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Synthesis and Characterization of Ammonium Benzilate Bioactive Ionic Liquids and Their Antimicrobial Activity

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The ammonium benzilate Ionic Liquids (ILs) was synthesized by the neutralization reaction of different cations of ethanolamine, dimethylethanolamine, and triethanolamine with benzillic acid. The reaction is monitored by thin layer Chromatography. The synthesized compounds were purified. Then the synthesized ILs was characterization by FT-IR Spectroscopy and 1H Nuclear Magnetic Resonance (NMR) Spectroscopy. Antibacterial screening of the test ILs was carried out with six human pathogenic bacteria, such as *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa and Shigella dysenteriae* and two phytopathogenic fungi like *Aspergillus niger and Rhizopus azzahra*. The minimum inhibitory concentration (MIC) was determined to establish a good bioactive molecule.

Keywords: FT-IR; NMR and antimicrobial activity.

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1. INTRODUCTION

Ionic Liquids (ILs) was introduced almost a century ago by the observation of Paul Walden in a neutralisation reaction of ethanolammonium nitrate [1]. ILs are organic salts mainly composed of organic cations and inorganic anions, which are per definition liquid below 100°C and exhibit in most cases relatively low viscosities [2]. The definition allows distinguishing them from a classical molten salt, which is mostly high-melting, highly viscous, highly polar, very low vapour pressures, thermally stable, wide kinetic control high thermal conductivity, a large electrochemical window and very corrosive substance [3-5]. Conventionally, ILs typically contains bulky asymmetric organic cations, such as imidazonium, pyridinium, pyrrolidinium, quaternary ammonium or tetraalkylphosphonium with very low symmetry to lead weak intermolecular interactions and low charge densities [6-7]. Quaternary ammonium based ILs is the most expected properties of ILs at which organic amine base are used as cation sources to produce salts with different types of carboxylic acids or inorganic acid as anion sources. Much quaternary ammonium ILs could be prepared to ‘‘tune" the physical properties such as high melting point, water miscibility, conductivity and viscosity by selection and modification of the anion and cation combination of the IL. ILs have attracted much attention due to the widespread potential for practical applications such as heat transfer and storage medium in solar thermal energy systems as well as for many areas such as fuel cells, rechargeable batteries and ‘‘green solvents’’ in the chemical process [8].

In the race to synthesize new pharmaceutical drugs, ILs has attracted a great deal of attention due to their variety of potential pharmaceutical applications [8-9]. The strategy of ILs can take advantage of the dual nature (by controlling the alkyl length of cations [10,11,12]. Quaternary ammonium salts (quats) are an economically advantageous class of industrial compounds [13]. They have surface-active properties discrete ions) to realize enhancements which may include controlled solubility (e.g., both hydrophilic and hydrophobic) and are possible bioactivity [14]. The antimicrobial activity and toxicity towards both prokaryotic and eukaryotic micro-organisms are designed and changed [15]. ILs possesses anti-microbial activity known to be bioactive in nature [16-17]. The low melting point of ammonium salts was relatively rare in the literature of antimicrobial activity during the last decades. The encouraging results of preliminary toxicological studies of synthesized ILs provide good opportunities for the development of biomedical applications for drug discovery [18]. The third generation ILs is active pharmaceutical ingredients [19]. The synthesized third generation ILs provides good opportunities for the replacement of natural bioactive molecule acted as the synthetic bioactive molecule with the satisfactory of green chemistry [20-21].

2. EXPERIMENT

2.1 Materials and Reagents

All the chemicals were of research grade and used without further purification unless otherwise stated. All the solvents were obtained upon distillation before use. Ethanolamine (Merck KGaA), Dimethylethanolamine (Merck KGaA), Triethanolamine (MarckKGaA) and Benzillic acid (Merck KGaA) were analytical grade commercial products. Thin Layer chromatography powder (Merck KGaA), standard antibiotic Gentamycin were purchased for the reaction workup. FT-IR spectrophotometer, (SHIMADZU, Japan, range 600 -4500 cm-1) was used with KBr disc technique. The synthesis and the characterization were done at the Department of chemistry at University of Chittagong, and Chittagong-4331, Bangladesh. The antimicrobial activity was at the Department of Microbiology in University of Chittagong, and Chittagong-4331, Bangladesh. The 1H NMR Spectroscopy was done at Iwate University, Japan.

2.2 Ionic Liquids Synthesis and Purification

The synthesis of the alkanol or alkyl ammonium ILs consists in an acid-basic neutralization reaction [22]. The base, in this case, ethanolamine, dimethylethanolamine, and triethanolamine were added under stirring in a slow dropwise about 20-25 minute maintaining the temperature using ice bath from exothermic heat release on a glass flask with benzilic acid. Then the mixture was stirred for (24-28) hours at room temperature, to obtain a viscous clear liquid. The reaction was monitored by Thin layer chromatography (TLC).The reaction products are an ester and a salt of ammonium [13, 22-23]. The purification process consists of a strong

