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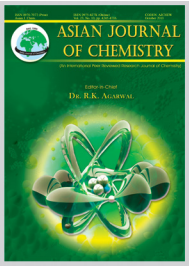

Antimicrobial Activities of the Cinnamoyl Amide of Amino Acid Derivatives

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Cinnamic acid derivatives are well known natural antimicrobial compounds. In present studies, 23 cinnamoyl amides of amino acid derivatives were synthesized and their antimicrobial activities against *Bacillus subtilis, Escherichia coli* and *Saccharomyces cerevisiae* were evaluated with benzoic acid as a reference. Most of the synthesized compounds were more active in inhibiting bacterial growth and all of them were more sensitive in controlling the growth of yeast *Saccharomyces cerevisiae* than cinnamic acid. Moreover, some of them such as cinnamoyl butyl glycinate(compound **4**) were more active than benzoic acid. The pH value influence on the antimicrobial activities of compound **4** was also investigated. Compared to benzoic acid, compound **4** was much more active when pH value was up to 7 and 7.5. These results gives us useful information for food preservatives.

Key Words: Antimicrobial, Cinnamic acid derivatives, Amino acid, Cinnamoyl amide.

INTRODUCTION

Cinnamic acid, hydroxy cinnamic acid (caffeic acid, ferulic acid, *p*-coumaric acid) and their ester, amide derivatives are widely distributed in plants and found in considerable amounts in propolis, fruits, vegetables and beverages of human diet[1-4]. It has been demonstrated that some cinnamic acid derivative, especially hydroxy cinnamate, including caffeic acid, *p*-coumaric acid and ferulic acid, have a wide range of therapeutical importance, such as, antioxidant activities[5-8], antitumor activities[9,10], antimelanogenic activities[11], hepato-protective activities[12], antiinflammatory activities[13], antiviral activities[14,15], antibacterial and fungicidal activities[16-25]. As one important kind of these derivatives, hydroxycinnamoyl amide of L-amino acids, namely, N-phenylpropenoyl-L-amino acids, has been recently identified as polyphenol/amino acid conjugates in plant constituent compounds such as in cococa, *etc.*[26-33]. Recently some new *trans*-cinnamic acid hydrazide derivatives were synthesized and some of them show excellent antimycobacterial activities[34]. Moreover, cinnamic acid modified peptides show potent activity against various gram-positive and gram-negative bacteria[35].

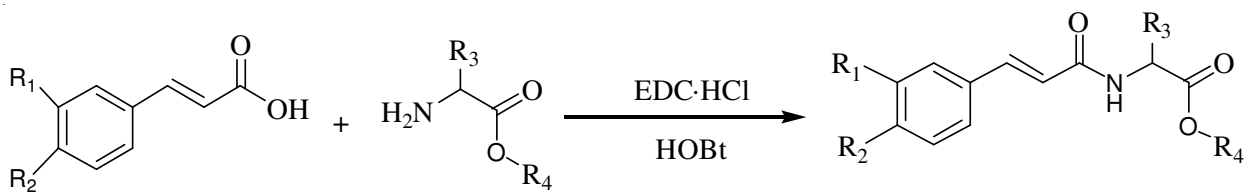
Food spoilage is an important safety issue for food processors and consumers and the usage of synthetic chemical food preservatives is one of the most common ways to control microbial growth. Recently, however, great concern has been raised about the negative effects among consumers and people expressed a strong desire for the reduction of chemical preservatives for preventing and controlling pathogenic microorganisms in foods[36], which have attracted researchers and food processors to explore natural food additives with a broad spectrum of antimicrobial activities[37].

Keeping this in mind, we have been interested in looking for new cinnamic acids analogues as food preservatives. Here, the synthesis of a series of cinnamoyl amide of L-amino acids derivatives as well as the results for evaluation of the antimicrobial activities of the synthesized compounds is reported.

EXPERIMENTAL

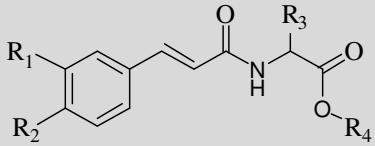
Methanol, cinnamic acid, caffeic acid, ferulic acid, *p*-coumaric acid, L-amino acids, potassium hydroxide, sodium phosphate and benzoic acid were obtained from Shanghai Reagent Co. IR spectra were recorded on a Avatar 330 infrared spectrophotometer (KBr, ν_{max}, cm^{-1}). ^1H NMR data were acquired at room temperature on a Bruker AV 400-MHz operating at 400 MHz. $CDCl_3$ or DMSO-d_6 was used as solvent; chemical shifts are expressed in δ (parts per million) values relative to tetramethylsilane (TMS) as internal reference. A Spectronic Genesys 8 UV/VIS spectrophotometer was used to record the UV.

General procedure for the syntheses of cinnamoyl amide of L-amino acid ester: The synthetic route used for the preparation of the title compounds is outlined in **Scheme-I**. The structure of all the synthesized compounds is given in Table-1.



