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ANTIMICROBIAL EVALUATION OF MALEIMIDE MONOMERS, HOMOPOLYMERS AND COPOLYMERS CONTAINING AZO, SULFONAMIDE AND THIAZOLE GROUPS

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ABSTRACT

New maleimide Monomers, homopolymers and copolymers containing **azo, sulfonamide** and **thiazole** groups were synthesized. The structures of newly synthesized compounds have been characterized on the basis of their spectroscopic data (FT-IR and ^{1}H-NMR). Antimicrobial studies of synthesized compounds were performed using agar well diffusion method. All the compounds showed pronounced activity against bacterial strains in comparison to activity against fungal strains.The **highest antibacterial** potency was exhibited by the **homopolymer** HPSPMI against **Escherichia coli, Klebsiella pneumoniae** and **Bacillus subtilis**. The activity of HPSPMI against later two was **more** pronounced than the **standard** drug used. Only Selected compounds showed antifungal activity. Appreciable results were found against Aspergillus fumigates but not with Candida albicans. HPSPMI showed highest activity against Aspergillus fumigates. Surprisingly after homo and copolymerization, maximum compounds exhibited not only pronounced antibacterial activity but also antifungal activity.

Keywords

maleimide monomers; homopolymers and copolymers; containing azo; sulfonamide and thiazole groups; antimicrobial activity

Academic Discipline And Sub-Disciplines

Chemistry and Polymer chemistry

TYPE (METHOD/APPROACH)

Synthesis of Monomers & polymers and antimicrobial assay

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INTRODUCTION

Antimicrobial polymer, also known as polymeric biocides is a class of polymers with antimicrobial activity or the ability to inhibit the growth of microorganisms. Significant advance in the past three decade have been made in the synthesis and application [1] of polymers to prevent microbial attack [2][3][4][5] and degradation for diverse end uses. This led to the discovery of a variety of new polymers with bioactive functional groups. Antimicrobial polymer coatings are intended for modification of packaging materials to inhibit spoilage of foodstuffs (bakery, confectionery, dairy, meat, fruit and vegetables), for which they are filled with antimicrobial components. Antimicrobial packages have been developed, Antimicrobial [6][7][8][9] [10][11] polymers are ideal for applications in hand-held water filters, surface coatings and fibrous disinfectants because they can be fabricated by various techniques and can be made insoluble in water. The design of insoluble polymers that can inactive, kill or remove target microorganisms by mere contact without releasing any reactive agents to the bulk phase being disinfected is desired. Applications of antimicrobial polymers are also going to exploit in textile industries. Antifungal agents (such as ketoconazole) are often found in anti-dandruff shampoos. The field of antimicrobial polymers [12][13][14] [15] has progressed steadily but slowly over the past years it appears to be on the verge of rapid expansion. The greater need for materials that fight infection will give encouragement for discovery and use of antimicrobial polymers. The use of antimicrobial polymers [16][17][18][19][20]offers promise for enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity and prolonging the lifetime of the antimicrobial agents. Research concerning the development of antimicrobial polymers represents a great challenge for both the academic world and industry. In this article polyimides are chosen as the polymeric substrate to describe antimicrobial properties.

EXPERIMENTAL

Procedure: Monomer Synthesis

Novel monomers N-[4-(Azophenyl) phenyl] maleimide monomer **(PAPMI)**, N-[4-(Sulfonamide) phenyl] maleimide monomer **(PSPMI)**, N-[4-(Phenyl) thiazole] maleimide monomer* **(PTHPMI)** were synthesized using a general procedure which has been described below (Sheme-1).

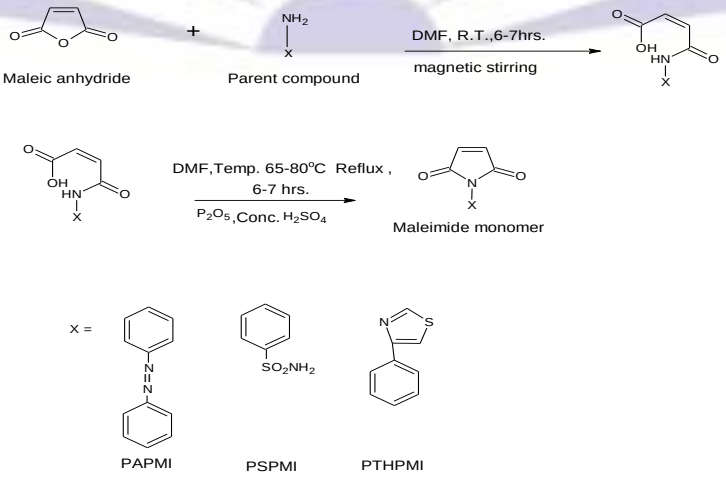
Step – I

The solution of maleic anhydride (0.1 mol) in DMF was gradually added over a period of 10-15 min. to a solution of parent compound (from which monomer has to be synthesized) in appropriate quantity of DMF solvent. The solution was stirred for 6-7 hrs at room temperature.

Step – II

It was again stirred for 6-7 hrs with 5 to 8 gm P_2O_5 and 4 to 6 drops of concentrated H_2SO_4 at 65 to 80°C temperature. The resulting solution was poured into crushed ice or cold water to precipitate monomer. The solution was filtered and washed with distilled water, sodium bicarbonate and again with distilled water. The remaining residue left behind was of crude monomer, which was filtered and dried in vacuum. On drying, crude monomer was crystallized with appropriate solvent to obtain pure monomer. The monomer synthesis was confirmed by FT-IR, ^{1}H-NMR and elemental analysis.

