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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF IMIDAZOLIUM BASED PROTIC IONIC LIQUIDS

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ABSTRACT

Six imidazolium-based room temperature protic ionic liquids (PILs) have been synthesized by the process of proton transfer from hydroxy acids (Bronsted acids) to substituted imidazole (Bronsted base). The PILs were characterized by UV, Infrared and Nuclear Magnetic Resonance and GC-MS spectroscopes. Their anti-microbial activities were determined using the well-diffusion method. All six PILs were toxic to *Ecoli streptomyces bacillus*, while 2-methyl imidazolium lactate showed high anti-microbial activity against a wide range of human pathogens

INTRODUCTION

Ionic liquids (ILs) are a class of solvents having negligible vapor pressure, good thermal and chemical Stabilities etc. ILs act as potential replacements for the conventional volatile and environmentally harmful conventional molecular solvents [1-2]. Ionic liquids (ILs) are generally divided into two categories, aprotic ionic liquids (AILs) and protic ionic liquids (PILs). Protic ionic liquids are formed by a variety of proton transfer between Bronsted acids and Bronsted bases under solvent-free conditions. The application of PILs in chemistry appears to be very high, and PILs have been explored in a number of areas such as novel electrolytes for fuel cells and batteries [3], biological application[4], organic synthesis[5], and chromatography[6]. Pernak and Skrzypczak reported the antibacterial activities of a series of imidazolium- and pyridinium-based ILs[7-12]. Pernak *et al.* found that antimicrobial activities of the protic ILs are strongly related to the length of the substituent and to the type of anion [13]. The long alkyl chain imidazolium salts with methyl and hydroxyethyl substitution in the 2 and 3 positions of the imidazolium ring have an efficient antimicrobial activity. In this paper there are six protic ionic liquids were synthesized and characterized and their antimicrobial activities are investigated using well diffusion method.

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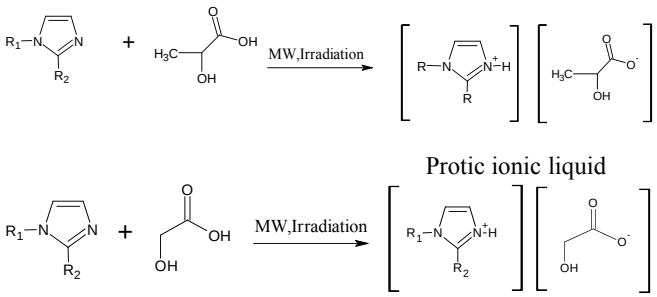
2. EXPERMENTAL

2.1Synthesis and characterization of various imidazolium PILs

The equimolar mixture of the precursor Bronsted acids (hydroxyl acids) and Bronsted bases (substituted imidazole) were taken in a reacting vessel and stirred for an hour using magnetic stirrer and then it was placed in a microwave and irradiated. The obtained ionic liquids are collected and characterized.



Protic ionic liquid
Where R_1-H,C_2H_5-,C_4H_9-. **R_2**-H,CH_3

Table 1. Reaction Condition for the Preparation of Various PILs

S.No	Bronsted bases	Bronsted acids	Protic Ionic liquids	MW power level (W)	Time (s)
1.	2-Methylimidazole	Lactic acid	2MIML	160	30
2.	1-Ethylimidazole	Lactic acid	1EIML	080	40
3.	1-Butylimidazole	Lactic acid	1BIML	240	20
4.	2-Methylimidazole	Glycolic acid	2MIMG	160	40
5.	1-Ethylimidazole	Glycolic acid	1EIMG	160	20
6.	1-Butylimidazole	Glycolic acid	1BIMG	240	20

2.2 Measurement

The solubility of imidazolium based PILs was tested for various substances. The conductivity of the ionic liquid was systematically measured with an ELICO conductivity meter and a standard conductivity cell. Densities of ionic liquids were measured by using Haursh's method. The viscosities of the ILs were measured using a Ostwald viscometer .For each IL, the experimental viscosity was obtained by averaging three to five flow time measurements. The refractive index of the ILs was measured using a commercial Spectrometer from Atago (model PAL-RI). The UV –visible spectra were recorded using Perkin Elmer Lambda35 Spectrometer and the FT-IR spectra were recorded using the Jasco (FT-IR 460) spectrometer. The proton nuclear magnetic resonance spectra were recorded in a Bruker 300 MHZ NMR Spectrometer using $CDCl_3$ as solvent and the GC-MS were recorded using the JEOL GC MATE-II data system with maximum resolution of 6000.

2.2.1 Antimicrobial activity

Well Diffusion Method

The test organisms used in the study were ***E. coli, streptomyces &bacillus*** the entire test cultures were obtained from the Biotechnology Laboratory of Bishop Heber College, cultures was checked before use. The cultures were maintained at4^{o}C on Nutrient agar (HiMedia) slants. The antibacterial of the selected Sample preparations were performed by agar well diffusion method. 20 ml of sterile Muller Hinton agar (Hi Media) was poured in sterile Petri dishes. The plates were allowed to solidify and used. 10 ml of sterile, Muller Hinton agar medium (seed agar) was seeded with organisms (about 0.2 ml according to 0.5 McFarland's standard), in semi hot conditions and was poured uniformly on the base agar. 8mm bores were made each equal distant from one another on the medium using sterile borer and 100µl ml of the different urine preparation were added to respective bore. The plates were incubated at 37^0 C for 24 hrs and zone of inhibition was measured. A reference standard of streptomycin (100µg/ml) was also used to compare with the obtained results in the study. For each test, three replicates were performed.