Scheme-I: Synthetic route for the preparation of the title compounds

TABLE-1
FORMATION OF THE SYNTHESIZED COMPOUNDS



Compd.	R_1	R_2	R_3	R_4
1	H	H	H	CH_3
2	H	H	H	C_2H_5
3	H	H	H	*n*-C_3H_8
4	H	H	H	*n*-C_4H_9
5	OH	OH	H	C_2H_5
6	OCH_3	OH	H	C_2H_5
7	H	OH	H	C_2H_5
8	H	H	CH_3	CH_3
9	H	H	CH_3	C_2H_5
10	H	H	CH_3	*n*-C_3H_8
11	H	H	$CH_3(CH_3)CHCH_2$	CH_3
12	H	H	$CH_3(CH_3)CHCH_2$	*n*-C_3H_8
13	H	H	$CH_3(CH_3)CHCH_2$	*i*-C_3H_8
14	H	H	$CH_3CH_2(CH_3)CH$	CH_3
15	H	H	$CH_3CH_2(CH_3)CH$	C_2H_5
16	H	H	$CH_2C_6H_5$	CH_3
17	H	H	$CH_2C_6H_5$	C_2H_5
18	H	OH	$CH_2C_6H_5$	C_2H_5
19	OCH_3	OH	$CH_2C_6H_5$	C_2H_5
20	OH	OH	$CH_2C_6H_5$	C_2H_5
21	H	H	$CH_2CH_2SCH_3$	C_2H_5
22	H	H	$CH_3(CH_3)CH$	C_2H_5
23	OCH_3	OH	$CH_3(CH_3)CH$	C_2H_5

The synthesis method was exemplified by the preparation of compound **5**. To a solution of caffeic acids 0.99 g (5.5 mmol) in THF (15 mL, cooled to 0 ºC) was added successively ethyl glycinate hydrochlorides 0.75 g (5.5 mmol) and solution of triethylamine (0.56 g, 5.6 mmol) in THF (5 mL), 1-hydroxybenzotriazole (HOBt, 0.74 g, 5.5 mmol) in THF (10 mL) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride 1.05 g, (EDC·HCl, 5.5 mmol) in THF (10 mL). After stirring for 1 h at 0 ºC, the mixture was warmed to room temperature and stirred for 18 h. After filtration of insoluble material from the mixture, 200 mL water was added into the filtrate and then extracted with ethyl acetate (3 × 150 mL). The ethyl acetate solution was washed with water, 10 % aqueous solution of $NaHCO_3$ and finally, with 20 % brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether = 9:1) to provide caffeoyl amide of ethyl glycinate (compound **5**). Others compounds were synthesized by the similar way to compound **5**.

Spectral data of the newly synthesized compounds were listed as following and the data for known compounds were listed in the supporting information. About 23 cinnamoyl amides of amino acid were produced and their antimicrobial activities were evaluated.

Cinnamoyl amide of butyl glycinate (compound 4): Yield 83.6 %, pale yellow solid, m.p. 93-94 ºC. ^{1}H NMR (400 MHz, $CDC1_3$), 7.662 (d, 1H, *J* = 15.6, ph-CH=), 7.519-7.499 (m, 2H, Ar-H), 7.379-7.365 (m, 3H, Ar-H), 6.458 (d, 1H, *J* = 15.6, C=CH-CO), 6.054 (brs, 1H, -NH), 4.820 (m, 2H, CH_2), 4.175 (t, 2H, *J* = 6.8, -OCH_2), 1.670-1.593 (m, 2H, -OCH_2CH_2), 1.460-1.369 (m, 2H, -CH_2-CH_3), 0.996-0.928 (t, 3H, *J* = 13.6, -CH_3). IR (KBr, ν_{max}, cm^{-1}): 3382 (N-H), 3060 (Ar-H), 3027 (C=C-H), 1768 (C=O), 1701 (O=C-N), 1632 (C=C), 1208 (C-O-C). MS (m/z, %): 261.9 ($M^{+\cdot}$, 100).

Caffeoyl amide of ethyl glycinate (compound 5): Yield 95.4 %, pale yellow solid, m.p. 170-171 ºC, ^{1}H NMR (400 MHz, DMSO-d_6), 9.415 (S, 1H, OH), 9.170 (S, 1H, OH), 8.414 (br s, 1H, NH), 7.282 (d, 1H, *J* = 15.6, ph-HC=C), 6.964 (S, 1H, ArH), 6.870 (d, 1H, *J* = 8.0, ArH), 6.757 (d, 1H, *J* = 8.0, ArH), 6.419 (d, 1H, *J* = 15.6, C=CH), 4.113 (d, 2H, *J* = 6.8, CH_2), 3.930-3.946 (qd, 2H, *J* = 6.8, -OCH_2), 1.217-1.181 (t, 3H, *J* = 7.6, CH_3). IR (KBr, ν_{amx}, cm^{-1}): 3503 (HO-), 3365 (N-H), 3046 (Ar-H), 3027 (C=C-H), 1728 (C=O), 1654 (O=C-N), 1605 (C=C). MS (m/z, %): 264.7 ($M^{+\cdot}$, 75.0), 219 (76.5), 55 (100).

