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SYNTHESIS AND ANTIBACTERIAL ACTIVITIES EVALUATION OF WATER-SOLUBLE CAFFEIC ACID AMMONIUM SALTS

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ABSTRACT

A series of water-soluble caffeic acid ammonium salts (compounds **1**–**28**) were synthesized by acid-base neutralization. Except compound **21**, all the compounds were first reported. The chemical structures of these compounds were confirmed by means of ^{1}H NMR, ESI MS and elemental analyses. All of the compounds were assayed for antibacterial (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525 and *Staphylococcus aureus* ATCC 6538) activities by MTT method. Compounds benzenaminium (*E*)-3-(3,4-dihydroxyphenyl)acrylate (**6**), dibutylammonium (*E*)-3-(3,4-dihydroxyphenyl)acrylate (**24**) and triethylammonium (*E*)-3-(3,4-dihydroxyphenyl)acrylate (**27**) showed considerable antibacterial activities. Generally, straight aliphatic ammonium salts were more powerful than branched aliphatic ammonium salts or aromatic ones, and tertiary ammonium salts had higher antibacterial activity than primary and secondary ammonium salts.

GRAPHIC ABSRACT

Synthesis and Antibacterial Activities Evaluation of Water-soluble Caffeic Acid Ammonium Salts

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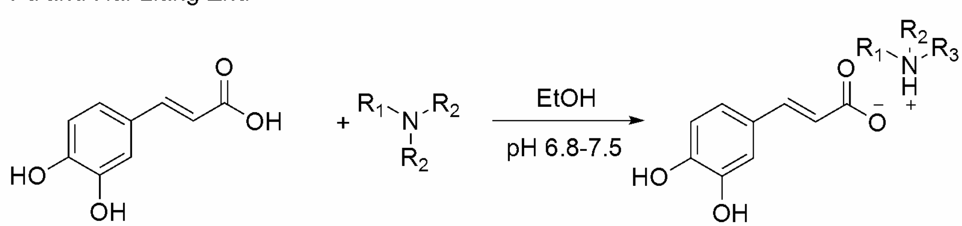

KEYWORDS

Synthesis, antibacterial, caffeic acid ammonium salt

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INTRODUCTION

Caffeic acid is one of the most widely distributed hydroxycinnamate and phenylpropanoid metabolites in plant tissues. This polyphenol is present in many dietary sources, including coffee beverages, blueberries, apples, and cider [1]. It acts as an antioxidant by scavenging oxygen-free radicals [2] and by chelating prooxidant metal ions, especially irons [3]. It has also been demonstrated to inhibit the growth of bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and some yeasts [4]. However, bad water solubility of caffeic acid limits the antibacterial activity [5, 6]. As we have known, many ammonium salts are surfactant and have good antibacterial activity [7]. Therefore, in this paper, a series of water-soluble caffeic acid ammonium salts were synthesized and their antibacterial activity against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas fluorescence* and *Staphylococcus aureus* were evaluated. The results of this study may be useful to researchers attempting to find new potential bactericides.

MATERIALS AND METHODS

Chemistry general

All chemicals (reagent grade) used were purchased from Aldrich (USA). ^{1}H NMR spectra were recorded at 300 MHz on ^{1}H-Varian-Mercury-300 spectrometers in DMSO-d_6, using TMS as internal standard. MS spectra were recorded with a Finnigan Trace MS spectrometer. Elementary analyses were performed on a Vario EL III elementary analysis instrument. Melting points were obtained by using an electrothermal digital melting point apparatus. TLC was run on the silica gel coated aluminum sheets (silica gel 60 GF254, E. Merck, Germany) and visualized in UV light (254 nm).

General synthesis method of caffeic acid ammonium salts

Equimolar quantities (1 mmol) of caffeic acid and amine in absolute alcohol (20 mL) were stirred for 1–4 h at room temperature. pH value was adjusted at 6.8–7.5. Then the solution was filtrated and left in air for a few days, yielding the crystal or powder caffeic acid ammonium salt.

