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Full Paper

Furoate Based Functionalised Ionic Liquid: Antimicrobial and Antioxidant Studies

R. Rama,[A,C] *S. Meenakshi,*[A] *J. Manjunathan,*[B] *G. Abirami,*[B] *and S. Karthikeyan*[A]

[A]Department of Chemistry, School of Basic Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India.
[B]Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India.
[C]Corresponding author. Email: rama.ragu10@gmail.com

A tetraalkyl ammonium cation and furoate anion based functionalised ionic liquid (FIL), *N*-methyl-*N*,*N*,*N*-trioctylammonium furoate ($[MTOA]^+[FA]^-$) has been synthesised and characterised using FT-IR, NMR, and UV spectroscopic techniques. The in vitro antimicrobial activity of the synthesised FIL against two types of Gram-positive bacteria, namely *Staphylococcus aureus* and *Enterobacter faecalis* as well as Gram-negative bacteria, namely *Escherichia coli* and *Pseudomonas aeruginosa* was tested using a well diffusion method. Similarly, an antifungal test was done against *Candida albicans*. $[MTOA]^+[FA]^-$ showed significant inhibitory effect against all the organisms tested as compared with the chosen standards. Antibacterial activity against gram-positive bacteria (zone of inhibition: 16 to 30 mm) was found to be higher than that of Gram-negative bacteria (zone of inhibition: 11 to 19 mm). Moreover, the antioxidant activity of the synthesised FIL from a ferrous ion (Fe^{2+}) chelating assay and its scavenging activity against 1,1-diphenyl-2-picrylhydrazyl, H_2O_2, and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical using spectrophotometric methods are reported. The FIL, $[MTOA]^+[FA]^-$ showed a moderate antioxidant activity.

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Introduction

The synthesis of novel types of antibacterial and antifungal agents is a focused research field due to the severe health problems arising from the resistance of numerous microorganism strains against diverse antibiotics.[1,2] Room temperature ionic liquids (RTILs) are receiving increasing attention in the pharmaceutical industry due to their interesting properties such as non-volatility, non-flammability, high thermal stability, and large liquid range, etc. and they may form novel substitutes to antibiotics.[3] There is a growing discovery of various types of RTILs. Moreover, studies on stability, toxicity, recyclability, and biodegradation investigations of ILs have been some of the main debated topics in IL-based research.[4] In the recent past, toxicological properties of several types of ILs have been reported mainly based on their effect on the growth inhibition of fungi, bacteria, and eukaryotic cells, etc.[5]

RTILs from antimicrobials with broad-spectrum activity show significant biological activity. Moreover, it was reported that the biological activities of ILs can be improved by having a cation with long alkyl substituents, a cation of an alcohol molecule, and anion derived from a drug molecule, etc.[2] Furan derivatives have been effectively used as antimicrobial agents and, in particular, furoic acid (FA) is an active pharmaceutical intermediate, anti-inflammatory, and antifungal agent. Chai et al. reported that FA shows a higher antibacterial activity than furfuryl alcohol and furfural.[6] Also, several types of FA derivatives have been reported with excellent antimicrobial properties. For example, Wen et al. reported 27 new types of furancarboxamide derivatives with a diphenyl ether moiety with high fungicidal activities against *R. solani* and *S. ampelimum*.[7] But furoate anion based ILs have not yet been studied for their biological activity.

Moreover, numerous quaternary ammonium ionic liquids have been investigated for various applications, and the biological activities of this type of ILs have been reported in the literature.[8–15] For example, Saadeh et al. reported the antimicrobial property of ILs prepared from the tetrabutylammonium (TBA) cation and various types of anions such as linoleate, formate, acetate, salicylate, and benzoate, etc.[9] The antioxidant activity of novel quaternary bis(ammonium) cation[12] and ascorbate anion[13] based ILs were reported by Czerniak et al. Hence, the RTIL derived from the quaternary ammonium cation, *N*-methyl-*N*,*N*,*N*-trioctylammonium (MTOA) and furoate anion was believed to exhibit significant biological activity.

In the present study, we synthesised a FIL, namely, *N*-methyl-*N*,*N*,*N*-trioctylammonium furoate ($[MTOA]^+[FA]^-$). The synthesised FIL has been characterised and its antimicrobial and antioxidant activities were examined for the first time.

Experimental

Materials

All the chemicals and solvents used in the present study were of AR grade. *N*-Methyl-*N*,*N*,*N*-trioctylammonium chloride ($[MTOA]^+[Cl]^-$) (97 %) was procured from Sigma Aldrich. Chloroform (99 %) and 2-furoic acid (98 %) were purchased from Sigma Aldrich (Bio Corporals, Chennai). Ethanol (98 %) and sodium hydroxide (97 %) were obtained from SD Fine Chemicals, Chennai.

