



# Synthesis and Characterization of Ammonium Ionic Liquids and Their Antimicrobial and Computational Overview

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## Authors' contributions

This work was carried out in collaboration between both authors. Author MIH designed the study, and managed the literature searches. Author AK performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Both authors read and approved the final manuscript.

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## ABSTRACT

The ammonium acetate and formate ionic liquids (ILs) were synthesized in the neutralization reaction with different cations of alkanol amine and alkyl amine with formic and acetic acid as an anion. Thin layer Chromatography monitored the reaction and the synthesized six ILs was characterization by FT-IR spectroscopy and <sup>1</sup>H, <sup>13</sup>C Nuclear Magnetic Resonance (NMR) spectroscopy. The spectroscopic analysis of the synthesized ILs gives the excellent agreement for the confirmation of their synthesis and purity. These compounds were tested in computing program of computational chemistry on two basis sets, Mm+ and PM3 for the calculation using molecular mechanics by Hyperchem<sup>R</sup> Release 8.0 (Hypercube, Inc.). Single point calculations were carried out to evaluate the data profile on the physical properties, such as total energy, binding energy, hydration energy, dipole moment, the heat of formation and energies for HOMO and LUMO. Furthermore, the synthesized ILs was screening antimicrobial activity against both of gram positive and gram negative bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*,

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*Salmonella typhi*, *Pseudomonas aeruginosa* and *Shigella dysenteriae* and two phytopathogenic fungi such as *Aspergillus niger* and *Rhizopus azzahra* to establish as a green and environmentally benign solvent.

**Keywords:** Ethanolamine; FT-IR; NMR; HyperchemR; antimicrobial activity.

## 1. INTRODUCTION

Ionic Liquids (ILs) was introduced almost a century ago by the observation of Paul Walden in a neutralization reaction of ethanolammonium nitrate [1]. They belong to a novel class of low temperature (typically <100°C) molten salts, consisted of discrete anions and cations [2]. They exhibit, in most cases, relatively low viscosities, thermally stabilities, high thermal conductivities, a large electrochemical window, highly polar and nonvolatility [3-4]. Millions of ILs can be synthesized and characterized with a variety of cations and anions in the theoretical study which is also known as the designer solvent due to their tunable physical and chemical properties [5]. Due to their unique properties [6], ILs have become one of the most important research targets in chemistry, physics, engineering and material science and technology [7].

There is a strong interest in ILs as alternatives for volatile organic solvents in the organic synthesis [8-9]. They can act as solvents for chemical reactions, including catalytic reactions [10-14]. ILs are found as the use in electrochemical applications [8-9,15-16], e.g. electrolytes in batteries [17], absorption of carbon dioxide [18], in photovoltaic devices [19], and a medium for electrodeposition of metals. ILs can also find applications in separation sciences as solvents for in depolymerization process of lignin [20], extraction processes [21], as a stationary phase for gas chromatography [22], as well as in mass spectrometry [23]. To explain the Molecular interactions between ammonium-based ionic liquids and molecular solvents, the thermodynamic parameters can be effectively used to gain valuable insights which would be most useful in various industries [24]. In other hands the fascinating physio-chemical properties of alkanolammonium based ILs including ion conducting, viscous, non-volatile and non-flammable, they are now used in various fields both of academia and industries as a solvent, catalyst, reaction media, pharmaceutical ingredient and solvent, polymerization reaction [25]. In addition, the biodegradability of ammonium cations is considerably enhanced