Ammonium (E)-3-(3,4-dihydroxyphenyl)acrylate (**1**)

White powder, yield 84%, mp: 183-184 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.27 (s, 4H), 7.17 (d, 1H, J = 15.8 Hz), 6.98 (d, 1H, J = 1.8 Hz), 6.81 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 6.71 (d, 1H, J = 8.0 Hz), 6.15 (d, 1H, J = 15.7 Hz). ESI MS: 198.1 $[M + H]^+$. Anal. Calc for $C_9H_{11}NO_4$: C, 54.82; H, 5.62; N, 7.10%. Found: C, 54.46; H, 5.49; N, 7.76%.

2-Hydroxyethanaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**2**)

Pale green crystal, yield 74%, mp: 189-200 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 8.01 (s, 3H), 7.17 (d, 1H, J = 15.7 Hz), 6.93 (d, 1H, J = 1.9 Hz), 6.79

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(dd, 1H, J_1 = 8.3 Hz, J_2 = 1.9 Hz), 6.71 (d, 1H, J = 8.1 Hz), 6.17 (d, 1H, J = 15.6 Hz), 4.16 (t 2H, J=6.6 Hz), 3.45 (t, 2H, J = 6.5 Hz). ESI MS: 242.1 $[M + H]^+$. Anal. Calc for $C_{11}H_{15}NO_5$: C, 54.77; H, 6.27; N, 5.81%. Found: C, 54.63; H, 6.38; N, 5.65%.

Propan-1-aminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**3**)

Pale green crystal, yield 72%, mp: 188-189 ºC, ^1H NMR (DMSO-d_6, δ ppm): 8.35 (s, 3H), 7.41 (d, 1H, J = 15.6 Hz), 7.05 (d, 1H, J = 2.0 Hz), 6.82 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.9 Hz), 6.78 (d, 1H, J = 8.3 Hz), 6.24 (d, 1H, J = 15.5 Hz), 3.35 (t, 2H, J=7.0 Hz), 2.02 (m, 2H), 1.01 (t, 4H, J = 5.5 Hz). ESI MS: 240.1 $[M + H]^+$. Anal. Calc for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16; N, 5.85%. Found: C, 60.36; H, 7.32; N, 5.54%.

Propan-2-aminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**4**)

Pale yellow crystal, yield 62%, mp: 187-188 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.93 (s, 3H), 7.32 (d, 1H, J = 15.5 Hz), 7.12 (d, 1H, J = 2.1 Hz), 6.80 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.9 Hz), 6.75 (d, 1H, J = 8.4 Hz), 6.22 (d, 1H, J = 15.5 Hz), 4.05 (q, 1H, J = 6.7 Hz), 1.25 (d, 6H, J = 6.5 Hz). ESI MS: 240.1 $[M + H]^+$. Anal. Calc for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16; N, 5.85%. Found: C, 60.61; H, 7.36; N, 5.65%.

Butan-1-aminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**5**)

Pale yellow crystal, yield 73%, mp: 188-189 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.83 (s, 3H), 7.23 (d, 1H, J = 14.7 Hz), 7.01 (d, 1H, J = 2.0 Hz), 6.76 (dd, 1H, J_1 = 8.2 Hz, J_2 = 1.8 Hz), 6.66 (d, 1H, J = 8.1 Hz), 6.15 (d, 1H, J = 15.1 Hz), 2.96 (t, 2H, J = 7.0 Hz), 1.41-2.05 (m, 4H), 0.92 (t, 3H, J = 5.2 Hz). ESI MS: 254.1 $[M + H]^+$. Anal. Calc for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53%. Found: C, 61.16; H, 7.62; N, 5.61%.

Benzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**6**)

Yellow crystal, yield 76%, mp: 200-201 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.67 (s, 3H), 7.41-7.50 (m, 5H), 7.26 (d, 1H, J = 14.9 Hz), 6.93 (d, 1H, J = 2.0 Hz), 6.74 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 6.59 (d, 1H, J = 8.0 Hz), 6.19 (d, 1H, J = 15.1 Hz). ESI MS: 274.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13%. Found: C, 65.63; H, 5.62; N, 5.16%.

2-Fluorobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**7**)

Pale yellow crystal, yield 72%, mp: 207-208 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.71 (s, 3H), 7.62 (m, 1H), 7.31-7.39 (m, 3H), 7.21 (d, 1H, J = 14.8 Hz), 6.91 (d, 1H, J = 2.0 Hz), 6.69 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 6.61 (d, 1H, J = 7.8 Hz), 6.18 (d, 1H, J = 15.0 Hz). ESI MS: 292.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{14}FNO_4$: C, 61.85; H, 4.84; F, 6.52, N, 4.81%. Found: C, 61.68; H, 5.01; F, 6.42; N, 4.91%.

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2-Chlorobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**8**)

Yellow powder, yield 75%, mp: 215-216 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.66 (s, 3H), 7.41-7.55 (m, 4H), 7.32 (d, 1H, J = 15.0 Hz), 6.94 (d, 1H, J = 2.0 Hz), 6.72 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.1 Hz), 6.64 (d, 1H, J = 8.0 Hz), 6.21 (d, 1H, J = 15.1 Hz). ESI MS: 308.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{14}ClNO_4$: C, 58.55; H, 4.59; Cl, 11.52, N, 4.55%. Found: C, 58.82; H, 4.90; Cl, 11.44; N, 4.51%.

2-Bromobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**9**)

Yellow powder, yield 61%, mp: 208-209 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 8.06 (s, 3H), 7.62 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.3 Hz), 7.43 (m, 2H), 7.35 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.0 Hz), 7.21 (d, 1H, J = 14.8 Hz), 6.91 (d, 1H, J = 1.9 Hz), 6.70 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.1 Hz), 6.61 (d, 1H, J = 8.1 Hz), 6.22 (d, 1H, J = 15.0 Hz). ESI MS: 352.0 $[M + H]^+$. Anal. Calc for $C_{15}H_{14}BrNO_4$: C, 51.16; H, 4.01; Br, 22.69, N, 3.98%. Found: C, 51.29; H, 4.10; Cl, 22.35; N, 4.08%.

4-Fluorobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**10**)

Pale yellow crystal, yield 74%, mp: 199-200 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.68 (s, 3H), 7.34-7.44 (m, 4H), 7.17 (d, 1H, J = 15.5 Hz), 6.86 (d, 1H, J = 2.0 Hz), 6.68 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 6.57 (d, 1H, J = 8.0 Hz), 6.21 (d, 1H, J = 15.2 Hz). ESI MS: 292.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{14}FNO_4$: C, 61.85; H, 4.84; F, 6.52, N, 4.81%. Found: C, 61.68; H, 4.80; F, 6.75; N, 4.97%.

4-Chlorobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**11**)

Pale yellow powder, yield 76%, mp: 195-196 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.71 (s, 3H), 7.44-7.55 (m, 4H), 7.19 (d, 1H, J = 15.0 Hz), 6.89 (d, 1H, J = 2.1 Hz), 6.69 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.0 Hz), 6.58 (d, 1H, J = 8.0 Hz), 6.24 (d, 1H, J = 15.1 Hz). ESI MS: 308.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{14}ClNO_4$: C, 58.85; H, 4.59; Cl, 11.52, N, 4.55%. Found: C, 58.78; H, 4.23; Cl, 11.74; N, 4.35%.