Synthesis of N-Methyl-N,N,N-trioctylammonium furoate ($[MTOA]^+[FA]^-$)

The procedure for the synthesis of the IL, $[MTOA]^+[FA]^-$ is shown in Fig. S1 (Supplementary Material) and it was carried out in two steps. In the first step, $[MTOA]^+[Cl]^-$ was dissolved in chloroform and it was equilibrated with 4 M NaOH for 1 h. The aqueous phase was then removed and the FIL phase was equilibrated again with a fresh solution of NaOH. This process was repeated at least 10 times until the chloride ion present in the aqueous and organic phase was found to be negligible (checked with acidified $AgNO_3$ solution). The chloroform was then evaporated and the obtained IL, $[MTOA]^+[OH]^-$ was dried.[16] In the second step, a mixture of 1 : 1.1 mol of $[MTOA]^+[OH]^-$ and 2-furoic acid was stirred in an ethanol medium for ~48 h.[17] After that, the IL phase was washed several times with distilled water. The solvent was evaporated and dried in a rotary evaporator at 70°C for ~10 h. A clear, viscous, and yellowish liquid was obtained. It was characterised by FT-IR, NMR, and UV spectroscopic techniques. The structure of $[MTOA]^+[FA]^-$ is shown in Fig. 1.

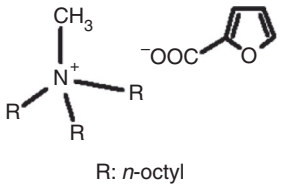

Fig. 1. Structure of *N*-methyl-*N*,*N*,*N*-trioctylammonium furoate ($[MTOA]^+[FA]^-$).

In Vitro Antimicrobial Assay

The in vitro antimicrobial activity of $[MTOA]^+[FA]^-$ against five human pathogens, *Staphylococcus aureus, Enterobacter faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* was tested using a well diffusion method.[18,19] The human pathogens were inoculated separately into 5 mL of sterile Mueller Hinton broth (MHB) and incubated at 37°C for 8 h (~0.5 O.D). Using sterile cotton swabs, the cultures were swabbed separately on the surface of sterile Mueller Hinton agar (MHA) plates. A sterilised cork borer (9 mm diameter) was used to prepare agar wells. The FILs were dissolved separately in sterile 10 % DMSO at a concentration of 1 mg mL^{-1} and different volumes of this solution (150, 100, and 50 µL) were added separately in each well on swabbed plates and incubated at 37°C. After 24 h, the growth of pathogens was observed and the inhibition zone was measured.[20] Positive controls, fluconazole for *C. albicans*, and streptomycin for Gram-positive and Gram-negative bacteria, were used. The study was performed in triplicate and the mean value was calculated.

Procedure for Antioxidant Assay

In this section, procedures followed for the free radical ($DPPH^•$, $ABTS^{•+}$, and H_2O_2) scavenging and ferrous ion chelating activity studies are described.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

The DPPH assay for the synthesised FIL was carried out as described in the literature.[21] In a test tube covered with aluminium foil, 4.3 mg of DPPH was dissolved in 3.3 mL of methanol. The DPPH solution (150 µL) was then introduced to 3 mL of methanol and the absorbance was noted at 517 nm for a control reading. Samples (50 µL) of various concentrations (20–100 µg mL^{-1}) as well as the standard compound (quercetin) were taken in 150 µL of methanol. Each sample was then again diluted with 3 mL of methanol and 150 µL of DPPH solution was added. Absorbance was noted using methanol as a blank after 15 min at 517 nm. The percentage of inhibition was calculated using Eqn 1:

$$\text{Percentage of inhibition}\,(\%) = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

where A_0 = absorbance of the control and A_1 = absorbance of FIL or standard.

Ferrous Ion (Fe^{2+}) Chelating Assay

The ferrous iron chelating assay was carried out according to the procedure given by Dinis et al.[22] The FIL (0.5 mL) at different concentrations (20–100 µg mL^{-1}) was added to 0.05 mL of 0.2 mM $FeCl_2$ solution and then 5 mM ferrozine (0.2 mL) was added to initiate the reaction. After vigorous shaking of the mixture, it was left at room temperature for 10 min. The ferrozine reacted with the Fe^{2+} ions and formed stable magenta complex species that were highly soluble in water. The absorbance of the solution was noted spectrophotometrically at 562 nm. The percentage inhibition of the ferrozine–Fe^{2+} complex formation was calculated by Eqn 2:

$$\text{Metal chelating effect}(\%) = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

where A_0 is the absorbance of the control (control contained $FeCl_2$ and ferrozine; complex formation molecules), and A_1 is the absorbance of the FIL or standard used (i.e., EDTA disodium salt dihydrate (Na_2-EDTA)).