when hydroxyl groups are incorporated. To make in utilizing as the green and promising eco-friendly solvents in the separation process, ILs can serve as nonvolatile entertainers to break zoetrope and enable a more energy efficient. [26]. Ammonium-based ILs especially ethanolammonium ILs are easy to prepare and the raw materials are mostly available with cheapness. To investigate, the study of alkanolammonium based ILs, three cations such as different types of a cation such as an ethanolamine, diethylamine, and triethanolamine were used to form ILs with the anion as formate and acetate. To ensure their use as a solvent, the physiochemical properties are the greatest goal. Estimation the physio-chemical properties are a process of the time consuming and the waste of chemical materials. To save the time and chemicals, the computer programming e.g. Hyperchem<sup>R</sup> Release 8.0 (Hypercube, Inc.) was used to calculate the total energy, binding energy, Heat of formation, HOMO, LUMO, LUMO~HOMO and different vibrational stretching of bonds [27-29]. Different cations and anions were used to make a comparison effects in physio-chemical properties through computer programming and antimicrobial toxicity. Most of these are highly polar and well soluble in water. So there is a large scope to pollute water body and environment [30]. The antimicrobial toxicological profile of these ILs was not well documented and the information is not enough for further applications. Due to the short lifespan, bacteria and fungi are the best starting point to investigate and estimate the toxic bio-data of synthesized formate and acetate ammonium ILs. The bacterial toxicity level in the form of antimicrobial activity against gram positive and gram negative bacteria were screened and evaluated for confirming their use in specific fields [31-32].

## 2. EXPERIMENT

### 2.1 Materials and Reagents

All the chemicals were of research grade and used without further purification unless otherwise stated. All the solvent were obtained upon distillation before use. Formic acid (Merck

KGaA), Acetic acid (Merck KGaA), 2-aminoethanol (Merck KGaA), Ethylamine (Merck KGaA), Triethanolamine (Merck KGaA) was 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<sup>-1</sup>) was used with KBr disc technique. The synthesis, computer programming, and the characterization were done at the department of chemistry in University of Chittagong, and Chittagong, Bangladesh. The antimicrobial activity was at Department of Microbiology in University of Chittagong, and Chittagong, Bangladesh. The <sup>1</sup>H, <sup>13</sup>C NMR Spectroscopy was done at Iwate University, Japan.

## 2.2 Ionic Liquids Synthesis and Purification

The synthesis of the alcohol or alkyl ammonium ILs consists in an acid-basic neutralization reaction[33]. The base, in this case,

ethanolamine, diethylamine, 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 the acid (formic and acetic acid respectively).

Then the mixture was stirred for 24 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 ethanolamine [33-35] (The ILs purification process consists in a strong agitation and slight heating, at 323.15K, for the vaporization of impurities (residual non reacted and water) under the vacuum of 20 kPa. Humidity below 0.1% was obtained after this purification process [33,35] (Alvarez et al., 2010; Iglesias et al. 2010), 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. The structure of synthesized IL is shown in Fig. 1.