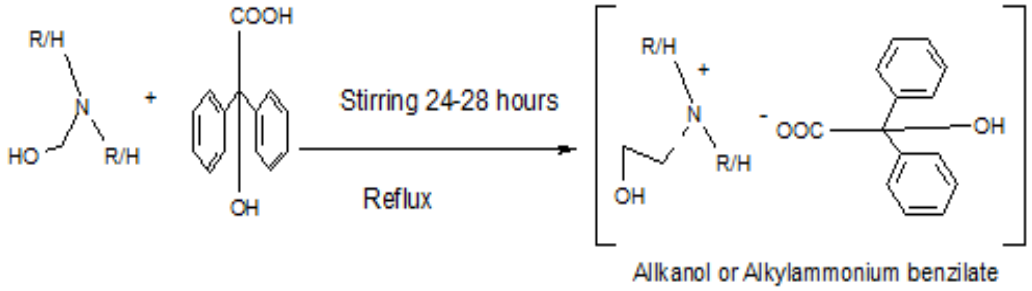

Fig. 1. General reaction scheme for synthesis of ILs

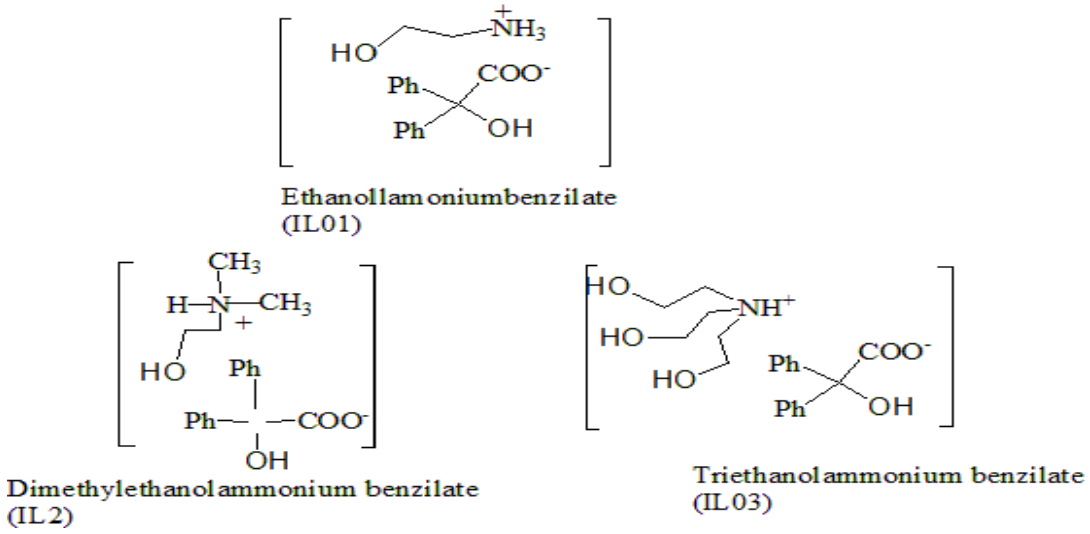

Fig. 2. Synthesized ammonium based IL

agitation and slight heating, at 323.15K, for the vaporization of impurities (residual non-reacted and water) under a vacuum of 20 kPa. Humidity below 0.1% was obtained after this purification process[22-23] and the liquids presented a limpid and viscous appearance. The ammonium salt formation was proved in by FT-IR spectroscopy by using a Shimadzu IR and 1H NMR spectroscopy. The structure of synthesized IL is shown in Fig. 2.

3. RESULTS AND DISCUSSION

3.1 FTIR Analysis

The presence of carboxylate (COO-) peak is at ~1597 cm^{-1} and ~1338 cm^{-1} by N-H vibrations. The broad absorption around ~3335-3441 cm^{-1} can be assigned the presence of OH groups. Then the C-O bond has also confirmed the peak of 1260-1000 cm^{-1} in the carbonate salts and 2995 cm^{-1} is for the stretching of Ar-H bond. The presence of Ph-COO group has confirmed the peak at ~1724 cm^{-1}. The broad peak of ammonium in 3500 to 2400 cm^{-1} confirms the formation of ammonium salt as ammonium based ILs.

Ethanolammonium benzilate (IL01), [C_2OHNH_3] [$Ph_2C(OH)COO$], M.W.: 289.15, Yield (%): 91.0.

Found: %C = 66.40- 66.76, %H=6.62-6.98, % N= 4.78-5.03

Calculated: FT-IR (KBr) in cm^{-1}: 3414, 3001, 2947, 2885, 1620, 1558, 1377, 1068, 1018

1H-NMR chemical shifts: 2.0 (s, 1H, OH), 2.0 (s, 1H, OH), 3.52 (t, 2H, NCH_2), 4,27 (t, 2H, OCH_2), 7.0 (s, 3H, NH_3), 7.11-7.39 (m, 10H, 2Ph)

Dimethylethanolammoniumbenzilate (IL02), $[(CH_3)_2(C_2OH)NH_2]$ $[Ph_2C(OH)COO]$, M.W. 318.19, Yield (%); 93.0.

Found: %C = 67.88- 67.93, %H=7.60- 7.46, % N= 4.40- 4.34

Calculated: FT-IR (KBr) in cm^{-1}: 3441, 3012, 2881, 1809, 1485, 1300, 1053, 752, 698

1H-NMR chemical shifts: 2.0 (s, 1H, OH), 2.0 (s, 1H, OH), 2.80 (s, 6H, $2_{\times}NCH_3$), 3.14 (t, 2H, NCH_2), 3.66 (t, 2H, OCH_2), 7.0 (bs, 2H, NH_2), 7.27-7.30 (m, 10H, 2Ph)

Triethanolammoniumbenzilate (IL03), $[(C_2OH)_3NH]$ $[Ph_2C(OH)COO]$, M.W. 377.0, Yield (%); 68.0.

Found: %C = 63.62-63.23, %H=7.21-7.41, % N= 3.71-3.99

Calculated: FT-IR (KBr) in cm^{-1}: 3356, 2970, 2931, 2129, 1562, 1485, 1404, 1292, 1076.

1H-NMR chemical shifts: 2.0 (s, 3H, $3_{\times}OH$), 2.00 (s, 1H, OH), 3.43 (t, 6H, $3_{\times}NCH_2$), 3.77 (t, 6H, $3_{\times}OCH_2$), 7.10 (s, 1H, NH), 7.19-7.24 (m, 10H, 2Ph).

4. EXPERIMENT WITH ANTIMICROBIAL ACTIVITY

4.1 Preparation of IL Solutions in Different Concentrations

The required amount of the sample was measured in Digital balance with highly carefully so that no impurities were obtained. At first 20% solution was prepared to investigate the antimicrobial activity. Then by serial dilution process, 1000 mili-mole per Litre (mM/L), 750 mM/L,500 mM/L, 250 mM/L and 100 mM/L solution was prepared and used to determine the MIC.