H_2O_2 Radical Scavenging Assay

The hydrogen peroxide (H_2O_2) scavenging activity of FIL was determined according to the method described by Ruch et al.[23] In the first step, a 2 mM solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). Different concentrations (20–100 µg mL^{-1}) of the FIL were added to the H_2O_2 solution (0.6 mL). At 230 nm, the absorbance for H_2O_2 was noted against a blank containing phosphate buffer without H_2O_2. Ascorbic acid was used as a standard and the percentage of inhibition was calculated using Eqn 1 (see above).

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Radical Scavenging Assay

This assay was done as described in the literature:[24] stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were prepared. The two stock solutions were mixed in equal

quantities and allowed to react in the absence of light at room temperature for 12 h. The solution was diluted by mixing 1 mL of $ABTS^{\bullet+}$ solution with 50 mL of methanol to obtain an absorbance of ~0.70 at 734 nm. The FIL was diluted with methanol, treated with $ABTS^{\bullet+}$ solution, and made up to a total volume of 1 mL. Absorbance was measured using the spectrophotometric method at time intervals of 10 min after the addition of 20–100 µg mL^{-1} concentrations of FIL. The assay was first done on ascorbic acid which was used as a standard. The results of the assay were specified relative to ascorbic acid. The assay was performed in triplicate and the scavenging effect of ABTS calculated using Eqn 3.

$$\text{ABTS scavenging effect}(\%) = ((A_0 - A_1/A_0) \times 100) \quad (3)$$

where A_0 = absorbance of control and A_1 = absorbance of the FIL or standard.

Results and Discussion

Characterisation of $[MTOA]^+[FA]^-$

FT-IR Spectroscopy

An FT-IR spectrum of the synthesised FIL, $[MTOA]^+[FA]^-$ is shown in Fig. S2 (Supplementary Material). Broad and strong vibration peaks for asymmetric and symmetric C–H stretching of the methylene and methyl units are observed at 2863 and 2929 cm^{-1} respectively. The C–H stretching frequency in the furan ring was obtained at 3120 cm^{-1}. The C=O asymmetric and symmetric stretching of the –COO group were assigned to IR peaks observed at 1713 and 1376 cm^{-1} respectively. The peak at 1477 cm^{-1} corresponds to a vibrational peak of a CH_2–N^+ group and asymmetric stretching at the C=C double bond was noted at 1583 cm^{-1}.

NMR Spectroscopy

The ^1H NMR spectrum (400 MHz, $CDCl_3$) of $[MTOA]^+[FA]^-$ is shown in Fig. S3 (Supplementary Material) and the chemical shift (δ, ppm) values obtained for protons present in the FIL are 0.85 (9H, m), 1.21–1.32 (30H, m), 1.68 (6H, m), 3.2 (3H, s), 3.37 (6H, m), 6.45, 7.10–7.68 (furan ring). The ^{13}C NMR spectrum of $[MTOA]^+[FA]^-$ is shown in Fig. S4 (Supplementary Material) and the chemical shift values (δ, ppm) obtained for carbon atoms present in the FIL are 14.0 (CH_3C), 22.52, 26.27, 28.99, 29.0, 29.38 31.59 (various CH_2), 49.02 (CH_3N), 61.61 (CH_2N), 111.91, 118.50, 144.90, 146.42. (aromatic *C*), 161.16 (*C*OO).

UV Spectroscopy

The UV spectrum of $[MTOA]^+[FA]^-$ was recorded and it is shown in Fig. S5 (Supplementary Material). There was an absorption between 240 and 280 nm for the IL, $[MTOA]^+[FA]^-$.

Antibacterial Activities of $[MTOA]^+[FA]^-$ Using the Well Diffusion Method

Antimicrobial properties of various types of ILs are reported in the literature[8–11] and the antimicrobial activity of the FIL, $[MTOA]^+[FA]^-$ is investigated here. Well diffusion (zone of inhibition) results of Gram-positive and Gram-negative bacteria are shown in Figs 2 and 3, respectively. The zone of inhibition as a function of FIL concentration against Gram-positive and Gram-negative bacteria is presented in Figs 4 and 5 respectively. The positive control, streptomycin, resulted in 16 and 20 mm