3. RESULTS AND DISCUSSION

3.1 Characterization of the synthesized PILs

The structure of the synthesized PILs were confirmed by UV,FT-IR,1HNMR and GC-MS Spectral studies and the data were given below and the spectra were given in Fig.1-24

2-Methylimidazoliumlactate

Λmax-233nm.**IR**-3426cm^{-1}(N-H^{+}),1589cm^{1}(lactateion),1417(lactateion),1122cm^{-1}(ring deform). NMR chemical shift (δ)values:8.05(1H(N-H in im)) 6.9 (1H.im-H,s),7.29(N-H^{+}),2.48(C-CH_3,3H,d),1.48(m,-CH_3 in lactate),4.04(-CH in lactate),1.94(-CH(OH)COO-in lactate) .**m/z values:**171.29,170.09,81.29.

1-Ethylimidazoliumlactate
Λmax-228nm.**IR**-3444cm^{-1}(N-H^{+}), 1599cm^{1}(lactateion),1393(lactateion),1152cm^{-1}(ring deform). **NMRchemicalshift(δ)values**:7.6(1H.im-H,s), 7.28(N-H^{+}),3.875(-CH2,2H,q),0.903(-CH3,3H,t),6.88(1H,d), 7.02(1H,d),1.397(-CH_3,3H,m,d,inlactateion),3.92(-CH,1H,q,in lactate),4.15(-CH in lactate) 1.755(-CH(OH)in lactate), .**m/z values** - 185.03,184.23,95.23.

1-Butylimidazoliumlactate
Λmax-236.**IR**-3413cm^{-1}(N-H^{+}),1594cm^{1}(lactateion),1458(lactateion),1149cm^{-1}(ring deform).**NMR chemical shift (δ)values**:7.25(1H.im-H,s),7.29 (N-H^{+}),4.226(-CH_2,2H,q),1.8(-CH_2,2H,m), 1.2(CH_2,2H,m), 0.921(-CH_3,3H,t),7.026(1H,d),7.25(1H,d),6.990(1H,im,m), 1.427(-CH_3,3H,m,d in lactateion),4.26(-CH ,1H,q,in lactate), 4.025(-CH(OH)in lactate), 1.56(CH(COO^{-}),1H,s).**m/z values**-.213.21,211.98,123.21.

2-Methylimidazoliumglycolate
Λmax-232 **IR**-3432cm^{-1}(N-H^{+}),1599cm^{1}(glycolateion), 1418 (glycolateion),1169cm^{-1}(ring deform).**NMR chemical shift (δ)values**: 8.15(1H(N-H in im))6.893 (1H.im-H,s),7.1(N-H^{+}), 2.38(C-CH_3,3H,d),3.96(m,-CH_2in glycolate),2.16(CH(COO-) in glycolate). **m/z values-**158.91,157.91,82.77.

1-Ethylimidazoliumglycolate
Λmax-.**IR**-3424cm^{-1}(N-H^{+}),1598cm^{1}(glycolateion),1418(glycolateion),1160cm^{-1}(ringdeform). **NMRchemicalshift(δ)values**:7.626(1H.im-H,s),7.3(N-H^{+}),3.86(-CH2,2H,q),1.19(-CH3,3H,t),6.89(1H,d), 7.038(1H,d), 3.96(m,-CH_2in glycolate),*2.16(-CH2(COO-) in glycolate)***m/z values-**171.49,170.28,95.26.

1-Butylimidazoliumglycolate
Λmax--.**IR**-3416cm^{-1}(N-H^{+}),1598cm^{1}(glycolateion),1408(glycolateion),1169cm^{-1}(ring deform).**NMR chemical shift (δ)values**:7.209(1H.im-H,s),7.215(N-H^{+}),4.13(-CH2,2H,q),1.769(-CH_2,2H,m),1.208 (CH_2,2H,m),0.916(-CH3,3H,t),6.883(1H,m),7.065(1H,d),3.94 (-CH2(OH),2H,s in glycolate ion), **m/z values**-204.91,203.79,128.72