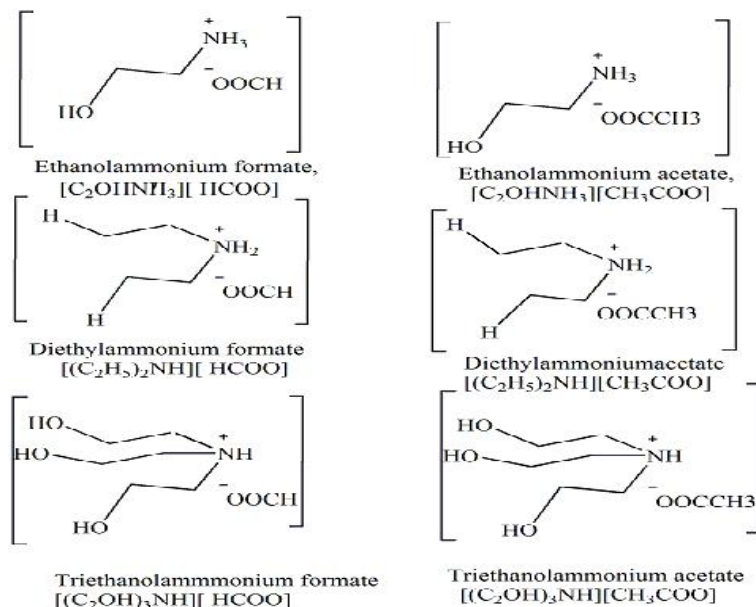


Fig. 1. Synthesized ammonium based IL

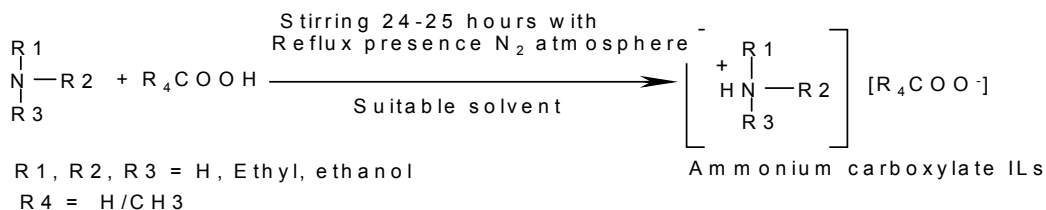


Fig. 2. Reaction scheme for synthesis

**Table 1. Data of NMR, Elementary analysis, FTIR and Molecular weight of synthesized ILs**

Name	Abbreviation	Yield %,	Elementary analysis	Calculated			Molecular weight
				<sup>1</sup> H NMR	<sup>13</sup> C NMR:	FTIR	
Ethanolammonium formate (IL01)	[C <sub>2</sub> OHNH <sub>3</sub> ][HCOO]	92 %	<b>Found:</b> %C = (33.62-34.02), %H= 8.47-8.23; %N= 13.08-12.96	2.0 (s, 1H, OH), 3.52(t, 2H, NCH <sub>2</sub> ), 4.27 (t, 2H, OCH <sub>2</sub> ), 7.0 (bs, 3H, NH <sub>3</sub> ), 9.6 (s, 1H, CH)	63.3 (-CH <sub>2</sub> ), 42.9 (-CH <sub>2</sub> ), 47.0 -CH <sub>3</sub> . FTIR: 3572 (-OH), 3147 (N-H)	Asymmetry, 3012 (C=C) in benzene ring, 2966 (N-H) symmetry, 2931 (C-H) asymmetry, 2897 (C-H) symmetry, 2360, 1589 (C-O) asymmetry, 1485 (-CO) symmetry cm <sup>-1</sup>	107.11gm
Ethanolammonium acetate (IL02)	[C <sub>2</sub> OHNH <sub>3</sub> ][CH <sub>3</sub> COO]	83.0 %	%C = 39.64-39.43, %H= 9.16- 9.51, % N= 11.56- 11.27	2.0 (s, 1H, OH), 2.20 (s, 3H, CH <sub>3</sub> ), 3.52 (t, 2H, NCH <sub>2</sub> ), 4.21 (t, 3H, OCH <sub>2</sub> ), 7.0 (bs, 3H, NH <sub>3</sub> )	63.3 (-CH <sub>2</sub> ), 42.9 (-CH <sub>2</sub> ), 56.0 (CH <sub>2</sub> ), 17.0 (-CH <sub>3</sub> )	3437 (-OH), 3414(N-H asymmetry), 3390 (N-H) asymmetry, 2939 (C-H) asymmetry, 2893 (C-H) symmetry, 1697 (C-O) symmetry, 1723 (C-O) asymmetry, cm <sup>-1</sup>	121.09 gm
Diethylammonium formate (IL03)	[(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> NH][HCOO],	93.0 %,	%C = 50.38 – 50.22, %H= 11.00- 11.76, % N= 11.75- 12.03	1.61 (t, 6H, 2×CH <sub>3</sub> ), 3.27 (q, 4H, 2×CH <sub>2</sub> ), 7.0 (bs, 2H, NH <sub>2</sub> ), 9.2 (s, 1H, CH)	11.08 (-CH <sub>3</sub> ), 42.2 (-CH <sub>2</sub> ), 30.6 (-CH <sub>3</sub> ), 47.0 (-CH <sub>3</sub> ), 6.8 (-CH <sub>3</sub> ), 6.8 (-CH <sub>3</sub> )	FTIR: 3433(N-H) asymmetry, 3059(N-H) symmetry, 2993 (C-H) asymmetry, 2951 (C-H) symmetry, 2885 and 2796 alkyl C-H stretch asymmetry and symmetry of ethylamine, 1650 (C-O) asymmetry, 1589 (C-O) symmetry, cm <sup>-1</sup>	119.10 gm