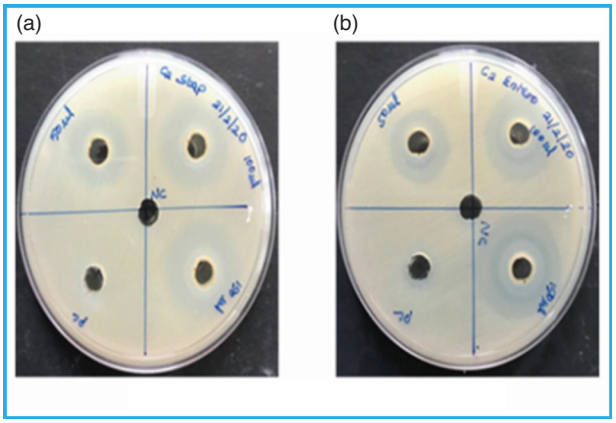

Fig. 2. Photograph of well diffusion results for $[MTOA]^+[FA]^-$ against Gram-positive bacteria: (a) *Staphylococcus aureus*. (b) *Enterobacter faecalis.*

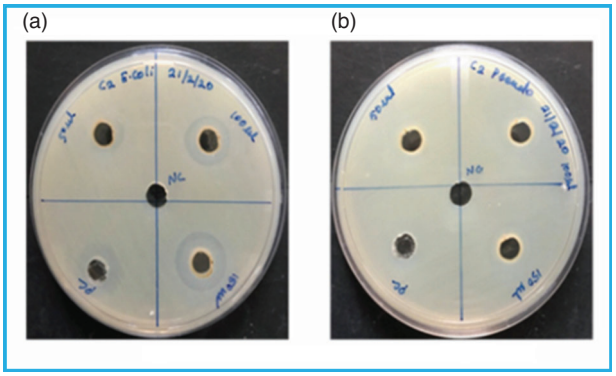

Fig. 3. Photograph of well diffusion results for $[MTOA]^+[FA]^-$ against Gram-negative bacteria: (a) *Escherichia coli.* (b) *Pseudomonas aeruginosa.*

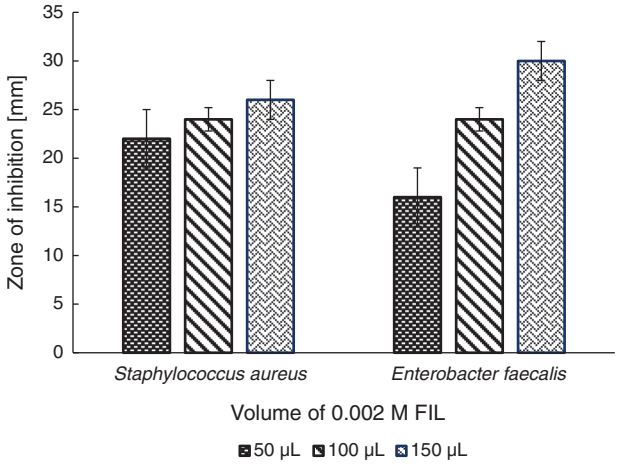

Fig. 4. Zone of inhibition for various concentrations of $[MTOA]^+[FA]^-$ against Gram-positive bacteria.

zones of inhibition for *E. coli* and *Enterobacter* respectively. For the negative control (10 % DMSO), there was no inhibition. $[MTOA]^+[FA]^-$ showed significant antibacterial activity as compared with the standard.

2-Furoic acid is reported as a bactericide and fungicide in the literature. Chai et al.[6] studied the antimicrobial activity of FA

against *bacillus subtilis* (Gram-positive) and *Salmonella bacteria* (Gram-negative). A zone of inhibition of > 20 mm at concentrations of 0.7 and 1.4 M was reported. In the present study, the furoate IL ($[MTOA]^+[FA]^-$) at lower concentrations (150, 100, and 50 µL of 0.002 M solution of FIL) showed significant antibacterial activity with a zone of inhibition of >20 mm against the Gram-positive bacteria, *Staphylococcus aureus and Enterobacter faecalis.* But the zone was found to be slightly less against the Gram-negative bacteria, *Escherichia coli,* and *Pseudomonas aeroginosa.* The higher zone of inhibition offered by $[MTOA]^+[FA]^-$ towards Gram-positive bacteria compared with Gram-negative bacteria could be attributed to the hydrophobic alkyl chains present in the FIL which can more readily embed into the porous cell of the Gram-positive bacteria and disturb the peptidoglycan layer which results in the cell death.

The $[MTOA]^+[FA]^-$ gave better results as compared with the cholinium-based ILs reported by Jumbri et al.[15] synthesised using five types of carboxylic acids. None of these cholinium-carboxylate ILs showed activity against the Gram-positive bacteria, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa.* In contrast $[MTOA]^+[FA]^-$ which contains the carboxylate anion of FA has provided remarkable antibacterial activity. Similarly, Salman et al. reported several types of ILs based on the tetrabutylammonium (TBA) cation with various kinds of carboxylate anions such as acetate, salicylate, and benzoate, etc.[9] The antibacterial activity of these ILs are moderately comparable with that of $[MTOA]^+[FA]^-$. Hence, the furoate anion-based FIL, $[MTOA]^+[FA]^-$ is a promising antimicrobial ionic liquid.