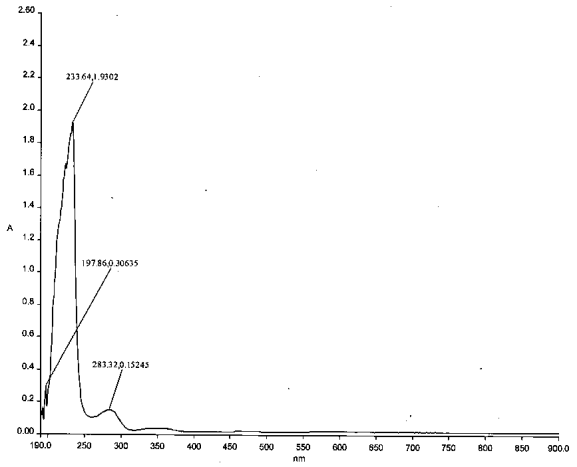


Fig.1. UV Spectrum of PIL1

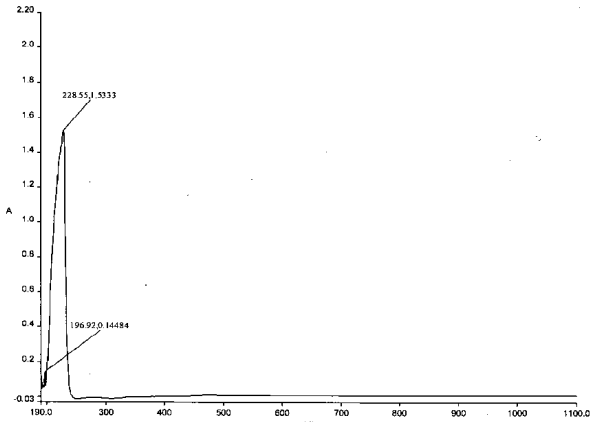


Fig.2. UV Spectrum of PIL2

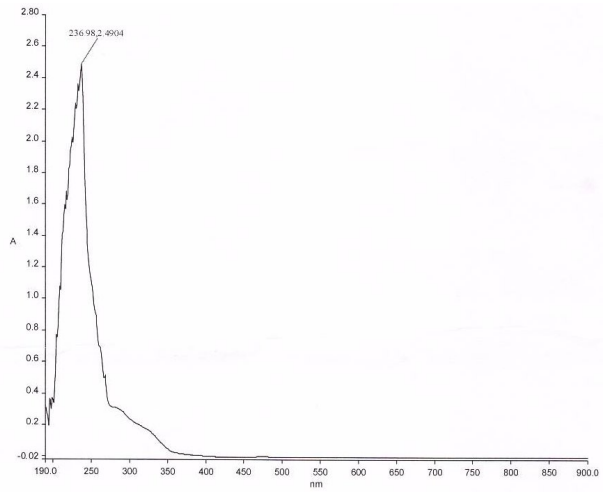
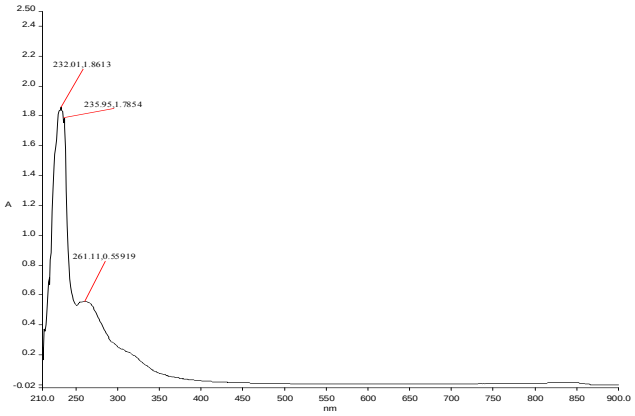
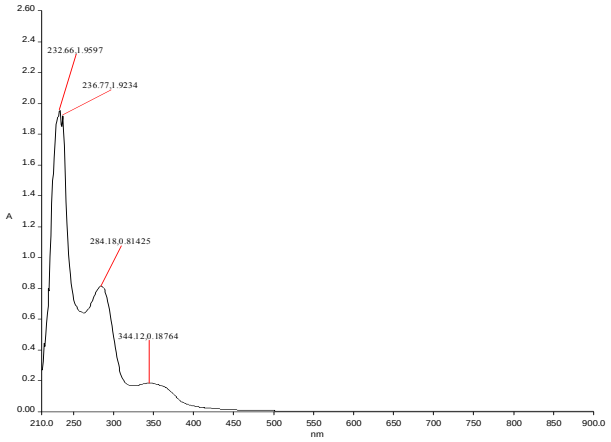


Fig. 3. UV Spectrum of PIL3



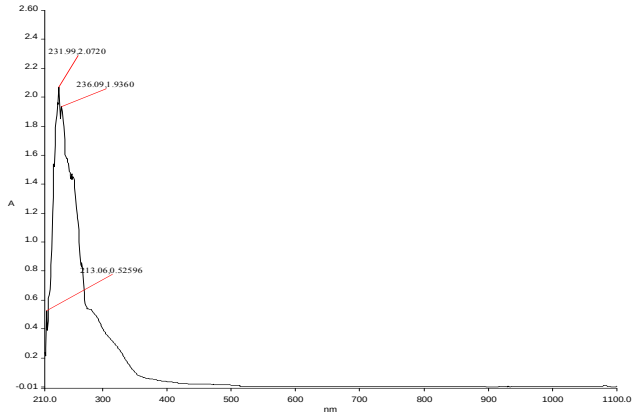
Instrument Model: Lambda 35 Scan Speed: 960.00 nm/min
Data Interval: 1.0000 nm