Diethylammonium acetate (IL04)	[(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> NH][CH <sub>3</sub> COO]	96.0 %,	%C = 54.09- 53.69, %H= 11.36- 10.96, % N= 10.52. 10.28	1.61 (t, 6H, 2×CH <sub>3</sub> ), 2.20 (s, 3H, CH <sub>3</sub> ), 3, 37 (q, 4H, 2×CH <sub>2</sub> ), 7.20 (bs, 2H, NH <sub>2</sub> )	11.08 (-CH <sub>3</sub> ), 42.2 (-CH <sub>2</sub> ), 30.6 (-CH <sub>3</sub> ), 56.0 (-CH <sub>3</sub> ), 17.0 (-CH <sub>3</sub> ), 6.8 (-CH <sub>3</sub> ), 6.8 (-CH <sub>3</sub> )	3437(N-H) asymmetry, 3414 (N-H) symmetry, 3035 (C-H) asymmetry, 2893 (C-H) symmetry, 2885 and 2796 alkyl C-H stretch asymmetry and symmetry of ethylamine, 1535 (C-O) asymmetry, 1500 (C-O) symmetry, cm <sup>-1</sup>	133.12 gm
Triethanolammonium formate (IL05)	[(C <sub>2</sub> OH) <sub>3</sub> NH][HCOO]	81 %,	%C = (45.90-45.70), %H= (9.44-9.66), % N= (7.65-7.40)	2.0 (s, 3H, 3×OH), 3.43 (t, 6H, 3×NCH <sub>2</sub> ), 3.77 (t, 6H, 3×OCH <sub>2</sub> ), 7.08 (s, 1H, NH), 9.62 (s, 1H, CH)	58.6 (-CH <sub>2</sub> ), 59.5 (-CH <sub>2</sub> ), 38.1 (-CH <sub>3</sub> ), 59.5 (-CH <sub>2</sub> ), 58.6 (-CH <sub>2</sub> ), 47 (-CH <sub>3</sub> ), 16.9 (-CH <sub>3</sub> ), 55.8(-CH <sub>2</sub> )	3417 for N-H bond of ammonium ion, 2947 (C-H) asymmetry, 2893 (C-H) asymmetry, 1666 (C-O) symmetry, 1593(C-O) symmetry	195.52 gm
Triethanolammonium formate(IL06)	[(C <sub>2</sub> OH) <sub>3</sub> NH][CH <sub>3</sub> COO]	84.0 %,	%C= (45.60- 45.28), H%= (9.74- 9.89), % N= (6.65- 6.52)	2.05 (s, 3H, 3×OH), 2.26 (s, 3H, CH <sub>3</sub> ), 3.21 (t, 6H, 3×NCH <sub>2</sub> ), 3.87 (t, 6H, 3×OCH <sub>2</sub> ), 7.0 (s, 1H, NH)	58.6 (-CH <sub>2</sub> ), 59.5 (-CH <sub>2</sub> ), 38.1 (-CH <sub>3</sub> ), 59.5 (-CH <sub>2</sub> ), 58.6 (-CH <sub>2</sub> ), 56.0 (-CH <sub>2</sub> ), 17.0 (-CH <sub>3</sub> ), 55.8 (-CH <sub>2</sub> ).	3363(N-H asymmetry), 3151(N-H asymmetry), 3016 (C-H) asymmetry, 2970 (C-H) symmetry, 2931, and 2897, alkyl C-H stretch asymmetry and symmetry of ethanolamine, 2839.22 C-H asymmetries and C-H symmetry, 1566 (C-O) asymmetry, 1485 (C-O) symmetry cm <sup>-1</sup>	210.52gm

### 3. RESULTS AND DISCUSSION

The NMR, Elementary analysis, FTIR and Molecular Weight of synthesized ILs are listed in Table 1.