4.2 Antimicrobial Assay

A preliminary investigation of the antibacterial activities of pure ILs was performed through measurements of primary screening both the gram-positive and gram-negative bacteria.Antibacterial screening of the 20 % *solution of ILs was carried out with six bacterial pathogens, such as Bacillus cereus, Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeroginosa* and *Shigella dysenteriae*. This method was carried

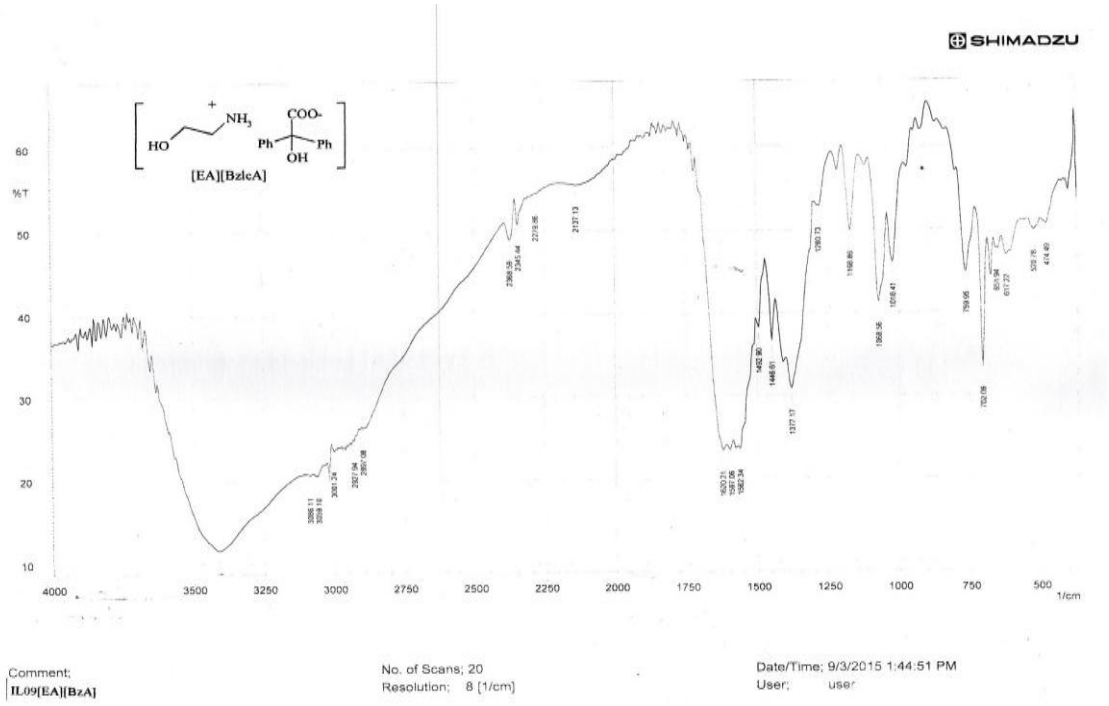

Fig. 3. FTIR peak of Ethanolammonium benzilate

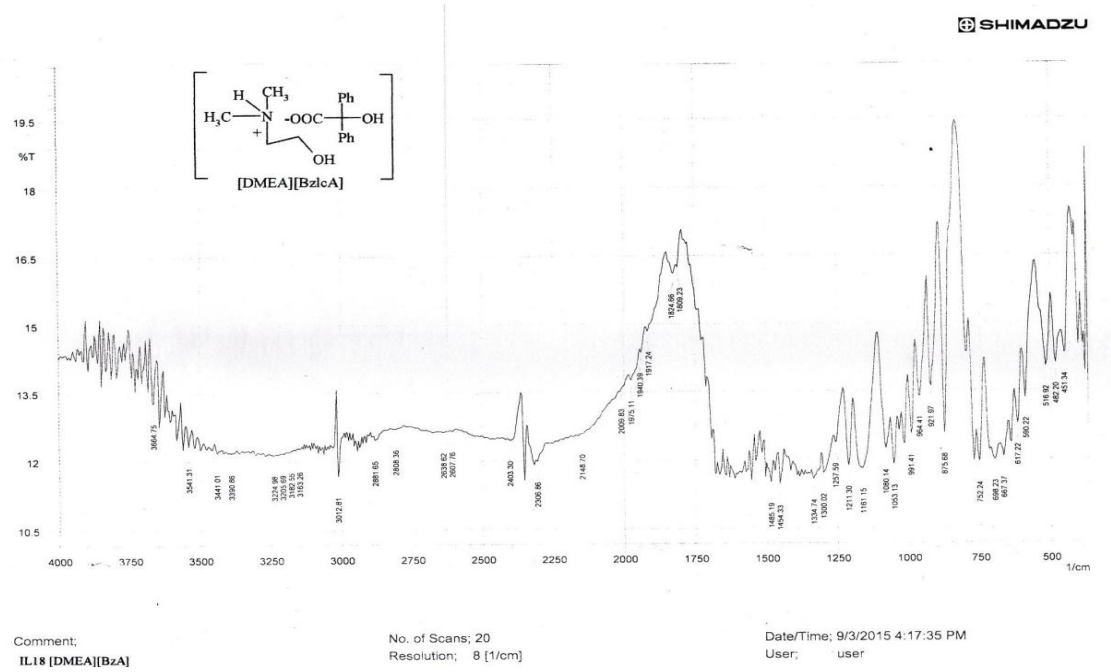

Fig. 4.