Fig. 4.UV Spectrum of PIL4



Instrument Model: Lambda 35 Scan Speed: 960.00 nm/min
Data Interval: 1.0000 nm

Fig.5. UV Spectrum of PIL5



Instrument Model: Lambda 35 Scan Speed: 960.00 nm/min
Data Interval: 1.0000 nm

Fig.6. UV Spectrum of PIL6

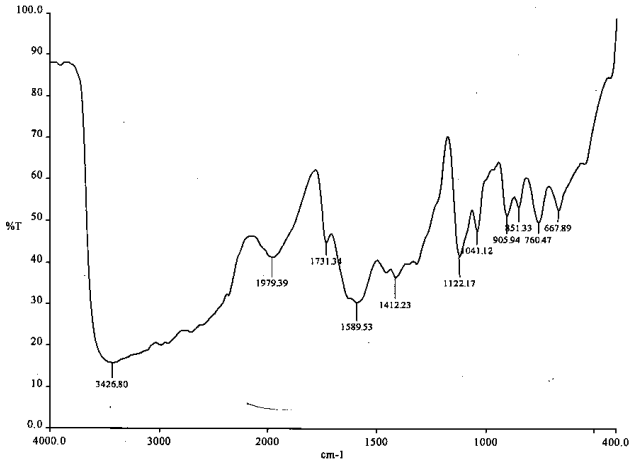


Fig.7. IR Spectrum of PIL1

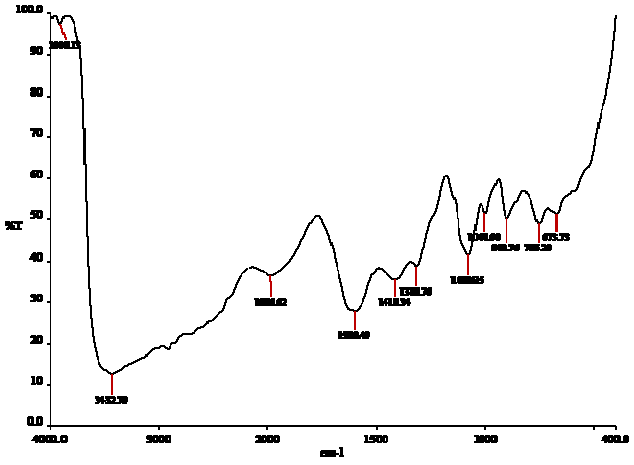


Fig.10. IR Spectrum of PIL4

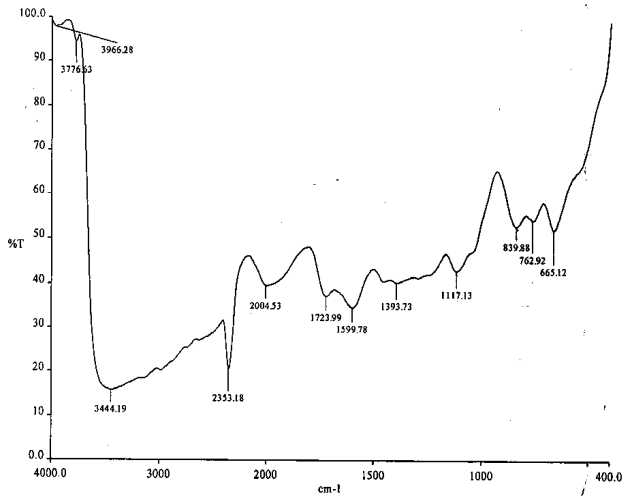


Fig.8. IR Spectrum of PIL2

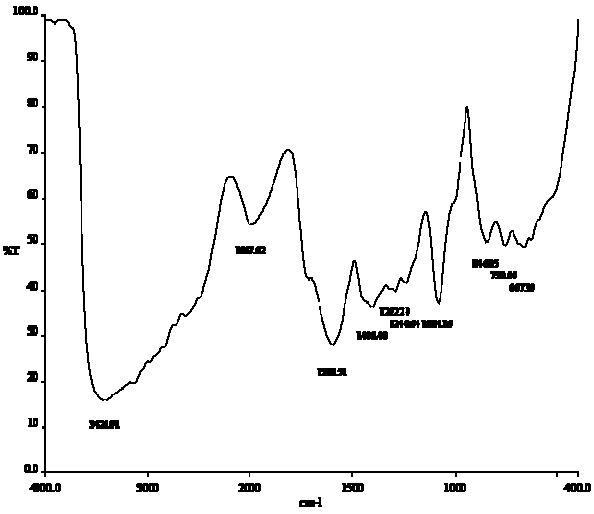


Fig.11. IR Spectrum of PIL5

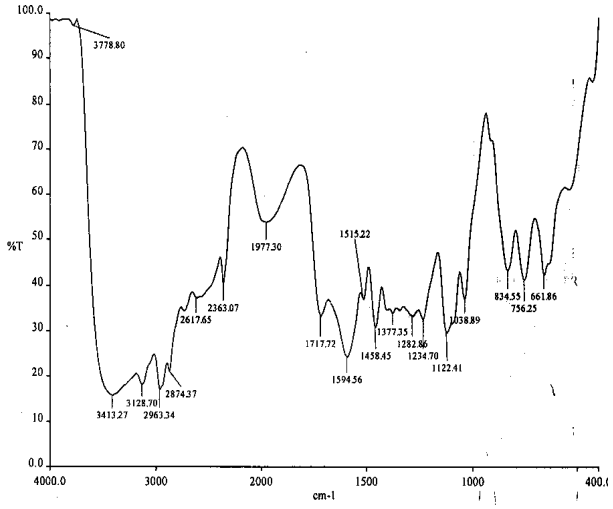


Fig.9. IR Spectrum of PIL3

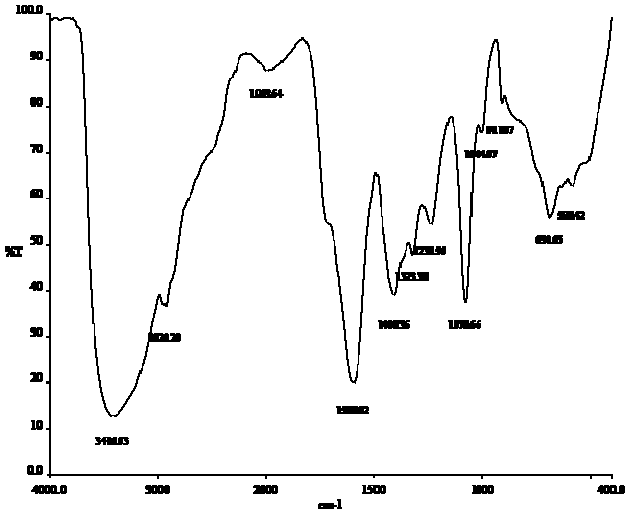


Fig.12. IR Spectrum of PIL6

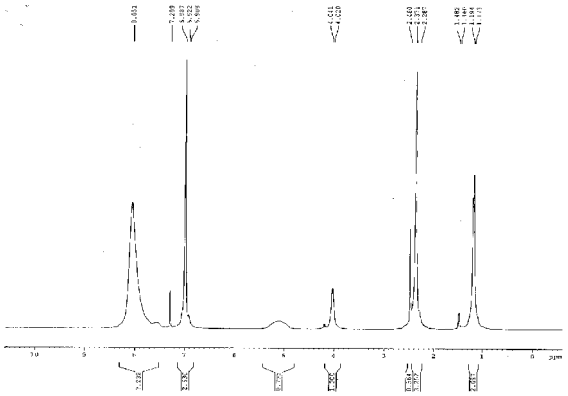


Fig.13.NMR Spectrum of PIL1

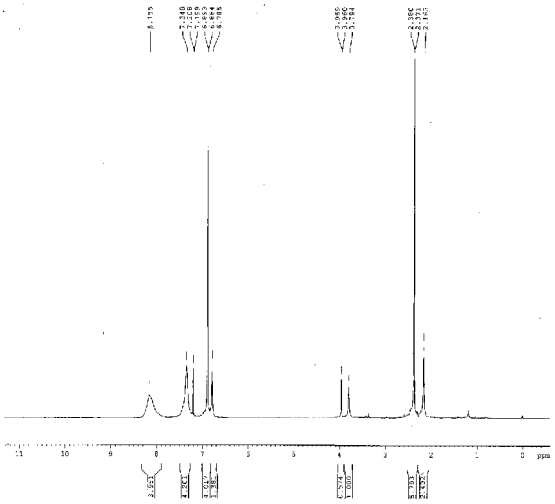