4-Bromobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**12**)

Yellow powder, yield 73%, mp: 206-207 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.85 (s, 3H), 7.64 (d, 2H, J = 7.9 Hz), 7.42 (d, 2H, J = 8.0 Hz), 7.16 (d, 1H, J = 15.0 Hz), 6.84 (d, 1H, J = 2.0 Hz), 6.63 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 6.55 (d, 1H, J = 8.0 Hz), 6.21 (d, 1H, J = 15.0 Hz). ESI MS: 352.0 $[M + H]^+$. Anal. Calc for $C_{15}H_{14}BrNO_4$: C, 51.16; H, 4.01; Br, 22.69, N, 3.98%. Found: C, 51.52; H, 4.31; Br, 22.75; N, 3.39%.

2,4-Difluorobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**13**)

Yellow crystal, yield 75%, mp: 208-209 ºC,

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^{1}H NMR (DMSO-d_6, δ ppm): 7.92 (s, 3H), 7.42 (d, 1H, J = 8.1 Hz), 7.21 (d, 1H, J = 14.8 Hz), 7.11 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 6.90 (d, 1H, J = 1.9 Hz), 6.81 (d, 1H, J = 8.1 Hz), 6.71 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.9 Hz), 6.59 (d, 1H, J = 7.8 Hz), 6.22 (d, 1H, J = 14.9 Hz). ESI MS: 310.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{13}F_2NO_4$: C, 58.25; H, 4.24; F, 12.29, N, 4.53%. Found: C, 51.36; H, 4.11; F, 12.42; N, 4.16%.

2,4-Dichlorobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**14**)

White powder, yield 78%, mp: 200-201 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 8.02 (s, 3H), 7.62 (d, 1H, J = 8.0 Hz), 7.51 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.9 Hz), 7.40 (d, 1H, J_1 = 8.0 Hz), 7.24 (d, 1H, J = 15.0 Hz), 6.85 (d, 1H, J = 2.0 Hz), 6.69 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.9 Hz), 6.58 (d, 1H, J = 7.9 Hz), 6.20 (d, 1H, J = 15.1 Hz). ESI MS: 342.0 $[M + H]^+$. Anal. Calc for $C_{15}H_{13}Cl_2NO_4$: C, 52.65; H, 3.83; Cl, 20.72, N, 4.09%. Found: C, 52.73; H, 3.52; Cl, 20.48; N, 4.25%.

2,4-Dibromobenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**15**)

Pale yellow crystal, yield 77%, mp: 207-208 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 8.05 (s, 3H), 7.91 (d, 1H, J = 8.1 Hz), 7.65 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 7.32 (d, 1H, J_1 = 8.1 Hz), 7.21 (d, 1H, J = 15.0 Hz), 6.81 (d, 1H, J = 2.1 Hz), 6.64 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 6.57 (d, 1H, J = 8.0 Hz), 6.16 (d, 1H, J = 15.0 Hz). ESI MS: 429.9 $[M + H]^+$. Anal. Calc for $C_{15}H_{13}Br_2NO_4$: C, 41.79; H, 3.04; Br, 37.07, N, 3.25%. Found: C, 41.61; H, 3.41; Br, 37.42; N, 3.02%.

4-Methylbenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**16**)

Pale yellow powder, yield 75%, mp: 205-206 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.73 (s, 3H), 7.34-7.42 (m, 4H), 7.12 (d, 1H, J = 15.0 Hz), 6.81 (d, 1H, J = 2.1 Hz), 6.62 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.1 Hz), 6.52 (d, 1H, J = 7.9 Hz), 6.17 (d, 1H, J = 15.0 Hz), 2.12 (s, 3H). ESI MS: 288.1 $[M + H]^+$. Anal. Calc for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88%. Found: C, 66.57; H, 5.32; N, 5.09%.

Phenylmethanaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**17**)

White powder, yield 86%, mp: 199-200 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.24-7.38 (m, 5H), 7.27 (d, 1H, J = 15.2 Hz), 6.99 (d, 1H, J = 1.8 Hz), 6.91 (dd, 1H, J_1 = 8.2 Hz, J_2 = 1.8 Hz), 6.74 (d, 1H, J = 8.2 Hz), 6.16 (d, 1H, J = 15.7 Hz), 3.80 (s, 2H). ESI MS: 288.1 $[M + H]^+$. Anal. Calc for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88%. Found: C, 67.02; H, 5.61; N, 5.01%.