*Before the synthesis of PTHPMI, it's parent compound 2-amino-4-phenyl thiazol was synthesized using following method: A mixture of 0.1 mole acetophenone, 0.1 mole iodine and 0.2 mole thiourea was heated on water both for 4-5 hrs. The resultant mixture was cooled and triturated with 100 c.c. diethyl ether. It was filtered and washed twice with 50 c.c. diethyl. It was filtered and washed twice with 50 c.c. diethyl ether and dried. After completely drying it was dissolved in hot water and immediate treated with concentrated NH4OH until the solution become alkaline. As the solution became alkaline we got precipitation. It was filtered and washed 3 to 4 times with cold water and dried.



Scheme-1

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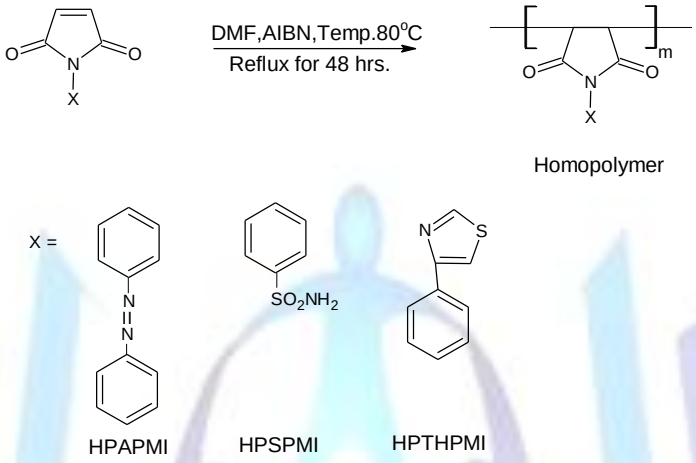
Procedure: Homopolymer Synthesis

These monomers were further used to synthesize homopolymers Poly-N-[4-(Azophenyl) phenyl] maleimide **(HPAPMI)**, Poly-N-[4-(Sulfonamide) phenyl] maleimide **(HPSPMI)**, Poly-N-[4-(Phenyl) thiazole] maleimide **(HPTHPMI)** via free radical polymerization using AIBN as initiator. A general procedure was used for homopolymerization, which has been described below (Scheme-2).

0.01 mole of monomer was taken in 40 to 50 ml DMF with 150 mg AIBN in a two necked round bottom flask and reflux at 80 to 85°C for 48 hrs. After polymerization for a given time, the reaction mixture was poured into 10% methanol water mixture to precipitate homopolymer. Homopolymer was filtered, purified by crystallization using appropriate solvent and dried under vacuum. Homopolymer synthesis was confirmed by FT-IR and ^{1}H-NMR.



Scheme-2

Procedure: Copolymer Synthesis

Above mentioned monomer units were used to synthesize following copolymers;

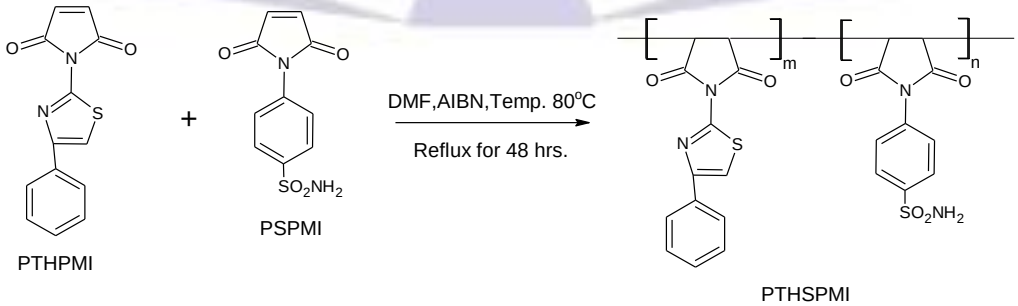
Poly(N-[4-(Azophenyl) phenyl] maleimide-co-N-[4-(Sulfonamide) phenyl] maleimide); **PAPSPMI**

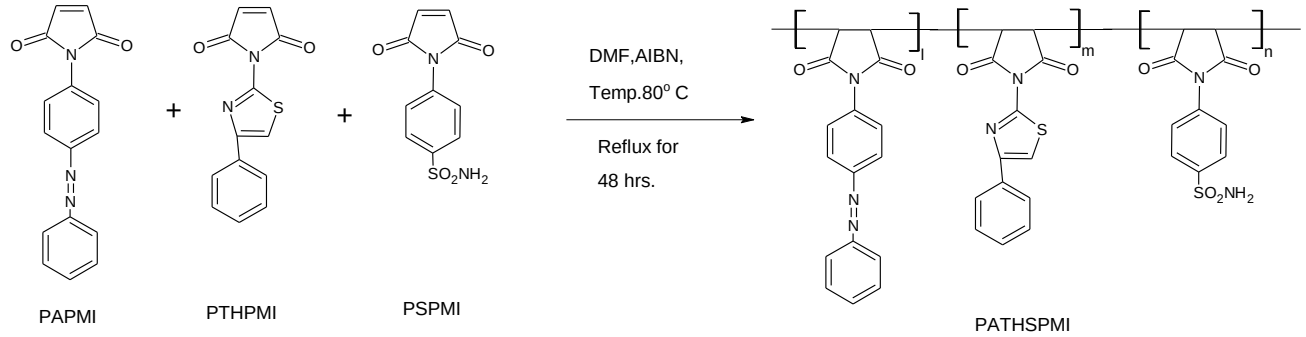
Poly(N-[4-(Azophenyl) phenyl] maleimide-co-N-[4-(Phenyl) thiazole] maleimide); **PATHPMI**

Poly(N-[4-(Azophenyl) phenyl] maleimide-co-N-[4-(Phenyl) thiazole] maleimide-co-N-[4-(Sulfonamide) phenyl] maleimide); **PATHSPMI**

A general procedure was used to synthesize all copolymers, which has been described below (Scheme-3,4).