Fig.16. NMR Spectrum of PIL4

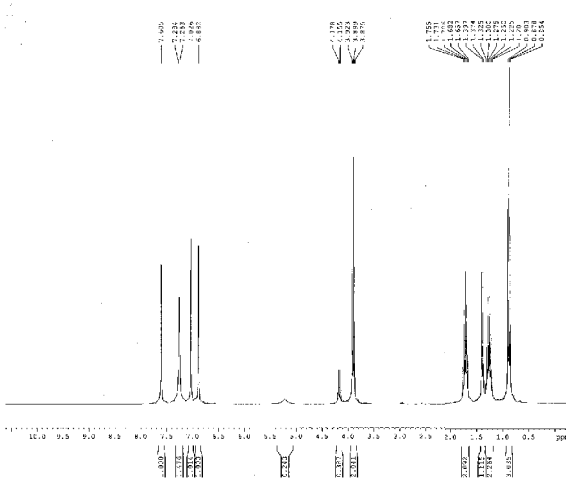


Fig14-NMR Spectrum of PIL2

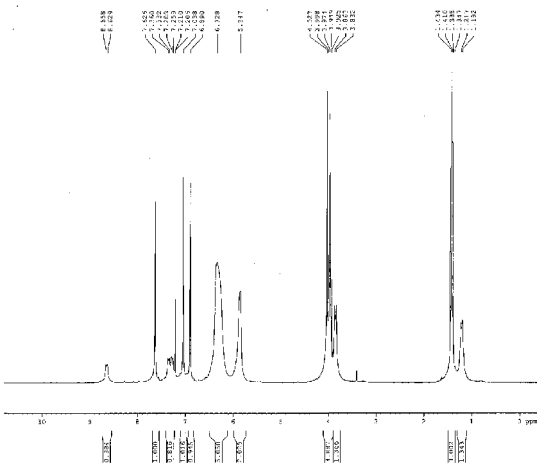


Fig17-NMR Spectrum of PIL5

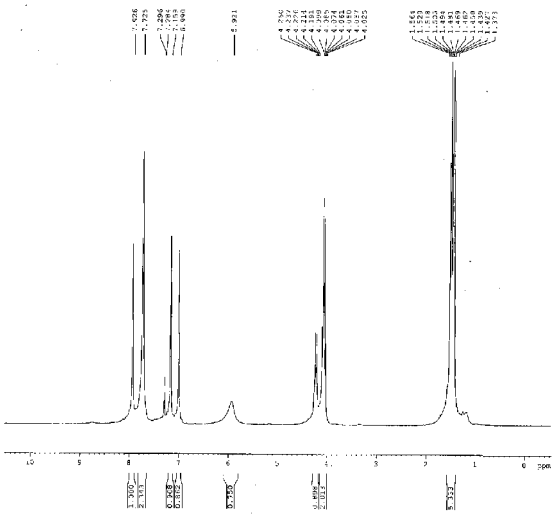


Fig. 15. NMR Spectrum of PIL3

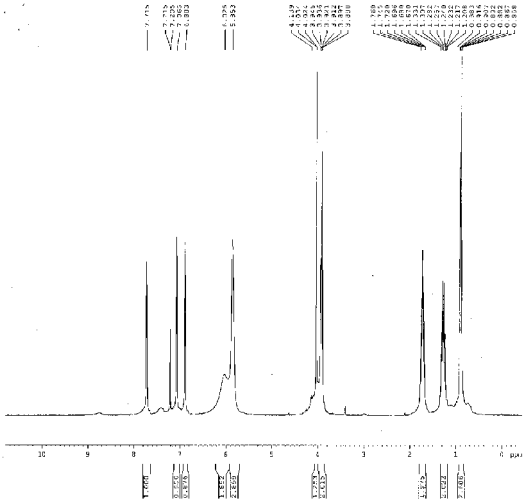


Fig.18. NMR Spectrum of PIL6

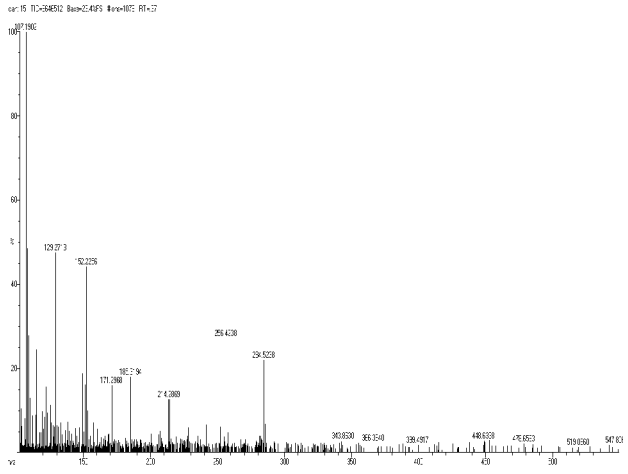


Fig.19. Mass Spectrum of PIL1

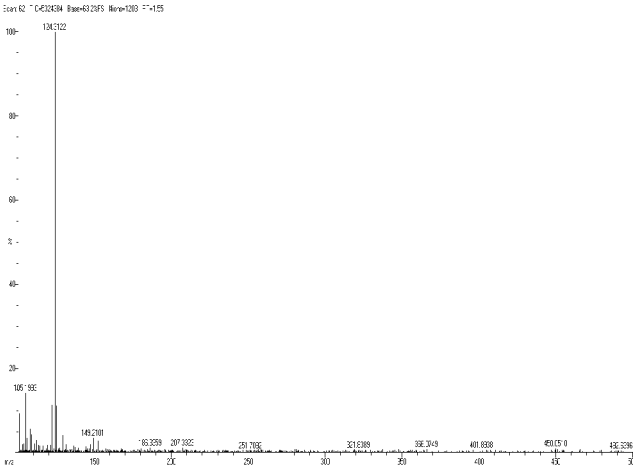


Fig. 22.Mass Spectrum of PIL4

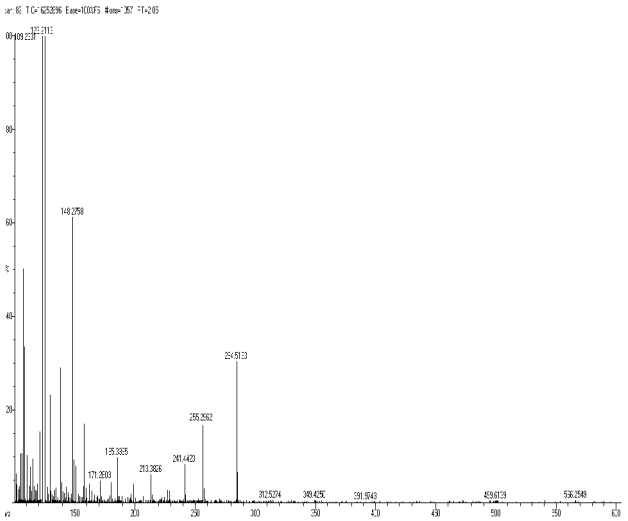


Fig.20.Mass Spectrum of PIL2

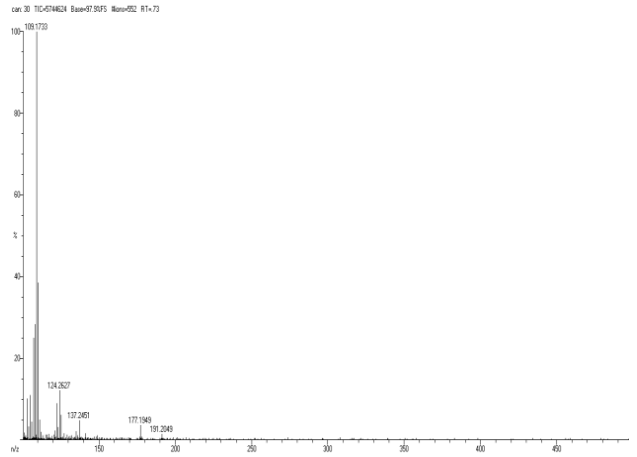


Fig. 23.Mass Spectrum of PIL5

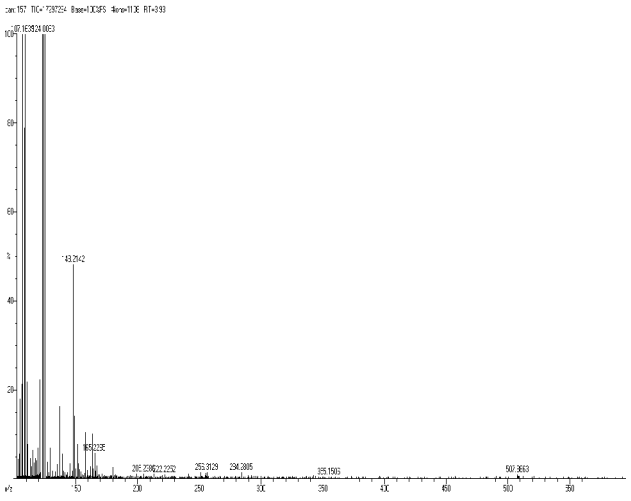


Fig.21. Mass Spectrum of PIL3

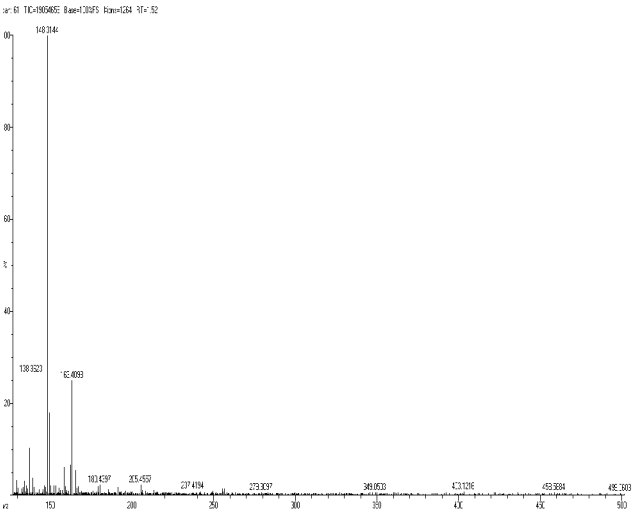


Fig. 24.Mass Spectrum of PIL6

Table 2. Physical properties of various PILs

S.No	Protic Ionic liquids	Conductance Sm^{-1}	Viscosity g/cm-3	Density cP	Refractive index	ΔpKa
1.	2- Methylimidazoliumlactate	6.64	1.1725	1158	1.4243	3.84
2.	1-Ethylimidazoliumlactate	5.51	1.0908	1193	1.4562	3.43
3.	1-Butylimidazoliumlactate	4.15	1.0503	1236	1.4922	3.23
4.	2-Methylimidazoliumglycolate	5.29	1.1023	1105	1.3962	3.92
5.	1-Ethylimidazoliumglycolate	4.49	1.0676	1178	1.4773	3.46
6.	1-Butylimidazoliumglycolate	3.61	1.0336	1216	1.4921	3.26

Table 3. Antibacterial activity PILs against pathogenic bacterial strains

S.No	Name of the Bacteria	Mean Zone of inhibition in mm					
		2MIML	1EIML	1BIML	2MIMG	1EIMG	1BIMG
1.	Ecoli	15	14	12	20	16	14
2.	Streptomyces	20	18	15	15	14	10
3	Bacillus	25	20	15	15	14	10