3,5-Dimethylbenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**18**)

Bottle green crystal, yield 77%, mp: 201-202 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.68 (s, 3H), 7.48 (t, 1H, J = 2.0 Hz), 7.25 (d, 1H, J = 15.1 Hz), 7.12 (d, 2H, J = 2.0 Hz), 6.97 (d, 1H, J = 1.9 Hz), 6.88 (dd, 1H, J_1 = 8.1 Hz, J_2 = 1.9 Hz), 6.72 (d, 1H, J = 8.1 Hz), 6.14 (d, 1H, J = 15.4 Hz), 2.32 (s,

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6H). ESI MS: 302.1 $[M + H]^+$. Anal. Calc for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65%. Found: C, 67.54; H, 6.62; N, 4.72%.

4-Isopropylbenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**19**)

Brown crystal, yield 74%, mp: 200-201 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.72 (s, 3H), 7.32-7.45 (m, 4H), 7.28 (d, 1H, J = 15.0 Hz), 6.99 (d, 1H, J = 2.0 Hz), 6.90 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 6.75 (d, 1H, J = 8.0 Hz), 6.16 (d, 1H, J = 15.2 Hz), 2.92 (q, 1H, J = 5.8 Hz), 1.12 (d, 6H, J = 5.5 Hz). ESI MS: 316.2 $[M + H]^+$. Anal. Calc for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44%. Found: C, 68.34; H, 6.38; N, 7.01%.

Naphthalen-1-aminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**20**)

Purple crystal, yield 72%, mp: 210-211 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.52-8.04 (m, 7H), 7.25 (d, 1H, J = 14.9 Hz), 6.96 (d, 1H, J = 2.0 Hz), 6.86 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.9 Hz), 6.71 (d, 1H, J = 8.0 Hz), 6.12 (d, 1H, J = 15.0 Hz). ESI MS: 324.1 $[M + H]^+$. Anal. Calc for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33%. Found: C, 70.35; H, 5.42; N, 4.25%.

Diethylammonium (E)-3-(3,4-dihydroxyphenyl)acrylate (**21**)

Pale yellow crystal, yield 71%, mp: 198-199 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.45 (s, 2H), 7.22 (d, 1H, J = 15.4 Hz), 6.96 (d, 1H, J = 2.0 Hz), 6.86 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 6.70 (d, 1H, J = 8.0 Hz), 6.12 (d, 1H, J = 15.0 Hz), 3.21 (m, 4H), 1.31 (m, 6H). ESI MS: 324.1 $[M + H]^+$. Anal. Calc for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53%. Found: C, 61.51; H, 7.33; N, 5.85%.

Dipropylammonium (E)-3-(3,4-dihydroxyphenyl)acrylate (**22**)

White powder, yield 80%, mp: 190-191 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.30 (s, 2H), 7.21 (d, 1H, J = 15.2 Hz), 6.94 (d, 1H, J = 2.0 Hz), 6.85 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 6.70 (d, 1H, J = 8.2 Hz), 6.14 (d, 1H, J = 15.3 Hz), 3.25 (m, 4H), 2.15 (m, 4H), 1.02 (m, 6H). ESI MS: 324.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98%. Found: C, 64.50; H, 8.05; N, 5.14%.

Diisopropylammonium (E)-3-(3,4-dihydroxyphenyl)acrylate (**23**)

White powder, yield 83%, mp: 191-192 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.54 (s, 2H), 7.20 (d, 1H, J = 15.9 Hz), 6.95 (d, 1H, J = 2.0 Hz), 6.84 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 6.71 (d, 1H, J = 8.0 Hz), 6.13 (d, 1H, J = 15.9 Hz), 3.10 (m, 2H), 1.10 (t, 12H, J = 6.3 Hz). ESI MS: 254.1 $[M + H]^+$. Anal. Calc for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98%. Found: C, 64.36; H, 8.44; N, 4.61%.