### 4. COMPUTATIONAL OVERVIEW OF THE SYNTHESIZED ILS

Computational chemistry is a branch of chemistry that uses computers to assist in solving chemical problems uses the results of theoretical chemistry, incorporated into efficient computer programs, to calculate the structures and properties of molecules and solids [36]. There are two broad areas within computational chemistry devoted to the structure of molecules and their reactivity as molecular mechanics (MM) and quantum mechanics (QM) [37]. They both perform the same basic types of calculations. Molecular mechanics uses the laws of classical physics to explain and interpret the structure and properties of molecules. Molecular mechanics methods are available in many computer programs, including MM3, HyperChem, Gaussian, Quanta, Sybyl and Alchemy [24,38].

The tested compounds were constructed using Hyperchem<sup>R</sup> Release 8.0 (Hypercube, Inc.) drawing platform and are geometrically optimized

for further calculations. For the calculation using molecular mechanics, these compounds were tested on two basis sets, Mm+ and PM3. Single point calculations were carried out to create the data profile on the physical properties, such as total energy, binding energy, hydration energy, dipole moment, a heat of formation and energies for HOMO and LUMO[27].

In the calculation, total energy and free energy for the compounds are same due to zero value of entropy. According to Gibb's equation,  $A = E - TS$  (where, A=Free energy, T=Temperature, E=Total energy, S=Entropy); If S=0, then  $A=E-0$  and  $A=E$ .

The properties estimated by the basis set Mm+ and PM3, can be used to explain the molecular properties and variations can be attributed to the hydrogen bonding, binding energy calculations. According to the results found by the software, it is obvious that the total energy is more for higher alkyl chain ILs compare to the lower alkyl chain ILs. Standard methods were employed for all the test and experiments. Lower carboxylate such as formate and acetate ILs were tested in molecular programming software Hyperchem<sup>R</sup> Release 8.0 and found a good result for properties calculation.

**Table 2. Properties calculation using optimized Mm+ set**

Chemicals	Calculation type	Total energy (kcal/mol)	Dipole moment (debyes)	Hydration energy (kcal/mol)
IL01	Mm+	0.50945	3.50600	3.900
IL02	Mm+	-0.69262	1.53409	-11.670
IL03	Mm+	3.74434	2.18200	-2.620
IL04	Mm+	7.14584	4.75700	-14.100
IL05	Mm+	14.66050	2.40697	-8.660
IL06	Mm+	17.62987	6.28500	-17.940

**Table 3. Properties calculation using optimized PM3 set**

Chemicals	IL01	IL02	IL03	IL04	IL05	IL06
Calculation type	PM3	PM3	PM3	PM3	PM3	PM3
Total energy (kcal/mol)	-34720.769	-38168.335	-48382.8	-51832.872	-61706.87	-65498.4
Binding energy (kcal/mol)	-1262.9246	-1542.4632	-1916.36	-2198.4278	-2533.345	-2855.42
Heat of formation (kcal/mol)	-93.863591	-98.308165	-137.554	-144.52623	-196.9071	-191.771
HOMO (eV)	-10.54155	-10.06375	-10.41844	-10.24847	-3.97568	-8.92506
LUMO (eV)	1.13607	0.90474	0.93535	0.94809	0.83742	-1.65566
LUMO~HOMO (eV)	11.677621	10.968487	11.353785	11.196557	4.813104	7.269402

## 5. EXPERIMENT WITH ANTIMICROBIAL ACTIVITY

### 5.1 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. Then the required 1.5 mL distilled water was added and well shake until well soluble.