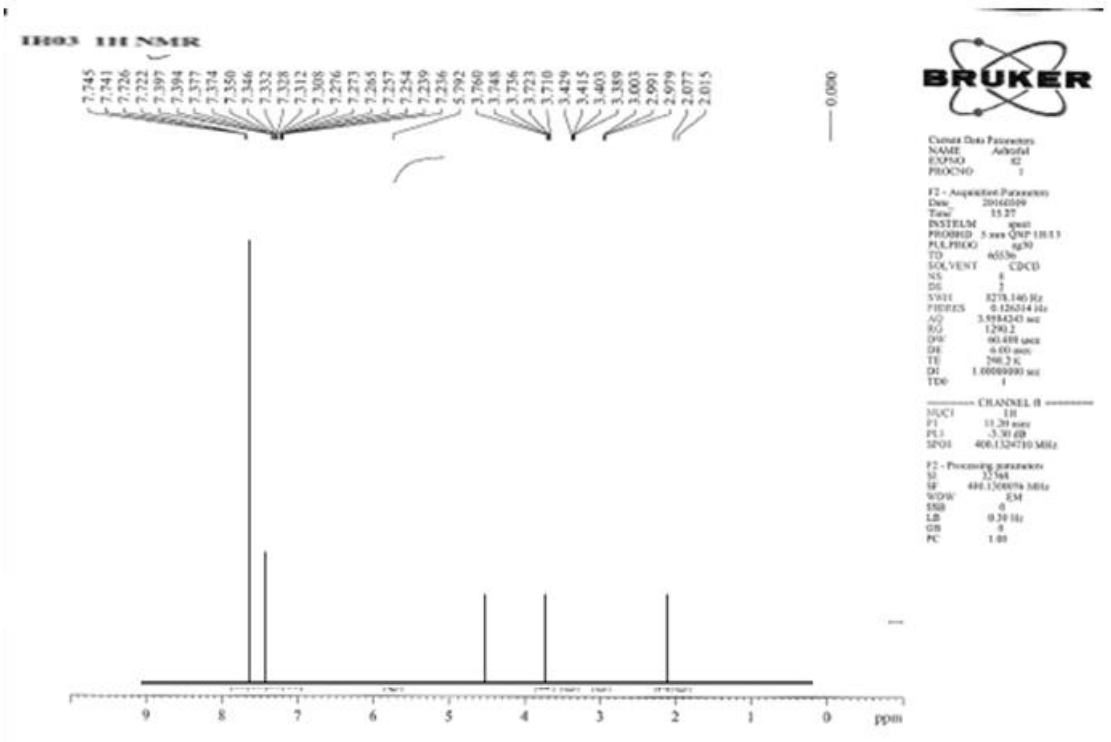

Fig. 5. 1H NMR for IL01

on via well diffusion method [24-25]. The bacterial inhibition zone (subtracting the well diameter 5.0 mm that is the diameter of cork borer) was measured in mm scale with

consideration ±1.0 with all taking value. All the measurements were done in triplicate and the averages were listed in Table 1. The concentration for MIC determination was maintained for all ILs in 1000 mM/L, 750 mM/L, 500 mM/L, 250 mM/L, 125 mM/L and 75mM/Lin distilled methanol through serial dilution technique. A control plate is always observed for the ILs if there is any significant inhibition occurred for the solvent. The results showed that all compounds had antimicrobial activity against bacterial pathogens used in this study.

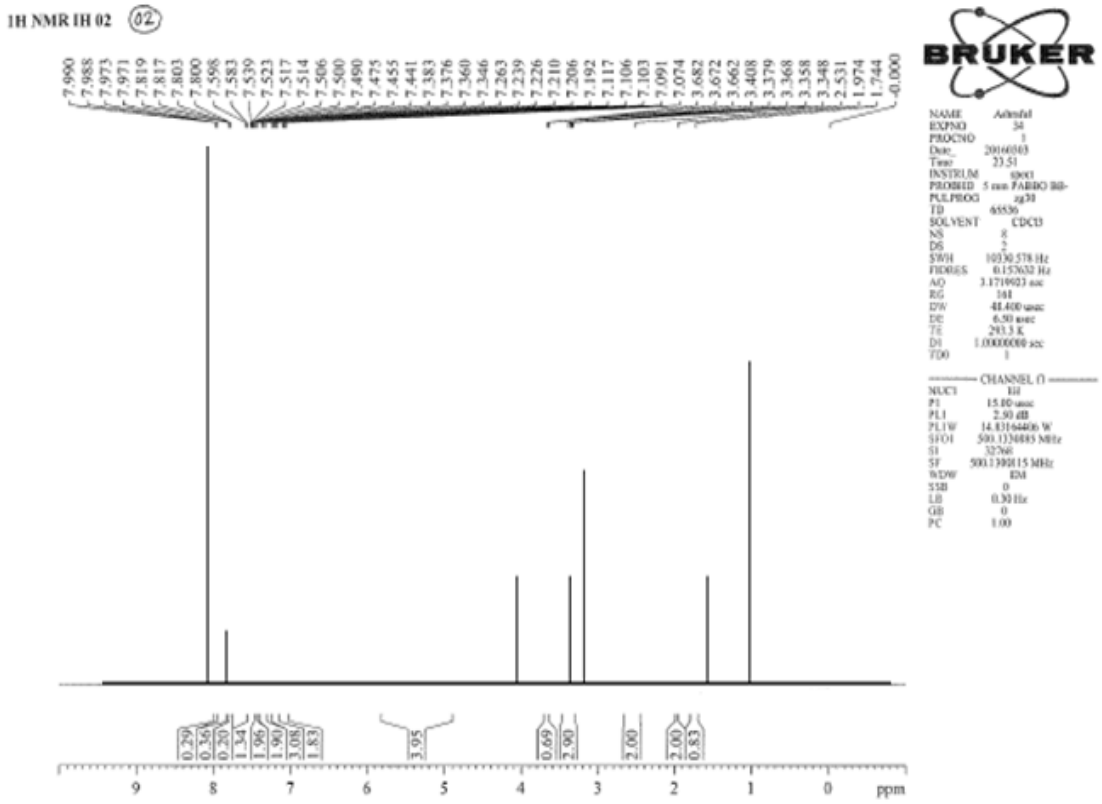

Fig. 6. 1HNMR for IL02

Table 1. Zone of inhabitation for 20% solution of ILs

List of pathogens and ILs	*B. cereus*	*S. aureus*	*E.. coli*	*S. typhi*	*P. aeroginosa*	*S. dysenteriae*
IL01	10.0±1.0	5.0±1.0	7.5±1.0	4.0±1.0	4.0±1.0	6.5±1.0
IL02	8.0±1.0	3.0±1.0	5.5±1.0	15.0±1.0	7.5±1.0	8.0±1.0
IL03	20.0±1.0	10.0±1.0	16.0±1.0	11.0±1.0	7.0±1.0	9.0±1.0