Monomer units were taken in equimolar quantity (0.01 mole) with 40 – 60 ml DMF solvent into a three necked round bottom flask. Compound should be dissolved completely. In the reaction solution 150 mg AIBN was introduced and shaked well to dissolve AIBN completely. Now the reaction mixture was refluxed at 80°C for 48 hrs. The reaction mixture was poured into 10% methanol water mixture to precipitate copolymer after polymerization for a given time. Copolymer was filtered, washed twice or thrice with distilled water and dried completely under vacuum. Crude copolymer was crystallized using appropriate solvent to get purity of sample. Synthesis of copolymer was confirmed by ^{1}H-NMR and FT-IR spectral analysis.





Scheme-3,4

SPECTRAL ANALYSIS

FTIR spectra of synthesized compounds were recorded on Perkin-Elmer Spectra RXI (4000 – 450 cm^{-1}) FTIR spectrophotometer using KBr pellet technique. ^{1}H-NMR spectra of samples were recorded on a Bruker DPX-300 spectrometer at 300 MH_Z with acetone as a solvent.

PAPMI & PSPMI: As reported in Int. J. of Chemistry and Chemical Engineering[21]

2-amino-4-phenyl thiazol: light yellow, 75 % Yield, mp: 144-146°C; **FTIR(CM^{-1}):** Ar. C=C and C=N 1604.1, 1522.1, 1439.5, 1334.4 , Ar. C–H str., 3020.1 , phenyl ring 762.2, 669.2, C–S str. 611.7 , NH_2 sym. and asym. Str. 3247.0 and 3392; **^{1}H-NMR** (300 MH_Z ,acetone, δ ppm): 4.85 (s, 2H, NH_2), 7.42 (s, 1H, thiazole), 7.48 - 8.18 (m, 5H, phenyl), Figure 1& 1.1

PTHPMI: Dark green, 83 % Yield, mp:162-163°C **; FTIR(CM^{-1}):** C=C Str. 1618.2, =C–H Str. 3098.2, C = O Sym. and asym. str. 1716.9 & 1782.2, Ar. C=C str. 1520.2, Ar. C–H str. 3021.3, Ar. C–N str. 1304.3, phenyl ring 671.1, 765.1, C–S Str. 627.0; **^{1}H-NMR (**300 MH_Z ,acetone , δ ppm**):** 7.59 (s, 2H, HC=CH), 7.21 (s, 1H, thiazole), 7.32-7.96 (m, 5H, phenyl), Figure 2 & 2.1

HPAPMI: Dark reddish brown, 85 % Yield, **FTIR(CM^{-1}):** C–C Str. 2951.2, – C–H Str. 1450.0, C = O Sym. and asym. 1714.2, 1780.0, Ar. C=C str. 1504.1, 1599.4, Ar. C–H str. 3019.4, Ar. C–N str.1302.2, phenyl ring 842.7, 688.1, 769.7; **^{1}H-NMR (**300 MH_Z ,acetone, δ ppm**):** 3.04 (s, 2H,HC – CH), 7.44 – 8.02 (m, 9H, phenyl), Figure 3 & 3.1

HPSPMI: Black, 67 % Yield, **FTIR(CM^{-1}):** C–C Str. 2972.7, – C–H Str. 1425.0, C = O Sym. and asym. 1713.2, 1778.5, Ar. C=C str. 1518.9, 1603.9, Ar. C–H str. 3020.1, Ar. C–N str., 1365.3, phenyl ring 797.7, S = O Str. 1027.5, SO_2 Sym. and asym. 1137.5, 1260.1. NH_2 Sym. and asym. 3250.3, 3342.7, C – S Str. 672.7; **^{1}H-NMR (**300 MH_Z ,acetone, δ ppm**):** 3.12 (s, 2H, HC – CH), 6.62 (s, 2H,NH_2), 6.74 - 7.28 (dublets, 4H, phenyl) Figure 4 & 4.1

HPTHPMI: Dark green ,72 % Yield, **FTIR(CM^{-1}):** C–C Str. 2962.8, – C–H Str. 1445.9, C = O Sym. and asym. 1725.8, 1776.2, Ar. C=C str. 1546.4, Ar. C–H str. 3020.0, Ar. C–N str. 1344.2, phenyl ring 670.2, 768.6, C–S Str. of thiazole ring (very weak) 631.2; **^{1}H-NMR (**300 MH_Z ,acetone, δ ppm**):** 2.94 (s, 2H, HC – CH), 7.32 -8.05 (m, 5H, phenyl) Figure 5 & 5.1

PAPSPMI: As reported in Int. J. of Chemistry and Chemical Engineering[21]

PTHSPMI: Dark Greenish black, 86 % Yield, **FTIR(CM^{-1}):** C–C Str. 2958.8, – C–H Str. of maleimide ring

1444.2, C = O Sym. and asym. Str. 1715.4, 1779.2, Ar. C=C str. 1506.0, 1593.1, Ar. C–H str. 3022.0, Ar. C–N str. 1334.3 phenyl ring 671.1, 764.3, S = O Str. 1023.6, SO_2 Sym. and asym. 1116.5, 1383.7, NH_2 Sym. and asym. 3233.9, 3298.2, C – S Str. 621.1, **^{1}H-NMR (**300 MH_Z ,acetone and $CDCl_3$, δ ppm**):** 2.95 (2 H, HC – CH), 7.19 (s,1H, thiazole), 6.67 (s,2 H, NH_2), 7.02 – 8.00 (m, 9 H,phenyl), Figure 6 & 6.1

PATHSPMI: Greenish black, 62 % Yield , **FTIR(CM^{-1}):** C–C Str. 2968.1, C – H Str. 1480.1, C=O Sym. and asym. 1718.8, 1776.1, Ar. C=C str. 1591.3, Ar. C=H str. 3021.0, Ar. C–N str. 1363.2, phenyl ring 673.1, 767.4, S=O Str. 1027.8, SO_2 Sym. and asym. Str. 1158.2, 1319.3, NH_2 Sym. and asym. 3353.7, 3288.5, C – S Str. 620.4, **^{1}H-NMR (**300 MH_Z ,acetone, δ ppm**):** 3.09 (2 H, HC – CH), 6.64 (s, 2 H,NH_2), 7.38 (s,1 H, thiazole), 6.76 – 8.01(m,18 H, phenyl), Figure 7 & 7.1