### 5.2 Antimicrobial Assay

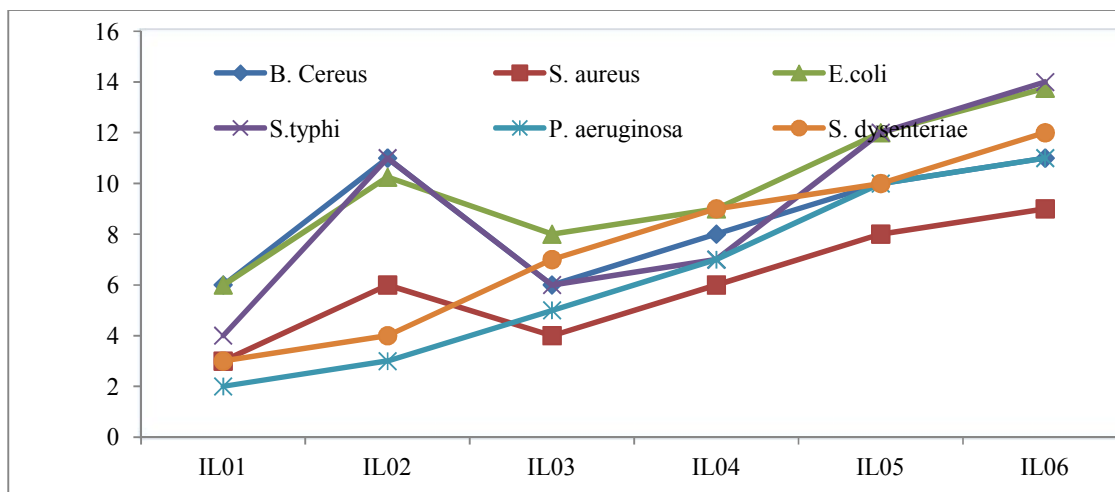
A preliminary investigation of the antibacterial activities of pure ILs was performed through measurements of primary screening both the gram-positive and gram-negative bacteria. Antibacterial screening of the test ILs was carried

out with six bacterial pathogens, such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Shigella dysenteriae*. This method was carried on via well diffusion method[39-40]. The bacterial inhibition zone (subtracting the well diameter 5.0 mm) was measured in mm scale with consideration  $\pm 1.0$  with all taking value. All the measurements were done in triplicate and the averages were listed in Table 4. The initial concentration was maintained for all ILs in 1000 mM/L, 500 mM/L and 100 mM/L in distilled water or methanol. 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.

From Table 4, a graph is plotted different ILs vs Zone of inhibition in Fig. 3.

**Table 4. Zone of inhibition (in mm) observed in three different concentrations (X=1000 mM/L, Y=500 mM/L, Z=100 mM/L)**

Pathogens and ILs	<i>B. cereus</i>			<i>S. aureus</i>			<i>E. coli</i>			<i>S.typhi</i>			<i>P. aeruginosa</i>			<i>S. dysenteriae</i>		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
IL01	6.0	0	0	3.0	0	0	6.0	0	0	4.0	0	0	2.0	0	0	3.0	0	0
IL02	11.0	0	0	6.0	0	0	10.25	6.0	0	11.0	5.2	0	3.0	0	0	4.0	0	0
IL03	6.0	0	0	4.0	0	0	8.0	0	0	6.0	5.0	0	5.0	0	0	7.0	0	0
IL04	8.0	0	0	6.0	0	0	9.0	0	0	7.0	0	0	7.0	0	0	9.0	0	0
IL05	10.0	6.0	0	8.0	6.0	0	12.0	8.0	0	12.0	0	0	10.0	0	0	10.0	14.0	0
IL06	11.0	0	0	9.0	0	0	13.75	0	0	14.0	0	0	11.0	0	0	12.0	0	0



**Fig. 3. A comparison study of toxicity for ammonium bases ILs**

From the above graph, it was shown that the antimicrobial toxicity of primary, secondary and tertiary is not same. In all case, primary ammonium based ILs has lower toxicity than secondary ammonium based ILs. Again secondary ammonium based ILs has higher toxicity than tertiary ammonium based ILs. But in all case, anion also has an activity on the toxicity. The ammonium formate has lower toxicity than ammonium acetate.

### 5.3 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 [41]. 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 were 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:

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

From the above data, it is found that the antifungal activity against *Aspergillus niger* and *Rhizopus azzahra* is a similar relation as like bacteria. The increasing order of antifungal activity is as of 1° < 2° < 3° ammonium based ILs. The anions have an effect on antimicrobial toxicity where acetate has higher antifungal activity than formate. The antifungal activity of *Rhizopus azzahra* is higher than *Aspergillus niger*.

