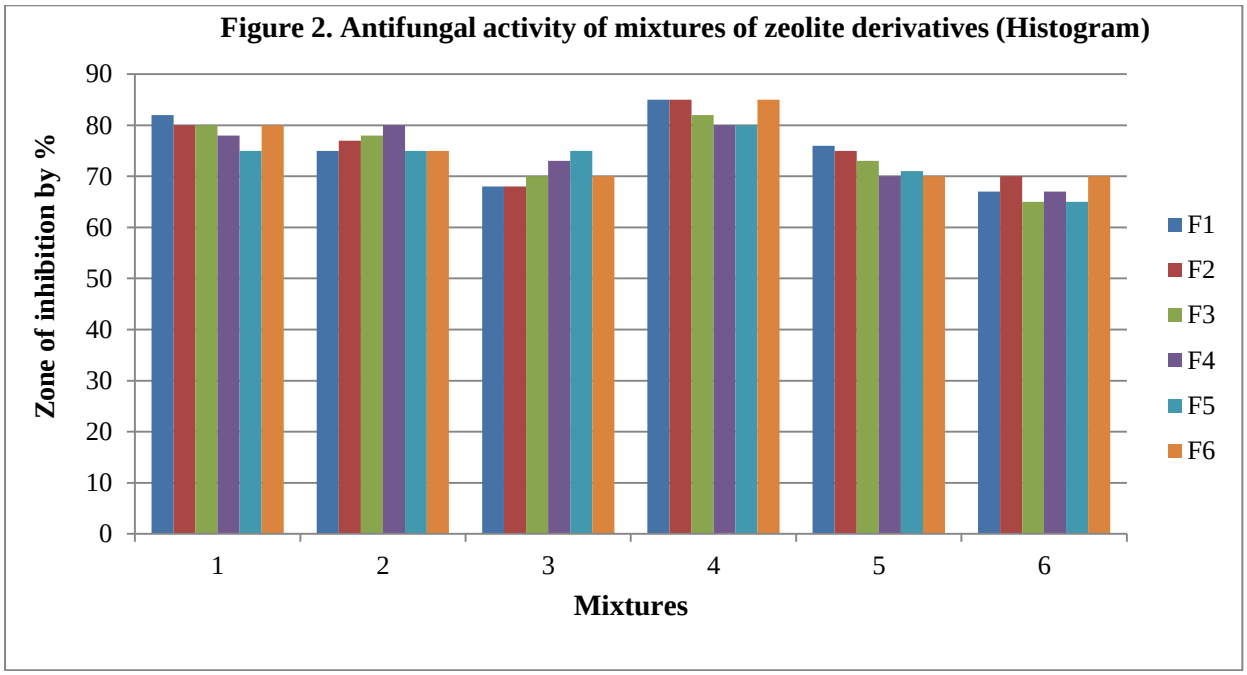
The present study is based on the antimicrobial activity of mixtures of zeolite derivatives already reported [16-18]. The aim is to investigate for effect of mixture on their toxicity against common pathogens. The study indicates that Propolis–zeolite (PZ), Silver–zeolite (SZ) and Silver–sulfadiazine (ASZ) all have good antimicrobial activity. The study covers composite, nanocomposite or coating form. The present study comprises the qualitative antimicrobial study of powder mixtures of PZ/SZ/ASZ zeolite derivatives.

Table 2 and Table 3 represent the antibacterial and antifungal activity of PZ/SZ/ASZ mixtures respectively. Examination of the antibacterial study (Table -1) revealed that all the mixtures have very good toxicity against Gram +ve and Gram –ve bacteria. The mixture No. 4 give highest antibacterial activity. This may be due to release increase in the quantity of antimicrobial agents i.e. Silver, Propolis, Silver – sulfadiazine. 50 mg of mixture was taken with sufficient amount of Silver, Propolis and Silver-sulfadiazine. The present zeolite derivatives mixtures showed broad antibacterial potency due to easy release of antibacterial agents. The results are also compared with standard drug i.e., ciprofloxacin with give highest inhibition zone.

The results of antifungal activity of all five mixtures of zeolite derivatives are shown in Table 3. The resultant data showed that all the mixtures have good toxicity for all six fungal strain. The mixture 3 is showed very good antifungal activity.



B1- Staphylococcus aereus, B2- Streptococcus pyrogenaes, B3- Escherichia coli, B4- Pseudomonas aeruginos.



F1-Aspergillus niger, F2-Aspergillus flavus, F3-Pennicillium expansum, F4-Candida albicans, F5-Rhizopus nigrieuns, F6-Botrydepladia thiobromine.

Conclusion: -

Zeolite derivatives viz. Silver – zeolite propolis, Silver – sulfadiazine – zeolite reported earlier were selected for present study. These derivatives were hybridized in various proportions. The in - vitro antibacterial and antifungal activities of these zeolite derivatives mixtures were evaluated by Agar disk diffusion method indicate that these derivatives are good toxicity agent bacteria and fungi mentioned in this paper. These zeolite derivatives can be of potential use for pharmaceuticals.

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