ANTIMICROBIAL STUDY

Antimicrobial activity of bioactive compounds against test microorganisms (bacteria and fungi) was evaluated using Agar Well Diffusion Assay (Lehrar *et al.*, 1991; Reddish 1929). The agar well diffusion assay was carried out by preparation of 4 to 6 wells of 10 mm diameter using a sterile cork borer per 90 mm agar plate aseptically (nutrient agar and sabouraud dextrose agar were used for antibacterial and antifungal activity respetively). A known quantity (10 µl) of microorganism was grown on agar plates. The agar cylinders were removed using a sterile loop. The wells were grouped as the test well and the control or standard well. The test wells were filled with 200 µl solution of sample compounds at 500 µg/ml concentration and the control well was filled with the same concentration and same volume of the standard. The wells were then sealed with agar and kept for 10 minutes. The plates were incubated at room temperature for 24 hours for antibacterial and 3 days for antifungal susceptibility. After incubation a zone referred to zone of inhibition developed around the well if the sample is sensitive towards the microorganism species. The inhibition zones were recorded in

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millimeters (mm) in the test well as the control well. An organism, which was placed on the agar, will not grow in the area around the well or in the zone of inhibition if it is susceptible to the sample compound.

Antibacterial Assay

Antibacterial susceptibility of above following compounds was tested by agar well diffusion assay using following microorganisms: **Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Bacillus subtilis** (Figure a&b). To perform antibacterial susceptibility, **ciprofloxacin** was used as standard or control to reveal the potency of synthesized compound. It is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein.

Table 1: Antibacterial Assay of Synthesized Compounds

Compound	Zone of Inhibition* (mm)			
	Escherichia coli	Klebsiella pneumoniae	Pseudomonas aeruginosa	Bacillus subtilis
PSPMI	5	23	10	2
PAPMI	11	23	11	14
PTHPMI	11	4	10	2
HPSPMI	**17**	**28**	16	**25**
HPAPMI	14	11	8	12
HPTHPMI	2	12	10	10
PAPSPMI	5	26	13	17
PTHSPMI	7	21	10	5
PATHSPMI	4	10	**18**	8
Standard	**25**	**23**	**27**	**24**

*Zone of inhibition is mentioned after subtracting the well diameter

Table 2: Maximum Zone of Inhibition with Corresponding Species

Compound	Species	Zone of Inhibition (mm)
PSPMI	**Klebsiella pneumoniae**	**23**
PAPMI	Bacillus subtilis	14
PTHPMI	Escherichia coli	11
HPSPMI	**Klebsiella pneumoniae**	**28**
HPAPMI	Escherichia coli	14
HPTHPMI	**Klebsiella pneumoniae**	**12**
PAPSPMI	**Klebsiella pneumoniae**	**26**
PTHSPMI	**Klebsiella pneumoniae**	21
PATHSPMI	Pseudomonas aeruginosa	18

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Table 3: Comparative Antibacterial Assay of Monomers, Corresponding Homopolymers and Copolymers

Compound		Zone of Inhibition (mm)			
		Escherichia coli	**Klebsiella pneumoniae**	**Pseudomonas aeruginosa**	**Bacillus subtilis**
M	PSPMI	5	23	10	2
H	HPSPMI	**17**	**28**	**16**	**25**
M	PAPMI	11	23	11	14
H	HPAPMI	**14**	11	8	12
C	PAPSPMI	5	**26**	**13**	**17**
M	PTHPMI	**11**	4	**10**	2
H	HPTHPMI	2	12	**10**	**10**
C	PTHSPMI	7	**21**	**10**	5

Here M = Monomer, H = Homopolymer, C = Copolymer

Antifungal Assay

Antifungal susceptibility of PSPMI, PAPMI, PTHPMI, HPSPMI, HPAPMI, HPTHPMI, PAPSPMI, PTHSPMI and PATHSPMI was performed using agar well diffusion assay using **Aspergillus fumigatus** and **Candida albicans** (Figure b).

To screen antifungal susceptibility of compounds, **fluconazole** was used as standard or control to expose the potency of the synthesized compounds. It is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections.

Table 4: Antifungal Assay of Synthesized Compounds

Compound	Zone of Inhibition* (mm)	
	Aspergillus fumigatus	**Candida albicans**
PSPMI	0	**1**
PAPMI	0	0
PTHPMI	0	**1**
HPSPMI	**16**	0
HPAPMI	13	0
HPTHPMI	0	**1**
PAPSPMI	15	0
PTHSPMI	6	0
PATHSPMI	0	0
Standard	**0**	**14**

* Zone of inhibition is mentioned after subtracting the well diameter

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Table 5: Comparative Antifungal Assay of Monomers, Corresponding Homopolymers and Copolymers

Compound		Zone of Inhibition (mm)	
		Aspergillus fumigatus	Candida albicans
M	PSPMI	0	**1**
H	HPSPMI	**16**	0
M	PAPMI	0	0
H	HPAPMI	13	0
C	PAPSPMI	**15**	0
M	PTHPMI	0	**1**
H	HPTHPMI	0	**1**
C	PTHSPMI	**6**	0

Here M = Monomer, H = Homopolymer, C = Copolymer

In the antibacterial and antifungal studies symbols 1 to 10 were used for the compounds and standard. Their details are:

Symbol	Compound	Symbol	Compound
1	PTHSPMI	6	PAPMI
2	PAPSPMI	7	HPTHPMI
3	HPSPMI	8	PTHPMI
4	HPAPMI	9	PSPMI
5	PATHSPMI	10	Standard

RESULT AND DISCUSSION

N-substituted maleimide monomers, homopolymers and copolymers were synthesized. Terpolymer synthesis was also carried out to find out the possibility of terpolymer formation. Antimicrobial study was done with all synthesized compounds.