Antifungal Property of $[MTOA]^+$ $[FA]^-$

The FIL, $[MTOA]^+[FA]^-$ was screened for antifungal activity by the well diffusion method using the human pathogenic fungal isolate namely *Candida albicans* and the result is shown in Fig. 6. The antifungal activity was evaluated by measuring the zone of inhibition of fungal growth surrounding the well. The zone obtained for the fungal organism at various concentrations of FIL is presented in Fig. 7. The antifungal activity shown by $[MTOA]^+[FA]^-$ is comparable with cholinium peracetate reported by Jumbri et al.[15] and higher than that of TBA fomate reported by Salman et al.[9] Moreover, a significant reduction in the growth of the fungal strain was shown by $[MTOA]^+[FA]^-$ as compared with the positive control, floconazole.

Antioxidant Studies of $[MTOA]^+$ $[FA]^-$

DPPH Radical Scavenging Activity

The DPPH free radical can react with antioxidant substances and the odd electron of DPPH will be paired with an electron donated by an antioxidant compound to result in decolouration. In the present study, the scavenging effects of $[MTOA]^+[FA]^-$ for the DPPH radical was analysed and the results are shown in Fig. 8.

With an increased concentration of the FIL, the scavenging activity increased. In the concentration range of 20–100 µg mL^{-1}, the scavenging activity of $[MTOA]^+[FA]^-$ for the DPPH radical was found to be 18.31–58.13 %. Moreover, $[MTOA]^+[FA]^-$ (half maximal inhibitory concentration, IC_{50}: 86.07 µg mL^{-1}) showed a moderate activity as compared with that of the standard (Fig. 9), quercetin (IC_{50}: 60.14 µg mL^{-1}). The antioxidant property of some FILs based on ammonium cations has been reported in the literature.[12,13,25] For example, Ahmad et al. reported the DPPH radical scavenging activity of

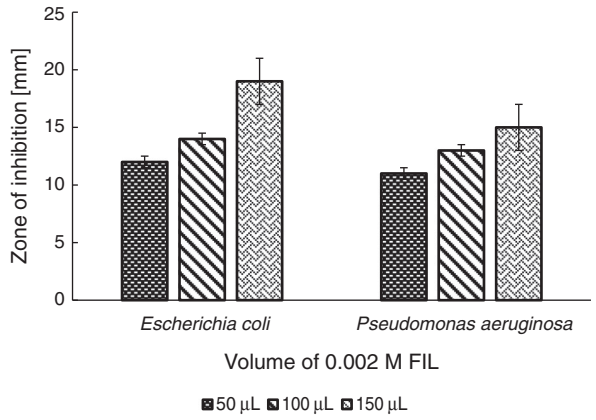

Fig. 5. Zone of inhibition for various concentrations of $[MTOA]^+[FA]^-$ against Gram-negative bacteria.

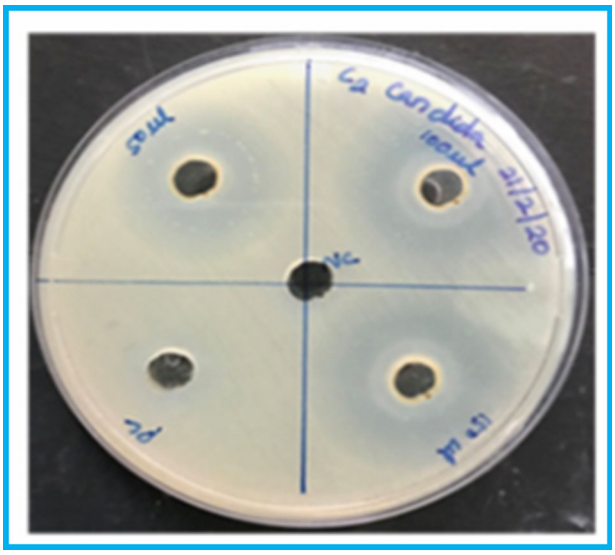

Fig. 6. Photograph of well diffusion results for $[MTOA]^+[FA]^-$ against fungal isolate.