Dibutylammonium (E)-3-(3,4-dihydroxyphenyl)acrylate (**24**)

White powder, yield 75%, mp: 199-200 ºC, ^1H NMR (DMSO-d_6, δ ppm): 7.42 (s, 2H), 7.21 (d,

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1H, J = 15.3 Hz), 6.96 (d, 1H, J = 2.0 Hz), 6.86 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 6.72 (d, 1H, J = 8.1 Hz), 6.15 (d, 1H, J = 15.2 Hz), 3.41 (m, 4H), 2.15 (m, 4H), 1.42 (m, 4H), 1.02 (m, 6H). ESI MS: 310.2 $[M + H]^+$. Anal. Calc for $C_{17}H_{27}NO_4$: C, 65.99; H, 8.80; N, 4.53%. Found: C, 65.62; H, 8.55; N, 4.72%.

N-Methylbenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**25**)

Pale red crystal, yield 75%, mp: 187-188 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.82 (s, 2H), 7.41-7.55 (m, 5H), 7.21 (d, 1H, J = 15.6 Hz), 6.95 (d, 1H, J = 2.1 Hz), 6.87 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 6.71 (d, 1H, J = 8.1 Hz), 6.16 (d, 1H, J = 15.5 Hz), 2.94 (s, 3H). ESI MS: 288.1 $[M + H]^+$. Anal. Calc for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88%. Found: C, 66.65; H, 5.62; N, 5.04%.

Diphenylammonium (E)-3-(3,4-dihydroxyphenyl)acrylate (**26**)

Pale yellow crystal, yield 71%, mp: 200-201 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.81 (s, 2H), 7.43-7.55 (m, 10H), 7.23 (d, 1H, J = 15.2 Hz), 6.96 (d, 1H, J = 2.1 Hz), 6.88 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.0 Hz), 6.68 (d, 1H, J = 8.1 Hz), 6.15 (d, 1H, J = 15.2 Hz). ESI MS: 350.1 $[M + H]^+$. Anal. Calc for $C_{16}H_{17}NO_4$: C, 72.19; H, 5.48; N, 4.01%. Found: C, 72.56; H, 5.65; N, 3.96%.

Triethylammonium (E)-3-(3,4-dihydroxyphenyl)acrylate (**27**)

White powder, yield 66%, mp: 195-196 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.34 (s, 1H), 7.21 (d, 1H, J = 15.8 Hz), 6.97 (d, 1H, J = 2.1 Hz), 6.86 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 6.69 (d, 1H, J = 8.2 Hz), 6.16 (d, 1H, J = 15.6 Hz), 3.21 (m, 6H), 1.38 (m, 9H). ESI MS: 282.2 $[M + H]^+$. Anal. Calc for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98%. Found: C, 64.42; H, 8.48; N, 4.71%.

N,N-Dimethylbenzenaminium (E)-3-(3,4-dihydroxyphenyl)acrylate (**28**)

Pale yellow crystal, yield 76%, mp: 199-200 ºC, ^{1}H NMR (DMSO-d_6, δ ppm): 7.82-7.98 (m, 5H), 7.21 (d, 1H, J = 15.8 Hz), 6.96 (d, 1H, J = 2.0 Hz), 6.88 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 6.71 (d, 1H, J = 8.2 Hz), 6.17 (d, 1H, J = 15.8 Hz), 3.02 (s, 6H). ESI MS: 302.1 $[M + H]^+$. Anal. Calc for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65%. Found: C, 67.64; H, 6.08; N, 4.91%.

Antibacterial activity

The antibacterial activities of the synthesized compounds were tested against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525 and *Staphylococcus aureus* ATCC 6538 using MH medium. The MICs of the test compounds were determined by a colorimetric method using the dye MTT [8]. A stock solution of the synthesized compounds (50 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified

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quantity of the medium containing the test compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtimicrotitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 ºC for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 lL of PBS containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm.