Antibacterial assay- Table 1 shows that all the tested compounds performed moderate to excellent antibacterial activity against the bacterial strains. It is evident from this table that the highest antibacterial activity was exhibited by the homopolymer HPSPMI against Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis. Surprisingly the activity of HPSPMI against Klebsiella pneumoniae and Bacillus subtilis was more pronounced than the standard drug used. The zone of inhibition was found higher for HPSPMI than standard against above species. This reveals higher potency of synthesized compounds towards antibacterial activity.

The data presented in table 2 indicates the maximum zone of inhibition for each compound with corresponding species. The results showed that the maximum zone of inhibition or highest antibacterial activity was exhibited against Klebsiella pneumoniae. Escherichia coli was next in these results.

Table 3 includes comparable data of monomers with their corresponding homopolymers and copolymers.

PSPMI and HPSPMI: It is evident from the table that after homopolymerization of PSPMI, the potency of HPSPMI towards antibacterial activity increased to great extent against all species.

PTHPMI, HPTHPMI and PTHSPMI: The effect of all these three on different species was not uniform except pseudomonas aeruginosa, all three gave the same spectrum with zone of inhibition 10 mm. PTHPMI was found the most effective against Escherichia coli. In case of Klebsiella pneumoniae PTHSPMI was the best and HPTHPMI was found the most effective against Bacillus subtilis.

Antifungal Assay – The data mentioned in table 4 indicates that compounds did not perform the antifungal potency as they performed against bacterial species. Surprisingly only few of them showed antifungal activity against selected fungi. The highest antifungal activity against Aspergillus fumigatus was exhibited by HPSPMI. The result of PAPSPMI was also

very appreciable and comparable to HPSPMI. The most wondering fact that can be observed from the table was all compounds that exhibited antifungal activity against Aspergillus fumigatus were more pronounced than standard drug fluconazole. PSPMI, PAPMI, PTHPMI, HPTHPMI and PATHSPMI did not show antifungal activity against Aspergillus fumigates.

The results against Candida albicans were shocking and contrary of the former discussed. The compounds that did not exhibit activity against Aspergillus fumigatus, most of them showed activity against Candida albicans and vice versa.

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PSPMI, PTHPMI and HPTHPMI showed very moderate antifungal activity against Candida albicans. In comparison to standard drug, the results were very poor.

Table 5 includes comparable data of monomers with their corresponding homopolymers and copolymers.

PSPMI and HPSPMI: Antifungal potency of homopolyer HPSPMI was pronounced over PSPMI against Aspergillus fumigatus. In case of Candida albicans contrary results were observed. HPSPMI did not exhibit antifungal activity whereas PSPMI gave moderate results.

PTHPMI, HPTHPMI and PTHSPMI: No antifungal activity was exhibited by monomer PTHPMI and its homopolymer HPTHPMI against Aspergillus fumigatus, only copolymer PTHSPMI exhibited antifungal potency. In case of Candida albicans just contrary results were found. PTHPMI and HPTHPMI showed moderate antifungal activity, however PTHSPMI did not exhibit such kind of activity.

CONCLUSION

All synthesized compounds showed excellent to moderate antimicrobial potency. The highest antibacterial and antifungal activity was exhibited by the homopolymer **HPSPMI** against Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis (bacterial species) and Aspergillus fumigates (fungal species). It again proves antimicrobial potency of **sulfonamide** group. The activity of HPSPMI against Klebsiella pneumoniae and Bacillus subtilis was more pronounced than the **standard** drug **Ciprofloxacin.** After homo and copolymerization, maximum compounds showed pronounced antibacterial as well as antifungal activity.

Synthesized compounds showing antimicrobial activity may be beneficial in numerous diseases where standard **Ciprofloxacin & Fluconazole** is used like typhoid fever, urinary tract, lower respiratory tract infections, acute sinusits, infectious diarrhea, bone and joint infections, skin and skin structure infections onychomycosis, candidiasis, Tinea corporis, Histoplasmosis etc. These results signify that the compounds are useful for antimicrobial applications.