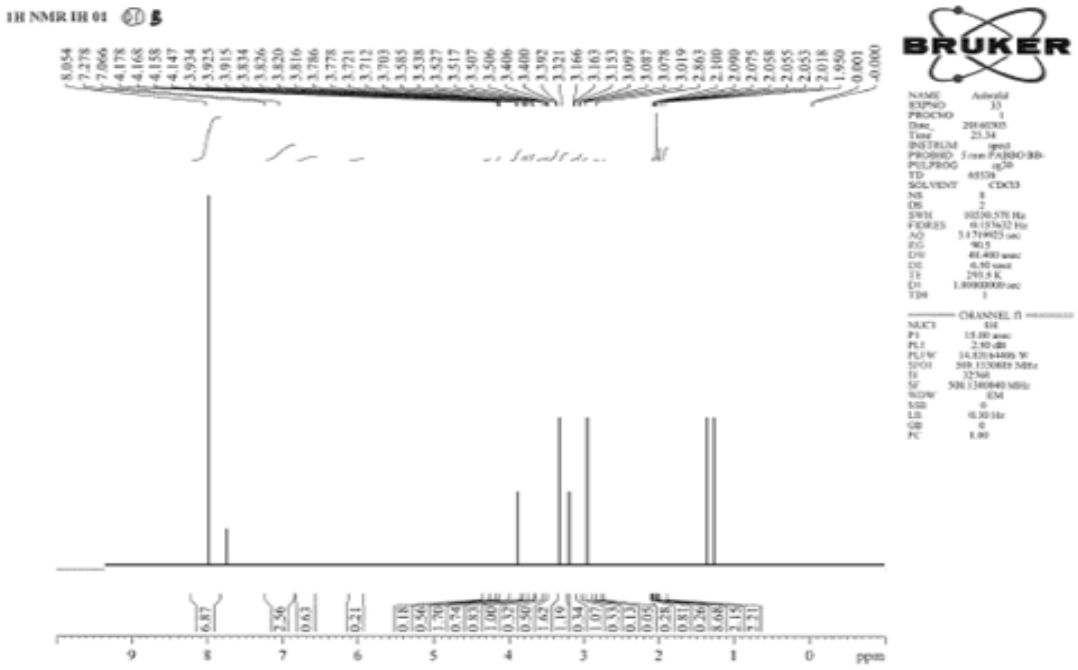

Fig. 7. 1H NMR for II03

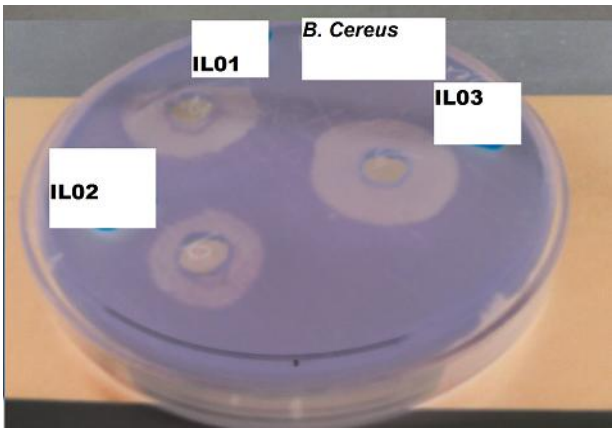

Fig. 8. Zone of inhabitation for 20% solution against *B. cereus*

4.3 Determination of MIC

All the data of Table 2 was placed in a graph in excel file. From the data and graph, the MIC can easily determine.

From the graph, it is easy to determine the MIC point for the IL01 sample at which the zone of inhabitation is zero. The obtained MIC of *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa and Shigella dysenteriae is* 250 Mm/L, 250 Mm/L, 75 Mm/L, 250 Mm/L, 250 Mm/L, 75 Mm/L respectively.

From the graph, it is easy to determine the MIC point for the IL02 sample at which the zone of inhabitation is zero. The obtained MIC of *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa and Shigella dysenteriae is 75.0 Mm/L, 250 Mm/L,125 Mm/L, 125 Mm/L, 500 Mm/L and 75 Mm/L.*

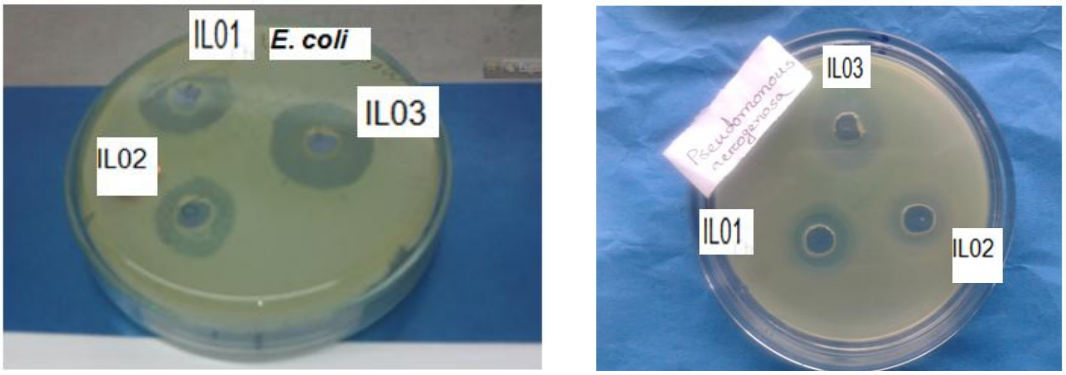

Fig. 9. Zone of inhabitation for 20% solution against *E. coli and P. aeroginosa*