RESULTS AND DISCUSSION

Chemistry

A series of water-soluble caffeic acid ammonium salts (compounds **1**–**28**) as potential antibacterial agents were synthesized by acid-base neutralization (Scheme 1). Except compound **21**, all the compounds were first reported. These compounds gave satisfactory elementary analyses (± 0.4%). ^{1}H NMR and ESI MS spectra were consistent with the assigned structures. Compound **1** was caffeic acid ammonium salt. Compounds **2**–**20** were caffeic acid primary ammonium salts. Compounds **21**–**26** were caffeic acid secondary ammonium salts. Compounds **27** and **28** were caffeic acid tertiary ammonium salts.

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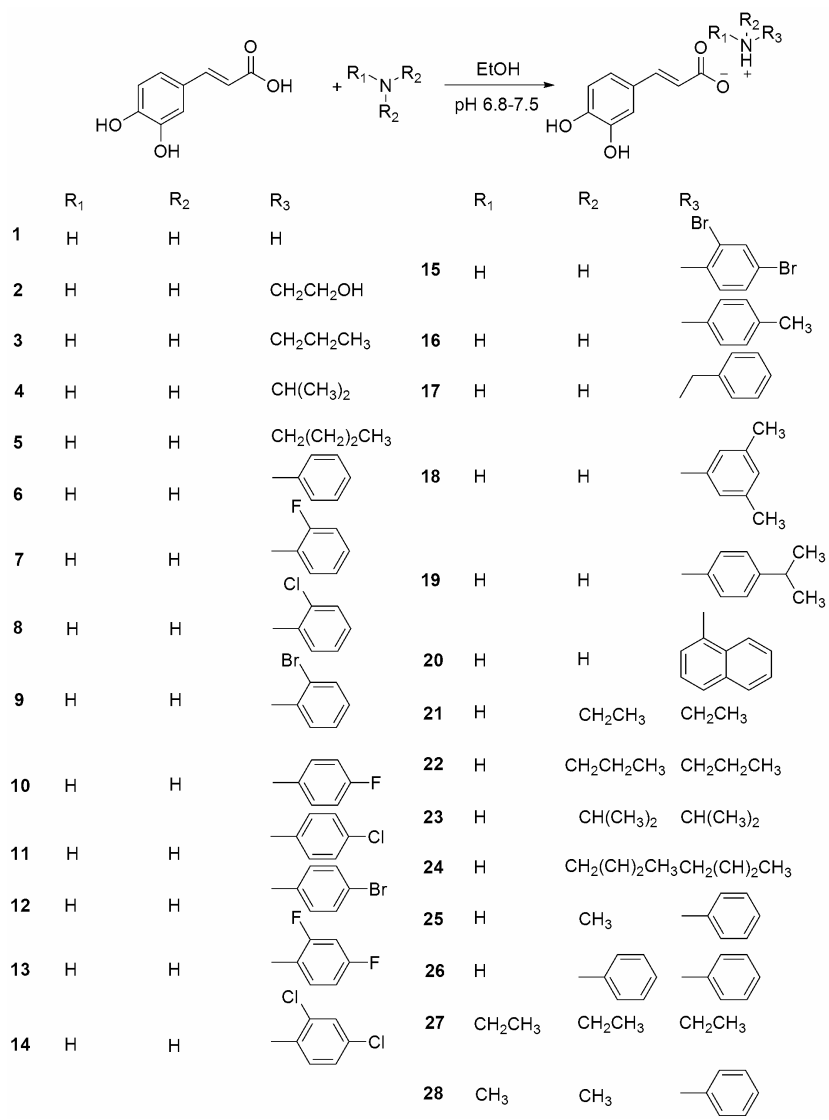

Scheme 1. Synthesis of caffeic acid ammonium salts.