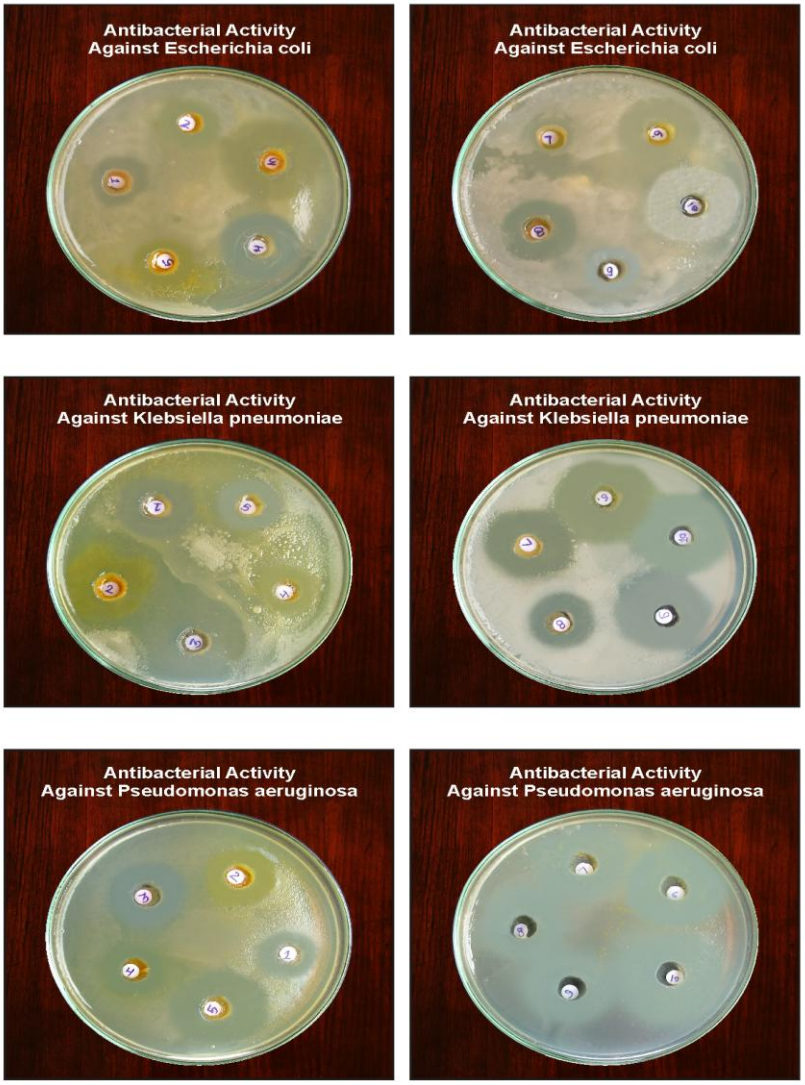

Figure a

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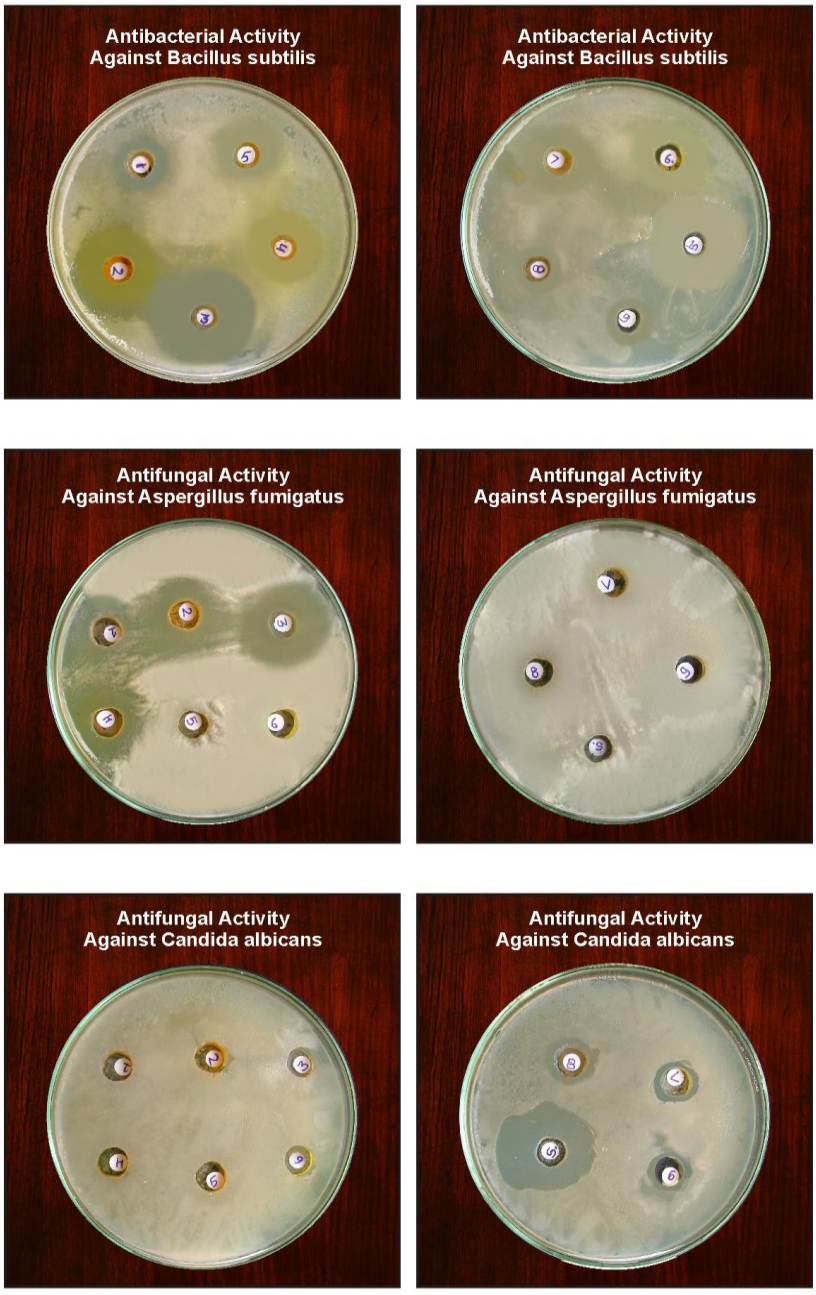