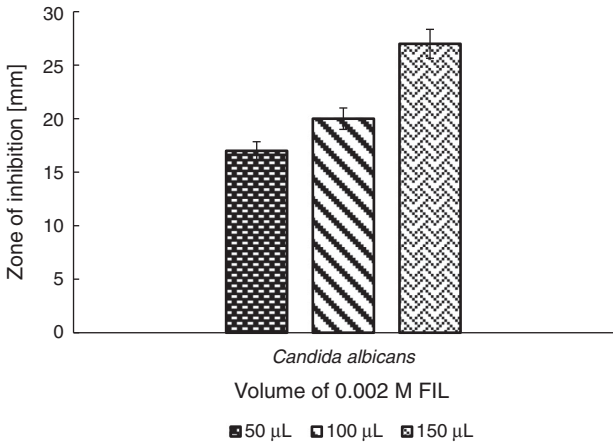

Fig. 7. Zone of inhibition for various concentrations of $[MTOA]^+[FA]^-$ against *Candida albicans.*

tertiary ammonium salicylate-based protic ionic liquids (PILs) namely, 3-dimethylamino-1-propanol salicylate (3DMAPS) and 3-diethylamino-1-propanol salicylate (3DEAPS). IC_{20}

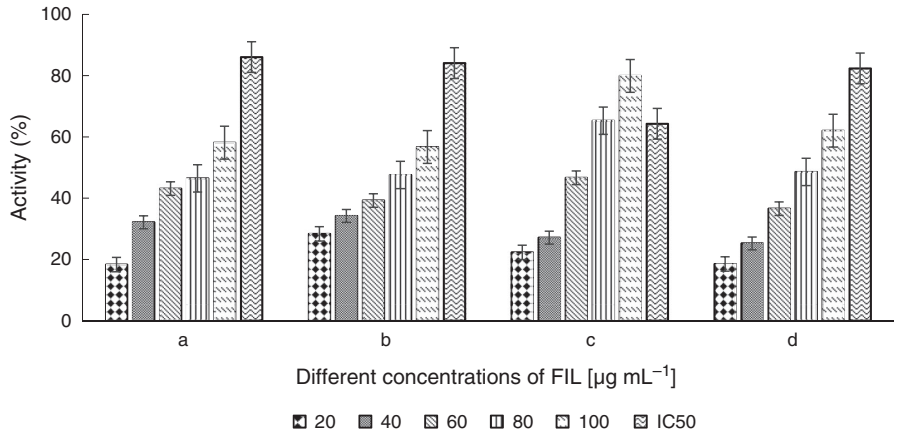

Fig. 8. Antioxidant activity of $[MTOA]^+[FA]^-$. (a) DPPH radical scavenging activity. (b) Ferrous ion chelating activity. (c) Hydrogen peroxide scavenging activity. (d) ABTS radical scavenging activity.

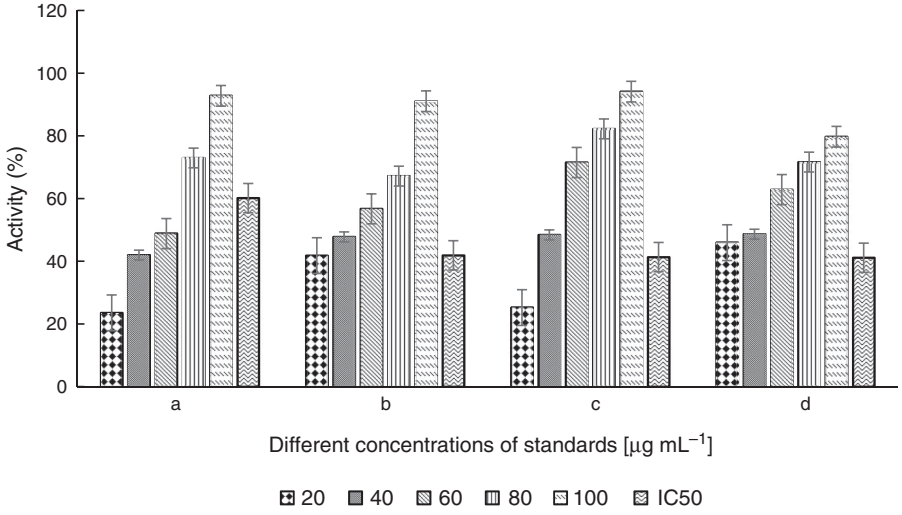

Fig. 9. Antioxidant activity of standards. (a) DPPH radical scavenging activity of quercetin. (b) Ferrous ion chelating activity of Na_2 EDTA. (c) Hydrogen peroxide scavenging activity of ascorbic acid. (d) ABTS radical scavenging activity of ascorbic acid.

values of 66.76 and 27.27 μM were reported for 3DMAPS and 3DEAPS respectively. The DPPH radical scavenging activity of the quaternary ammonium-based FIL synthesised in the present study is moderately comparable with the above-said results.[25] The scavenging activity shown by $[MTOA]^+[FA]^-$ could be due to the presence of the bioactive furoate anion and an ammonium cation with a larger alkyl chain.