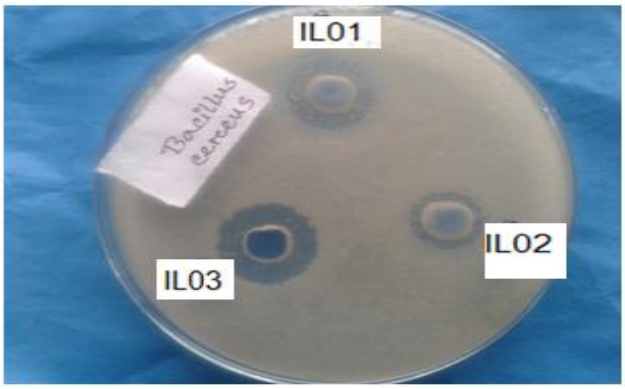

Fig. 10. Zone of inhabitation for 500 mM/L solution against *B. cereus*

Table 2. Data of 1000 mM/L, 750 mM/L, 500 mM/L, 250 mM/L, 125 mM/L and 75 mM/L against different Bacteria

		B. cereus	*S. aureus*	*E. coli*	*S. typhi*	*P. aeroginosa*	*S. dysenteriae*
L01	1000 mM/L	9.0±1.0	6.0±1.0	11.0±1.0	8.0±1.0	7.0±1.0	10.0±1.0
	750 mM/L	8.0±1.0	4.5±1.0	9.0±1.0	6.0±1.0	5.0±1.0	8.0±1.0
	500mM/L	6.0±1.0	1.5±1.0	6.5±1.0	3.0±1.0	3.0±1.0	5.0±1.0
	250mM/L	0.0	0.0	3.5±1.0	0.0	0.0	3.0±1.0
	125mM/L	0.0	0.0	1.5±1.0	0.0	0.0	1.5±1.0
	75 Mm/L	0.0	0.0	0.0	0.0	0.0	0.0
IL02	1000 mM/L	11.0±1.0	7.0±1.0	8.0±1.0	8.5±1.0	6.0±1.0	12.0±1.0
	750 mM/L	9.5±1.0	5.0±1.0	6.0±1.0	7. ±1.0	2.5±1.0	10.0±1.0
	500mM/L	3.5±1.0	2.0±1.0	3.0±1.0	5.0±1.0	0.0	7.0±1.0
	250mM/L	5.0±1.0	0.0	1.0±1.0	2.0±1.0	0.0	3.0±1.0
	125mM/L	3.0±1.0	0.0	0.0	0.0	0.0	1.5±1.0
	75 Mm/L	0.0	0.0	0.0	0.0	0.0	0.0
IL03	1000 mM/L	17.0±1.0	15. ±1.0	16.0±1.0	17.0±1.0	8.0±1.0	14.0±1.0
	750 mM/L	11.517.0±1.0	12.5±1.0	11.5±1.0	12.0±1.0	3.0±1.0	12.5±1.0
	500mM/L	7.0±1.0	11.0±1.0	7.0±1.0	4.0±1.0	2.5±1.0	11.0±1.0
	250mM/L	4.5 ±1.0	5.5±1.0	3.0±1.0	0.00	0.00	5.0±1.0
	125mM/L	3.0±1.0	0.0	0.00	0.00	0.00	0.00
	75 Mm/L	0.0	0.0	0.00	0.00	0.00	0.00