Figure b

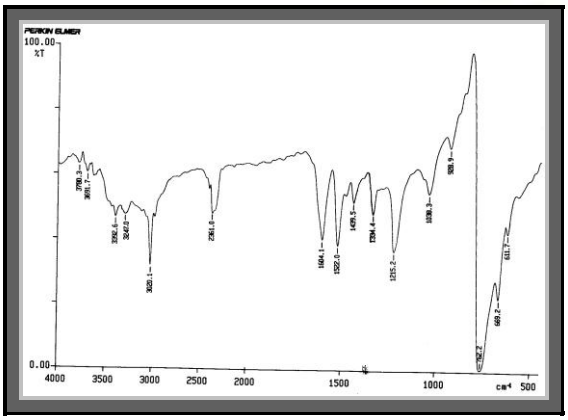

Figure1: FTIR Spectra of 2-amino-4-phenyl thiazole

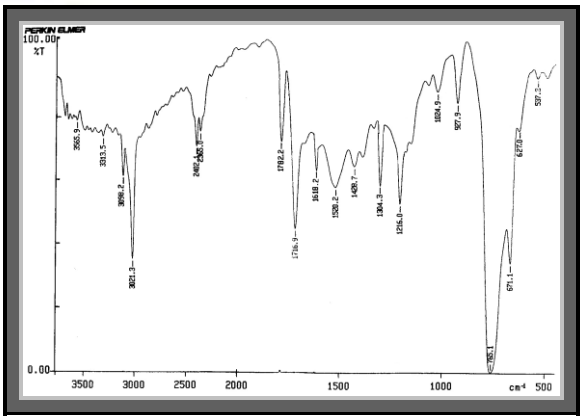

Figure2: FTIR Spectra of PTHPMI

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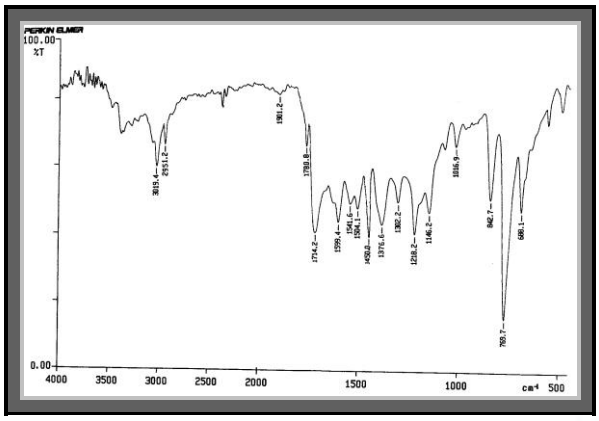