Ferrous Ion Chelating Ability

A ferrous ion chelating assay was carried out to determine the ability of the synthesised FIL to chelate Fe^{2+} ions. In this assay, the chelating agents disrupt the ferrozine–Fe^{2+} complex, thus decrease the red colour. The chelating activity of $[MTOA]^+[FA]^-$ at five different concentrations against ferrous ions was investigated in the present study. The chelating abilities of FIL increased with an increase in the concentration of the FIL and it is shown in Fig. 8. The FIL at concentrations of 20–100 μg mL^{-1} showed significant activity and the IC_{50} value (84.10 μg mL^{-1}) of the FIL was found to be higher than that obtained for the standard, 41.87 μg mL^{-1} (Fig. 9). It reveals that FIL shows a moderate ferrous ion chelating ability.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

H_2O_2 itself is not very reactive and is not a radical species that plays a role in oxidative stress, but it may sometimes be toxic to cells because it can give rise to hydroxyl radicals in cells. The hydrogen peroxide scavenging activity of $[MTOA]^+[FA]^-$ effectively increases with the rise in concentration from 20 to 100 μg mL^{-1} (Fig. 8). The IC_{50} value of $[MTOA]^+[FA]^-$ was found to be 64.29 μg mL^{-1} (standard, $IC_{50} = 41.31$ μg mL^{-1}). It is understood that the H_2O_2 scavenging activity of the synthesised FIL is moderately comparable with that of the standard, ascorbic acid (Fig. 9).

ABTS Radical Scavenging Activity

The ABTS radical has a typical absorption at 734 nm and is reactive against most antioxidants which induces a colour change from blue to a colourless neutral form. The scavenging effects of the FIL, $[MTOA]^+[FA]^-$ against the ABTS radical increased when its concentration was increased from 20 to 100 μg mL^{-1} (Fig. 8). The extent of reduction or decolourisation is directly proportional to the concentration of the FIL and it shows moderate ABTS radical scavenging ability with an

IC_{50} value of 82.37 μg mL^{-1} (standard, ascorbic acid: IC_{50} = 41.11 μg mL^{-1} (Fig. 9)).

Conclusion

In the present study, quaternary ammonium and furoate based functionalised ionic liquid, $[MTOA]^+[FA]^-$ has been synthesised and characterised using UV, IR, and NMR spectroscopic techniques. Moreover, for the first time, the antimicrobial properties of $[MTOA]^+[FA]^-$ was analysed against five types of human pathogens and significant inhibitory effect was observed even at reduced concentrations (150 μL, 100 μL, 50 μL of a 1 mg mL^{-1} solution of $[MTOA]^+[FA]^-$). In particular, the zone of inhibition for gram-positive bacteria was found to be in the range of 16 to 30 mm. But in the case of Gram-negative bacteria, the zone of inhibition was slightly less than that of Gram-positive bacteria. As compared with the standard, streptomycin, excellent antibacterial activity was observed for the FIL. Similarly, a significant inhibitory effect against a fungal strain was shown by the FIL, $[MTOA]^+[FA]^-$ as compared with the positive control, floconazole. Moreover, the antioxidant activity was also investigated and the IC_{50} values for $DPPH^•$, $ABTS^{•+}$, and H_2O_2 scavenging and Fe^{2+} ion chelating activity was found to be 86.07, 82.37, 64.29, and 84.10 μg mL^{-1}, respectively. These results revealed that $[MTOA]^+[FA]^-$ is a capable antimicrobial ionic liquid and also shows moderate antioxidant activity.

Supplementary Material

The synthetic scheme of the functionalised ionic liquid, $[MTOA]^+[FA]^-$ and its characterisation details (IR, NMR, and UV spectra) are available on the Journal's website.

Conflicts of Interest

The authors declare no conflicts of interest

Acknowledgement

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References

[1] B. Bromberger, J. Sommer, C. Robben, C. Trautner, R. Kalb, P. Rossmanith, P. J. Mester, *Sep. Purif. Technol.* **2020**, *251*, 117309. doi:10.1016/J.SEPPUR.2020.117309

[2] A. Miskiewicz, P. Ceranowicz, M. Szymczak, K. Bartuś, P. Kowalczyk, *Int. J. Mol. Sci.* **2018**, *19*, 2779. doi:10.3390/IJMS19092779

[3] R. L. Tundis, G. Frattaruolo, B. Carullo, M. Armentano, M. R. Badolato, M. R. Loizzo, F. Aiello, A. R. Cappello, *Nat. Prod. Res.* **2019**, *33*, 162. doi:10.1080/14786419.2018.1440224