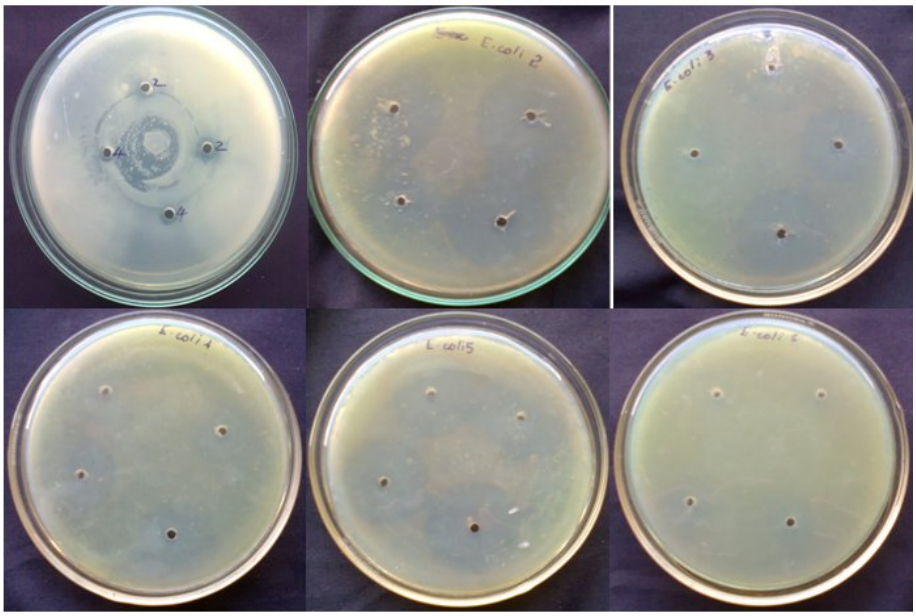


Fig. 25. Antimicrobial Activity of PILs against E.Coli

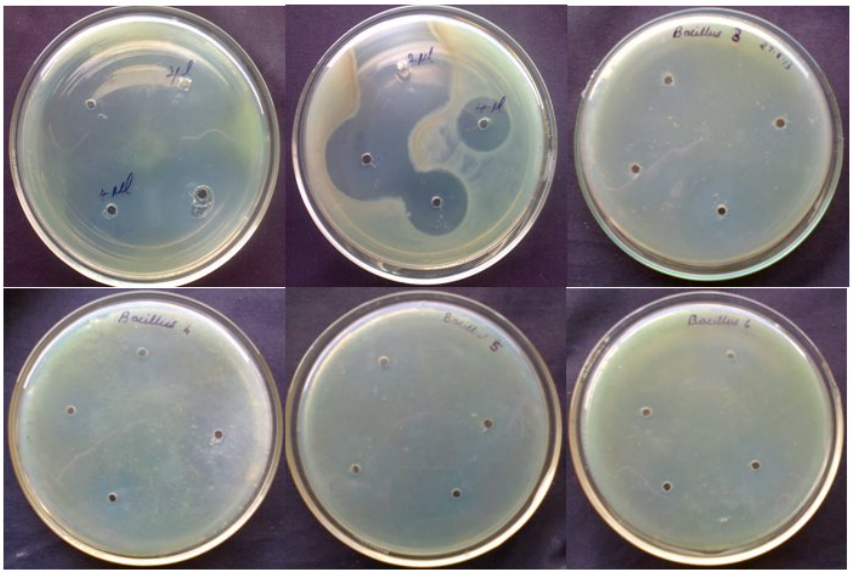


Fig.26. Antimicrobial Activity of PILs against Bacillus

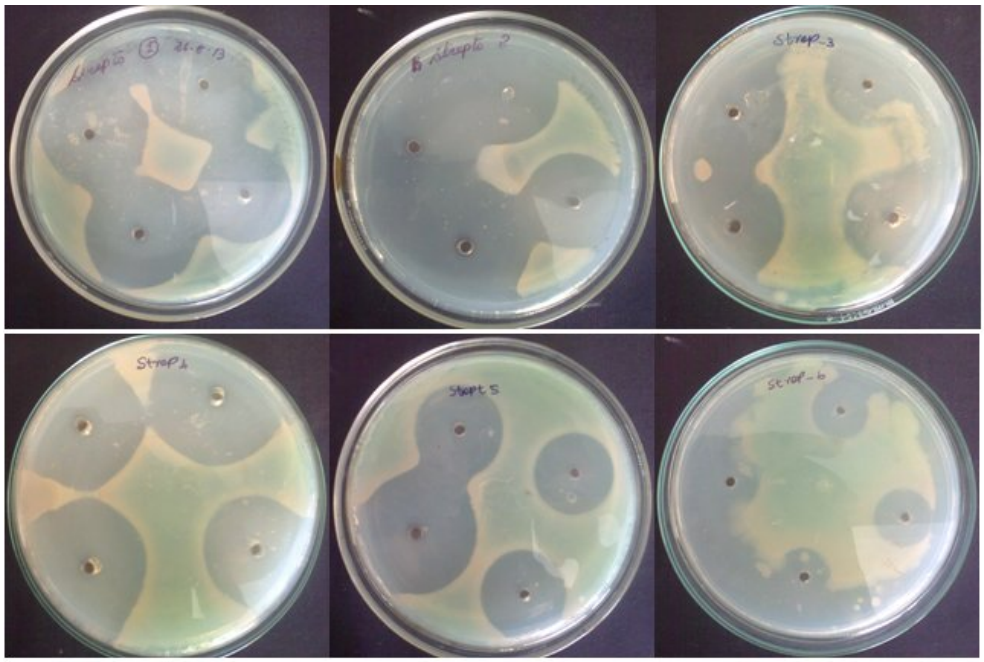


Fig. 27. Antimicrobial Activity of PILs against Streptomyces

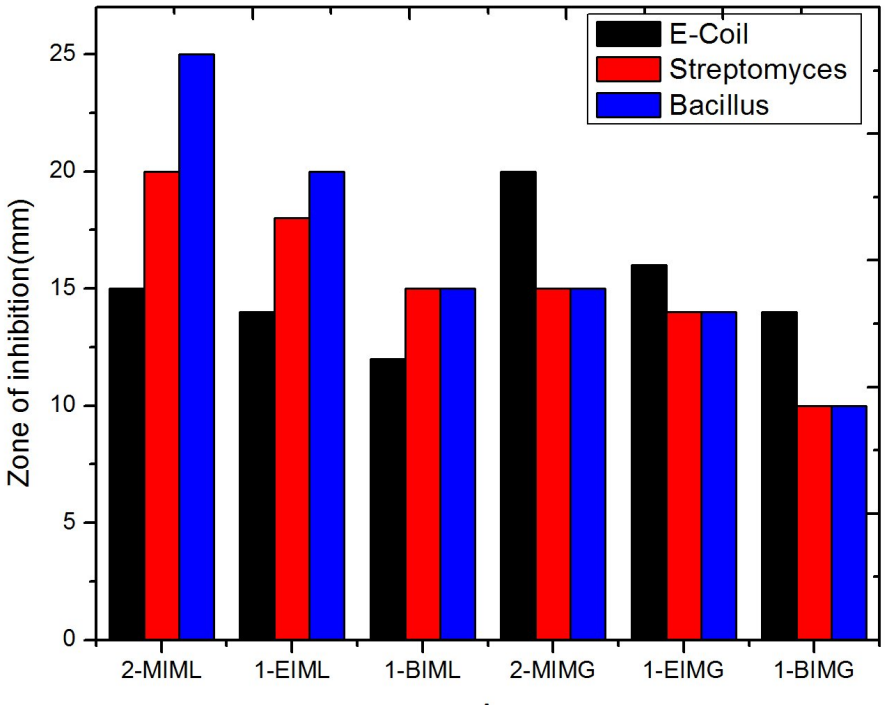


Fig. 28. Antimicrobial Activity of PILsagainst Ecoli, Bacillius,Streptomyces

3.2. Antibacterial activity PILs

The antimicrobial activities of the synthesized PILs were obtained by evaluating the inhibition zone on the agar plates. This zone is defined as the area on the agar plates where the growth of the microbe was prevented by the test compound (PILs). The results are summarized in table 3&fig 25-27. It has been noted that an increase in the chain length of alkyl substituent in PILs were correlated with their increased toxicity or the inhibition of the growth of microbes.

Conclusion

There are six PILs with alkyl substituted imidazolium cation were synthesized and characterized using UV,IR, ^{1}HNMR

&GC-MS spectral studies and their physical properties are measured using the appropriate technique. All these measurements showed that all the PILs had good physical properties like solubility, iconicity, conductivity etc. The antimicrobial activity of the PILs was measured using well diffusion method and show good antimicrobial activities on *Ecoli, Streptomyce and Bacillus.* The antimicrobial activities of the PILs were associated with the structure of the cation and the anion and the number of side chain groups. With the same anion, the more the alkyl chain length, lower are the diffusion zone.Thus 2-MIML and 2-MIMG posses higher antimicrobial activity. The significant antimicrobial properties observed in this research suggest that all the six PILs have potential applications in the modern biotechnology.

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