Figure 3: FTIR Spectra of HPAPMI

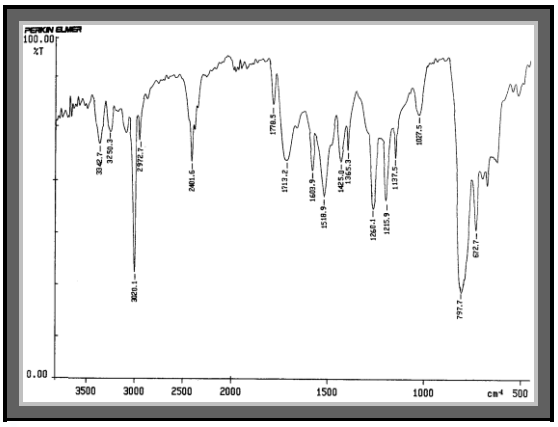

Figure 4: FTIR Spectra of HPSPM

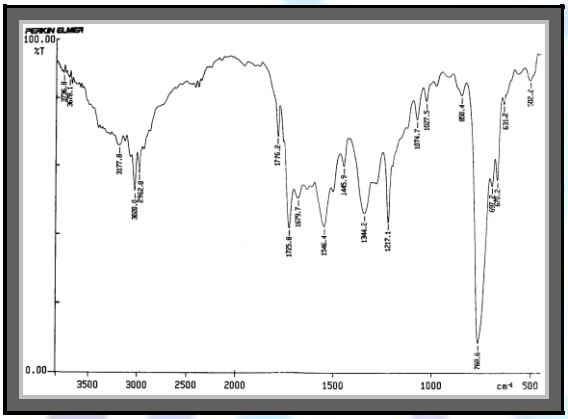

Figure 5: FTIR Spectra of HPTHPMI

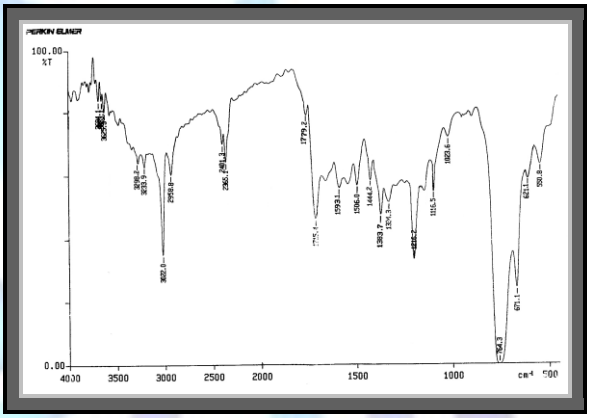

Figure 6 : FTIR Spectra of PTHSPMI

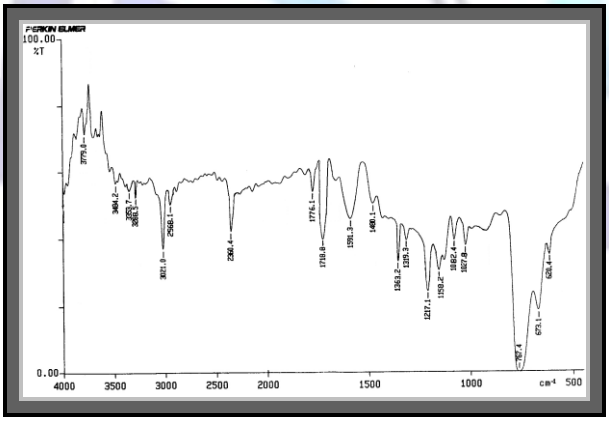

Figure7 : FTIR Spectra of PATHSPMI

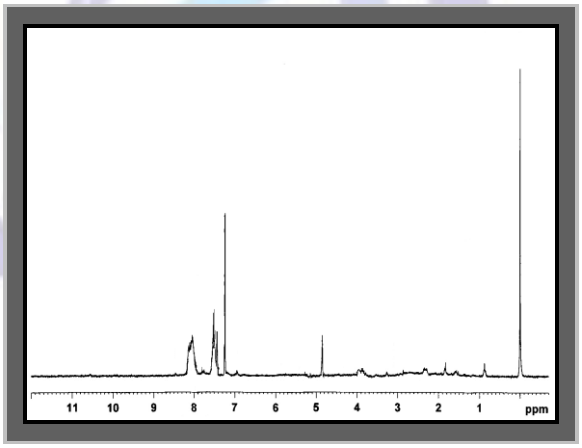

Figure 1.1: ^{1}H-NMR Spectra of 2-amino-4-phenyl thiazol

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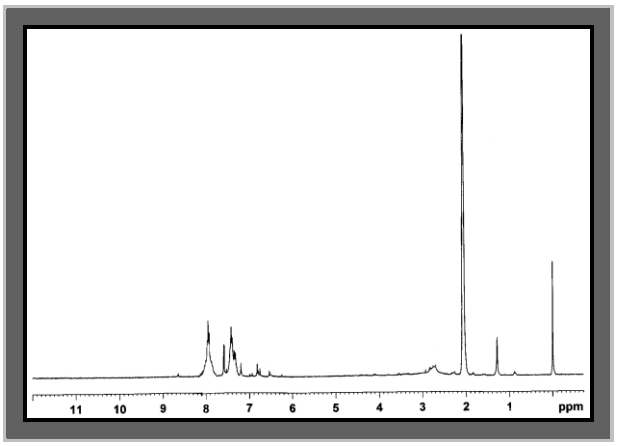

Figure 2.1: ^{1}H-NMR Spectra of PTHPMI

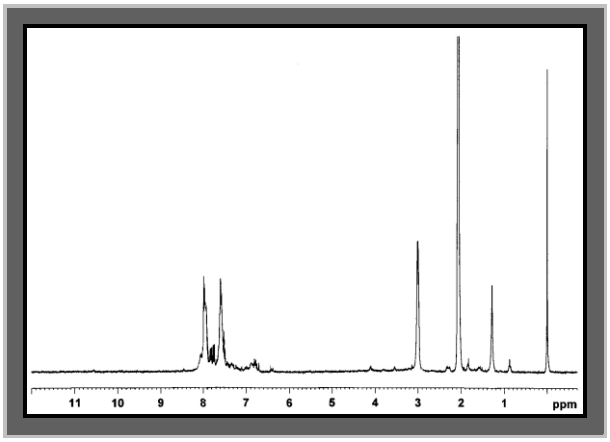

Figure3.1: ^{1}H-NMR Spectra of HPAPMI

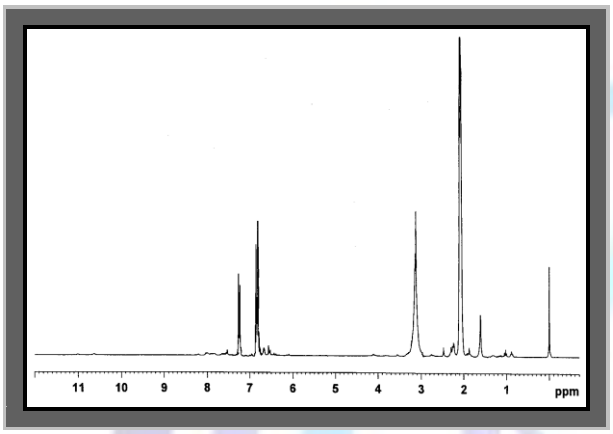

Figure 4.1: ^{1}H-NMR Spectra of HPSPMI

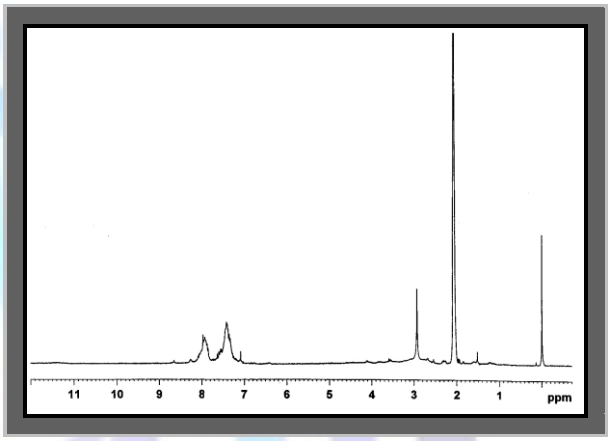

Figure 5.1: ^{1}H-NMR Spectra of HPTHPMI

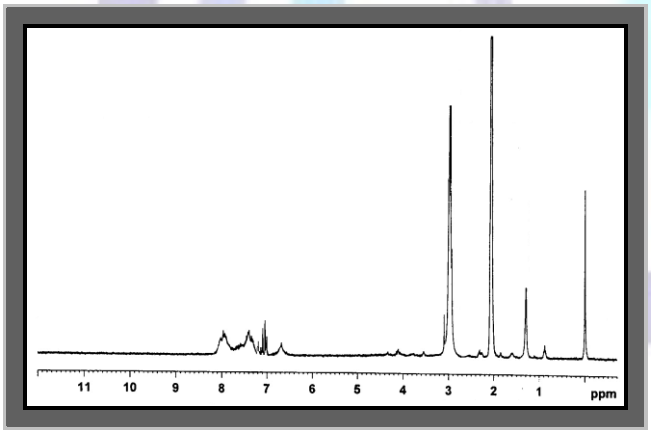

Figure 6.1: ^{1}H-NMR Spectra of PTHSPMI

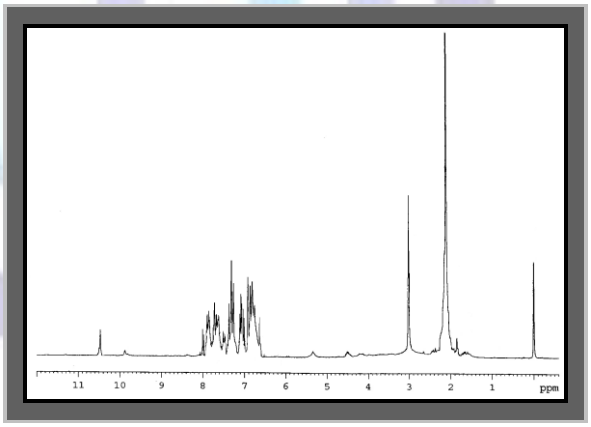

Figure6.1: ^{1}H-NMR Spectra of PATHSPMI

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