[4] J. M. Gomes, S. S. Silva, R. L. Reis, *Chem. Soc. Rev.* **2019**, *48*, 4317. doi:10.1039/C9CS00016J

[5] J. Yu, S. Zhang, Y. Dai, X. Lu, Q. Lei, W. Fang, *J. Hazard. Mater.* **2016**, *307*, 73. doi:10.1016/J.JHAZMAT.2015.12.028

[6] W. M. Chai, X. Liu, Y. H. Hu, H. L. Feng, Y. L. Jia, Y. J. Guo, H. T. Zhou, Q. X. Chen, *Int. J. Biol. Macromol.* **2013**, *57*, 151. doi:10.1016/J.IJBIOMAC.2013.02.019

[7] F. Wen, H. Jin, K. Tao, T. Hou, *Eur. J. Med. Chem.* **2016**, *120*, 244. doi:10.1016/J.EJMECH.2016.04.060

[8] W. L. Hough-Troutman, M. Smiglak, S. Griffin, W. M. Reichert, I. Mirska, J. Jodynis-Liebert, T. Adamska, J. Nawrot, M. Stasiewicz, R. D. Rogers, J. Pernak, *New J. Chem.* **2009**, *33*, 26. doi:10.1039/B813213P

[9] S. M. Saadeh, Z. Yasseen, F. A. Sharif, H. M. A. Shawish, *Ecotoxicol. Environ. Saf.* **2009**, *72*, 1805. doi:10.1016/J.ECOENV.2008.12.015

[10] R. Rama, S. Meenakshi, *J. Mol. Struct.* **2020**, *1204*, 127490. doi:10.1016/J.MOLSTRUC.2019.127490

[11] J. Cybulski, A. Wiśniewska, A. Kulig-Adamiak, L. Lewicka, A. Cieniecka-Rosłonkiewicz, K. Kita, A. Fojutowski, J. Nawrot, K. Materna, J. Pernak, *Chem. – Eur. J.* **2008**, *14*, 9305. doi:10.1002/CHEM.200800973

[12] K. Czerniak, F. Walkiewicz, *New J. Chem.* **2017**, *41*, 530. doi:10.1039/C6NJ02428A

[13] K. Czerniak, *Chemik* **2016**, *70*, 521.

[14] N. Muhammad, M. I. Hossain, Z. Man, M. El-Harbawi, M. A. Bustam, Y. A. Noaman, N. B. Mohamed Alitheen, M. K. Ng, G. Hefter, C. Y. Yin, *Chem. Eng. J.* **2012**, *57*, 2191.

[15] K. Jumbri, N. S. F. M. Noh, N. A. Ahmad, M. B. A. Rahman, H. Ahmad, *J. Adv. Res. Fluid Mech. Therm. Sci.* **2018**, *42*, 16.

[16] R. Rama, R. Kumaresan, K. A. Venkatesan, M. P. Antony, P. R. Vasudeva Rao, *Radiochim. Acta* **2014**, *102*, 1009.

[17] D. Deng, Y. Jiang, X. Liu, *New J. Chem.* **2017**, *41*, 2090. doi:10.1039/C6NJ03563A

[18] S. Magaldi, S. Mata-Essayag, C. H. De Capriles, C. Perez, M. T. Colella, C. Olaizola, Y. Ontiveros, *Int. J. Infect. Dis.* **2004**, *8*, 39. doi:10.1016/J.IJID.2003.03.002

[19] National Committee for Clinical Laboratory Standards (NCCLS), *Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated from Aquatic Animals: A Report*. NCClS document M42-R **2003** (NCCLS: Wayne, PA).

[20] National Committee for Clinical Laboratory Standards (NCCLS), *Performance Standards for Antimicrobial Disk Susceptibility Tests* **1999** (NCCLS: Wayne, PA).

[21] M. Ismail, M. Ibrar, Z. Iqbal, J. Hussain, H. Hussain, M. Ahmed, A. Ejaz, M. I. Choudhary, *Rec. Nat. Prod.* **2019**, *3*, 193.

[22] T. C. Dinis, V. M. Madeira, L. M. Almeida, *Arch. Biochem. Biophys.* **1994**, *315*, 161. doi:10.1006/ABBI.1994.1485

[23] R. J. Ruch, S. J. Cheng, J. E. Klaunig, *Carcinogenesis* **1989**, *10*, 1003. doi:10.1093/CARCIN/10.6.1003

[24] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radic. Biol. Med.* **1999**, *26*, 1231. doi:10.1016/S0891-5849(98)00315-3

[25] N. A. Ahmad, K. Jumbri, A. Ramli, N. Ghani, H. Ahmad, *Malays. J. Anal. Sci.* **2019**, *23*, 383.