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Antibacterial activity

All the compounds were evaluated for their antibacterial activities against four bacteria (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525 and *Staphylococcus aureus* ATCC 6538) and the results are shown in Table 1. The MIC values of the compounds differed greatly, ranging from 4.1 to 50 μg/mL. Compound **24** and **27** showed significant activity against *Bacillus subtilis* (4.1 μg/mL) while compounds **3**, **5**–**7**, **21**, **22** and **28** exhibited mild to moderate activity (13.0–46.7 μg/mL). Compounds **3**, **8**, **11**, **14**, **16**, **18**, **21**, **24**, **27** and **28** exhibited mild to moderate activity (12.1–47.4 μg/mL) against *Pseudomonas fluorescens*. Compound **6** showed highest activity against *Staphylococcus aureus* (7.5 μg/mL) while compounds **3**, **5**, **17**, **18**, **21**, **24** and **27** exhibited moderate activity (34.7–42.8 μg/mL). However, except compound **27**, no compound showed significant inhibition activity against *Escherichia coli*.

Some studies [9, 10] have reported that Gram-positive bacteria present higher sensitivity than Gram-negative bacteria to various polyphenols. This higher resistance can be explained on the basis of the different composition of the cell-wall membrane. Our results showed that the resistance of the tested bacteria supported this finding. The bacteria that were more sensitive were Gram-positive strain of *Bacillus cereus* followed by the Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas fluorescens*. The most resistant bacterium was Gram-negative *Escherichia coli*.

Compounds **3**, **7**, **21**, **24** and **27** showed higher antibacterial activity than lots of other compounds. This result disclosed that straight aliphatic ammonium salts were more active than branched aliphatic ammonium salts or aromatic ammonium salts. Moreover, compound **27** showed highest activity against the four bacterial strains, indicating that tertiary ammonium salts had higher antibacterial activity than primary and secondary ammonium salts. This founding is accordant with other report.[7] In addition, compounds **8**, **11** and **14** exhibited moderate activity against *Pseudomonas fluorescens*. This is probably related with the existence of chlorine atom. However, compound **6** was found to have highest activity against *Staphylococcus aureus* and the phenyl might play an important role.

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Table 1
Antibacterial activity of caffeic acid amine salts

Compound	Minimum inhibitory concentrations (μg/mL)			
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluorescens*	*Staphylococcus aureus*
1	>50	>50	>50	>50
2	>50	>50	>50	>50
3	21.2	>50	21.5	39.4
4	>50	>50	>50	>50
5	35.8	>50	>50	42.8
6	39.5	>50	>50	7.5
7	46.7	>50	>50	>50
8	>50	>50	39.2	>50
9	>50	>50	>50	>50
10	>50	>50	>50	>50
11	>50	>50	36.8	>50
12	>50	>50	>50	>50
13	>50	>50	>50	>50
14	>50	>50	25.6	>50
15	>50	>50	>50	>50
16	>50	>50	47.4	>50
17	>50	>50	>50	34.7
18	>50	>50	39.2	42.8
19	>50	>50	>50	>50
20	>50	>50	>50	>50
21	13.0	>50	12.1	39.4
22	23.5	>50	>50	>50
23	>50	>50	>50	>50
24	4.1	>50	15.4	39.4
25	>50	>50	>50	>50
26	>50	>50	>50	>50
27	4.1	45.2	12.1	36.9
28	29.6	>50	36.7	>50
Caffeic acid	>50	>50	>50	>50

Kanamycin	0.4	4.0	4.1	1.2
Penicillin	0.8	\	\	2.5

CONCLUSION

A series of water-soluble caffeic acid ammonium salts (**1**–**28**) were synthesized. To study the potential antibacterial activities of the synthesized compounds, screening experiments were performed for four bacteria strains. Compounds **6**, **24** and **27** showed considerable antibacterial activities. Generally, straight aliphatic ammonium salts were more active than branched aliphatic ammonium salts or caffeic acid aromatic ammonium salts and tertiary ammonium salts had higher antibacterial activity than primary and secondary ammonium salts.

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