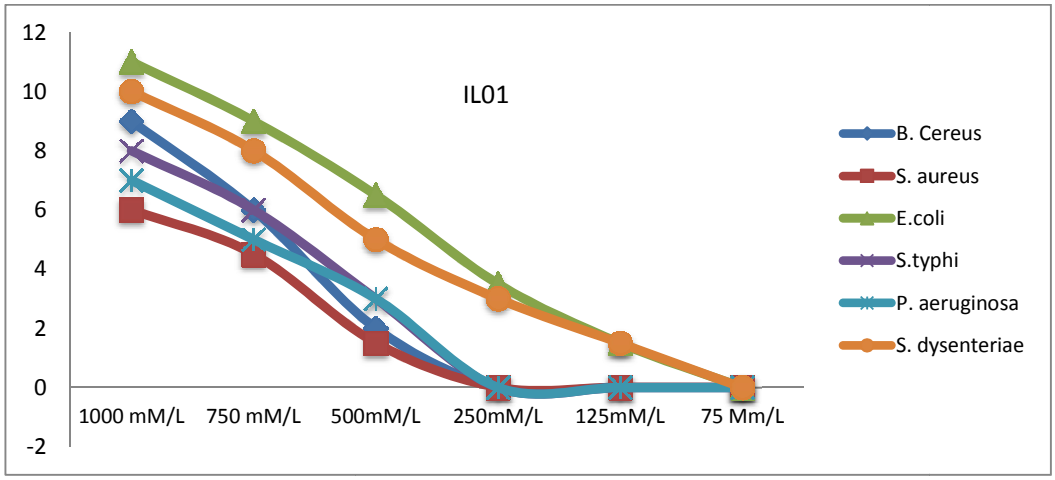

Fig. 11. Determination of MIC for IL01

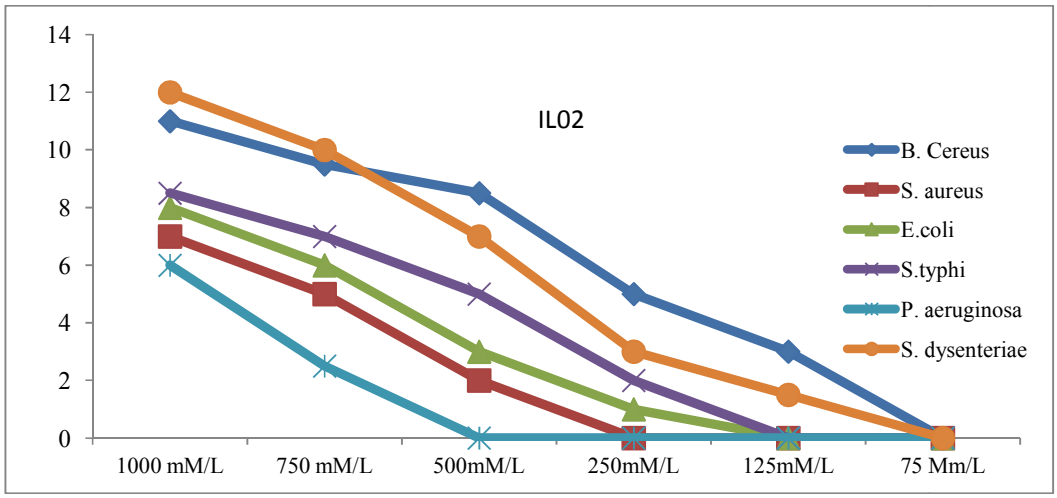

Fig. 4. Determination of MIC for IL02

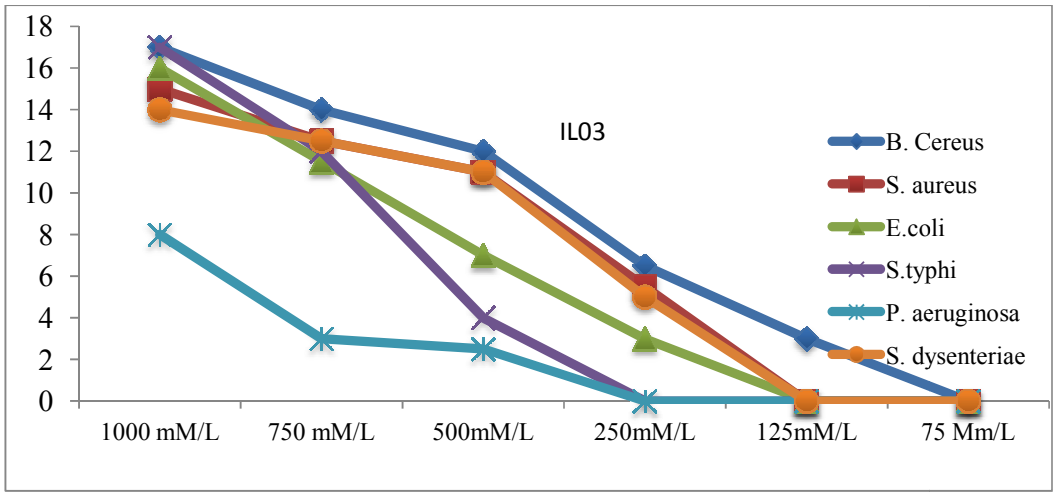

Fig. 5. Determination of MIC for IL03

From the graph, it is easy to determine the MIC point for the IL02 sample at which the zone of inhabitation is zero. The obtained MIC of *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa and Shigella dysenteriae is* 75 Mm/L, 125 Mm/L, 125 Mm/L, 250 Mm/L, 250 Mm/L and 125 Mm/L.

4.4 Antifungal Screening Test

Aspergillus niger and *Rhizopus azzahra* were used for evaluating the antifungal activity of all synthesized compounds. The antifungal activity was evaluated by Well diffusion method [26]. The media was altered Potato dextrose broth (abbreviated "PDB") is formulated identically to PDA, omitting the agar. Common organisms that can be cultured on PDB are molds such as *Aspergillus niger and Rhizopus azzahra*. All synthesized compounds were dissolved in water or methanol basis on their solubility for making the concentration 1000 mM/L. The 100 µL solution of ILs were taken in petri-plate. The Media of PDB was dispersed and solidified. A well of 5 mm was made in the middle of Petri-plate using cork-borer and the fungal lead was placed there. The plates were then kept in the incubator for 96 h at 37°C. After 3 days, the fungal growth in presence of ILs was measured and analyzed.

The antifungal test was completed and calculated the growth percentage compared with the control where the growth of control is 100% percent. The growth percentage is deduced by the following equation:

$$\%\,\text{Growth} = \frac{\text{Growth of fungi with ILs solution}}{\text{Growth of fungi without ILs solution as control}} \times 100$$

4.5 Comparative Study of Toxicity

To compare the antibacterial toxicity, all data was taken in Microsoft Excel to make bar graph.

From the Figs. 7 and 8, it is shown that in all concentration of 1000, 750 and 500 Mm/L, the IL03 can show high antimicrobial activity the IL02 and IL01 respectively. The antimicrobial activity of IL02 is also partially greater than IL01. From this study, it is written that the length of cation has an important rule on bioactivity. With increasing the length and number of alkyl group in cation, the antibacterial activity increases. In the other hand, the antifungal activity is greater in IL01 than IL02 and IL03.

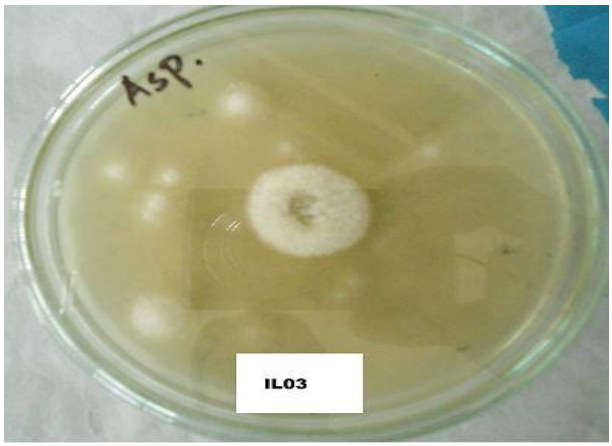

Fig. 6. Zone of inhabitation for antifungal activity of *Aspergillus niger*

Chemicals tested	Zone of growth (in mm)		Percent growth		Percentage of inhabitation	
	Aspergillus niger	***Rhizoplus azzahra***	***Aspergillus niger***	***Rhizoplus azzahra***	***Aspergillus niger***	***Rhizoplus azzahra***
Control	28.0±1.0	31.0±1.0	100%	100%	0.0%	0.0 %
IL01	17.0±1.0	21.5±1.0	60.71%	52.43%	39.29%	47.57%
IL02	26.5±1.0	26.0±1.0	92.85%	83.87%	7.15%	16.12%
IL03	24.0±1.0	25.0±1.0	85.7%	80.65%	14.30%	19.35%