Table 5. Result and data for antifungal test

Chemicals tested	Zone of growth (in mm)		Percentage of growth		Percentage of inhabitation	
	<i>Aspergillus niger</i>	<i>Rhizoplus azzahra</i>	<i>Aspergillus niger</i>	<i>Rhizoplus azzahra</i>	<i>Aspergillus niger</i>	<i>Rhizoplus azzahra</i>
Control	28 mm	41mm	100.0%	100.0%	--	--
IL01	20.5±1	29.2±1.0	78.57%	71.34%	21.25%	28.66%
IL02	19.0±1.0	25.2±1.0	67.80%	62.43%	32.20%	37.53%
IL03	23.0±1.0	28.5±1.0	82.14%	69.51%	17.86%	30.49%
IL04	19.7±1.0	27.0±1.0	70.53%	65.85%	29.47%	34.15%
IL05	24.0±1.0	30.5±1.0	85.00%	74.39%	15.00%	25.61%
IL06	16.2±1.0	24.0±1.0	57.85%	58.53%	42.15%	41.75%

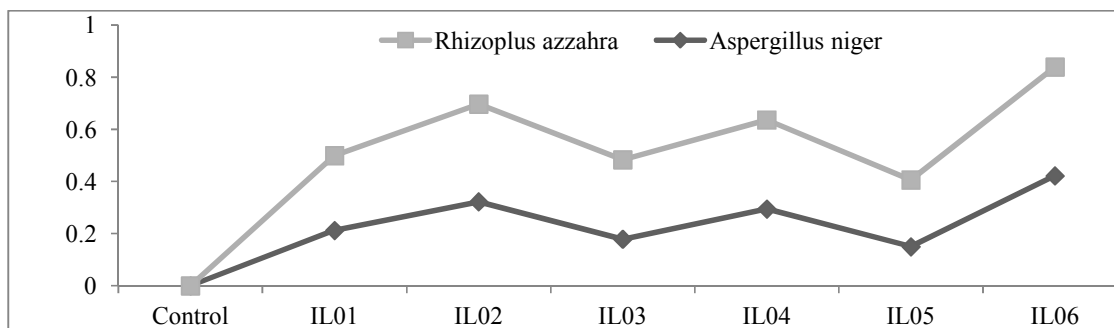


Fig. 4. Comparative study of 1°, 2° and 3° ammonium based ILs

## 6. CONCLUSION

The synthesized ILs was characterized by Fourier Transform Infrared Spectroscopy (FT-IR), Nuclear Magnetic Resonance (<sup>1</sup>H-NMR and <sup>13</sup>C NMR) Spectroscopy and elemental analysis. The synthesized quaternary ammonium formate and acetate-based ILs is the room temperature ionic liquids so it has a great goal to use in chemical, pharmaceutical, textile industries, organic synthesis, organic extraction and separation method as the solvent. Using HyperchemR computer programming, the HOMO-LUMO gap of IL01 to IL06 is 11.677621, 10.968487, 11.353785, 11.196557, 4.813104 and 7.269402 respectively that indicates the low reactivity of ILs. The low reactivity of ILs is the good agreements to use as solvents in the chemical process. To safe use in chemical industries, the antimicrobial toxicity profile is the most imperative key point of chemical substance. To evaluate the antimicrobial toxicity profiles, bacteria is the first choice due to having the short life. The antibacterial screening was conducted using the well-diffusion technique with ILs solutions of three different concentrations. The results were then used to select the initial higher concentration for dilution method, to obtain the concentrations as 1000, 500 and 100 mM/L and the antifungal activity was tested against two phytopathogenic fungi using well diffusion susceptibility test. All of synthesized ILs showed poor inhibition as potential antimicrobial agents, can proceed to future study. It also shows the information about the comparative study of primary, secondary and tertiary-based ILs. They have very near toxicity. It offers a new scope to evaluate the physio-chemical properties and application in the chemical process as an alternative to organic traditional solvent.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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