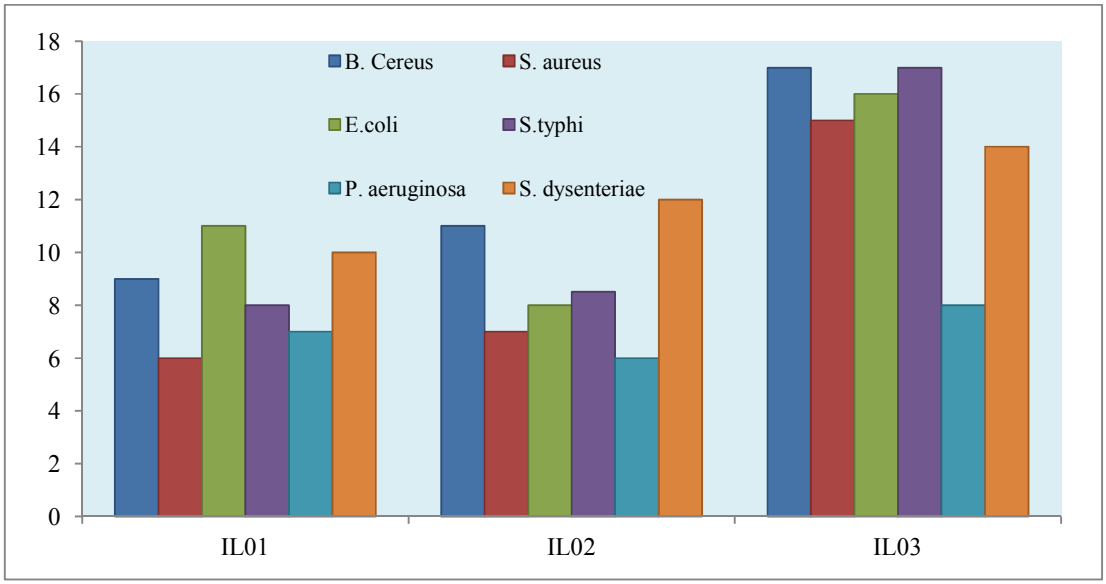

Fig. 7. Comparative antimicrobial studies for 1000 mM/L sample

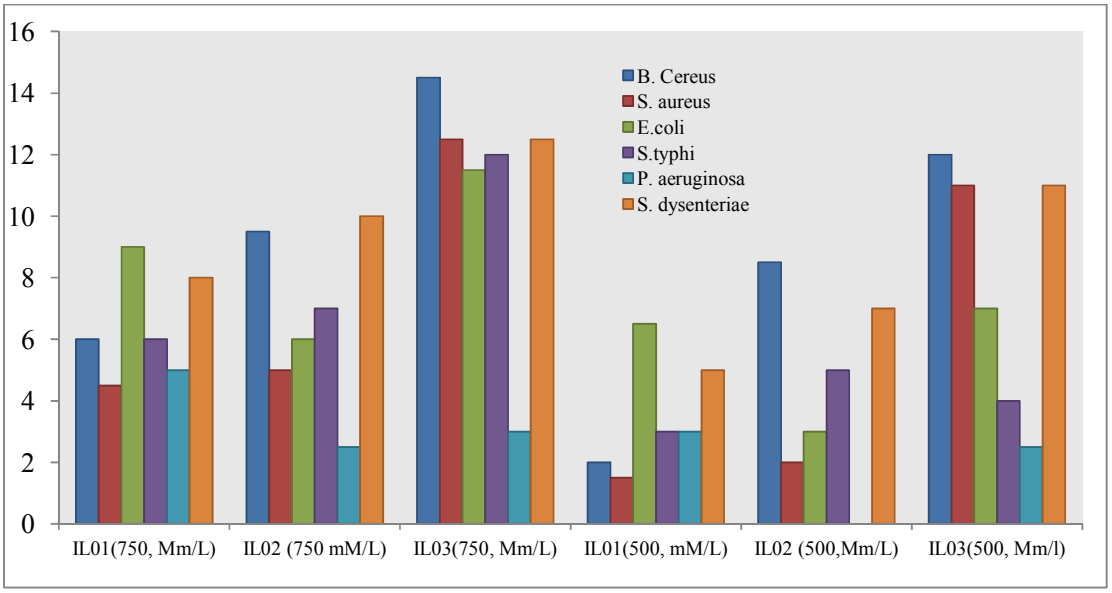

Fig. 8. Comparative antimicrobial studies for 750 mM/L and 500 mM/L sample

5. CONCLUSION

This study showed the three quaternary ammonium ILs and their synthesis with antimicrobial toxicity profiles. The synthesized ILs was characterized by Fourier Transform Infrared Spectroscopy (FT-IR), Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy and elemental analysis. An antibacterial screening was conducted using the well-diffusion technique with ILs solutions of 20% concentrations for initial screening and show the good response. It is a good effective result for using the bioactive molecule. To establish the bioactivity, the MIC was determined using serial dilution technique and show 250 to 75 Mm/L concentrations. It is different to different pathogens. The IL01 is more antifungal than IL03 and IL02 respectively. All the data of antibacterial and antifungal activity supports that the IL01, IL02, and IL03 are good bioactive synthesis ionic liquids molecules.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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