Feruloyl amide of ethyl glycinate (compound 6): Yield 91.3 %, pale yellow solid, m.p. 154-155 ºC, ^{1}H NMR (400 MHz, $CDCl_3$), 7.595 (d, 1H, *J* = 15.6, ph-HC=C), 7.083 (d, 1H, *J* = 8.0, ArH), 7.012 (S, 1H, ArH), 6.924 (d, 1H, *J* = 8.0, ArH), 6.329 (d, 1H, *J* = 15.6, C=CH), 6.118 (br s, 1H, NH), 5.877 (br s, 1H, OH), 4.270-4.252 (m, 2H, *J* = 7.2, -CH_2), 4.181 (d, 2H, *J* = 5.2, -CH_2), 3.9725 (s, 3H, OCH_3), 1.328-1293 (t, 3H, *J* = 7.2, -CH_2CH_3). IR (KBr, ν_{max}, cm^{-1}): 3503 (HO-), 3365 (N-H), 3046 (Ar-H), 3027 (C=C-H), 1728 (C=O), 1654 (O=C-N), 1605 (C=C). MS (m/z, %): 278 ($M^{+\cdot}$, 100.0).

***p*-Coumaroyl amide of ethyl glycinate (compound 7):** Yield 86.5 %, white solid, m.p. 169-170 ºC, ^{1}H NMR (400 MHz, $CDCl_3$), 7.609 (d, 1H, *J* = 15.6, ph-HC=C), 7.410 (d, 2H, *J* = 8.4, ArH), 6.849 (d, 2H, *J* = 8.4, ArH), 6.329 (d, 1H, *J* = 15.6, C=CH), 6.118 (br s, 1H, NH), 4.270-4.252 (d, 2H, *J* = 7.2, -CH_2), 4.181 (d, 2H, *J* = 5.2, CH_2), 1.328-1.292 (t, 3H, *J* = 7.2, -CH_3). IR (KBr, ν_{max}, cm^{-1}): 3503 (HO-), 3365 (N-H), 3046 (Ar-H), 3027 (C=C-H), 1728 (C=O), 1654 (O=C-N), 1605 (C=C), 1210. MS (m/z, %): 249 ($(M)^{+\cdot}$, 100.0).

Cinnamoyl amide of ethyl isoleucinate (compound 15): Yield 95.4 %, white solid, m.p. 92-92.5 ºC. ^{1}H NMR (400 MHz, $CDCl_3$), 7.663 (d, 1H, *J* = 15.6, phCH=), 7.522-7.502 (m, 2H, ArH), 7.398-7.359 (m, 3H, ArH), 6.489 (d, 1H, *J* = 15.6, C=CH), 6.229 (brs, 1H, -NH), 4.786-4.736 (qd, 1H, *J* = 4.8, -CH), 4.261-4.198 (m, 2H, *J* = 15.6, -OCH_2), 1.973-1.945 (m, 1H, -$CHCH_3$), 1.532-1.199 (m, 2H, -CH_2CH_3), 0.973-0.889

(m, 9H, -CH_3). IR (KBr, ν_{max}, cm^{-1}): 3292 (N-H), 3061 (Ar-H), 3034 (C=C-H), 1737 (C=O), 1656 (O=C-N), 1624 (C=C), 1194 (C-O-C). MS (m/z, %): 290.0 ($M^{+\cdot}$, 100.0).

***p*-Coumaroyl amide of ethyl phenylalaninate (compound 18):** Yield 94.2 %, white solid, m.p. 129-130 ºC, ^{1}H NMR (400 MHz, DMSO-d_6): 9.879 (S, 1H, OH), 8.444 (d, 1H, *J* = 7.6, NH), 7.395 (d, 2H, *J* = 8.4, ArH), 7.314 (d, 1H, *J* = 15.6, phCH=C), 7.269-7.210 (d, 5H, ArH), 6.794 (d, 2H, *J* = 8.4, ArH), 6.478 (d, 1H, *J* = 15.6, C=CH), 4.568 (d, 1H, *J* = 4.8, NCH), 4.081-4.028 (qd, 2H, *J* = 7.2, OCH_2), 3.075-2.927 (m, 2H, CH_2), 1.130-1.094 (t, 3H, *J* = 7.2, CH_3). IR (KBr, ν_{max}, cm^{-1}): 3503 (HO-), 3365 (N-H), 3046 (Ar-H), 3027 (C=C-H), 1728 (C=O), 1654 (O=C-N), 1605 (C=C). MS (m/z, %): 339.8 ($M^{+\cdot}$, 32.0), 129.9 (100.0).

Feruloyl amide of ethyl phenylalaninate (compound 19): Yield 87.3 %, pale yellow solid, m.p. 154-155 ºC, ^{1}H NMR (400 MHz, DMSO-d_6): 9.483 (S, 1H, OH), 8.411 (d, 1H, NH), 7.314-7.273 (m, 6H, HC=C, ArH), 7.119 (S, 1H, ArH), 6.996 (d, 1H, *J* = 8.0, ArH), 6.795 (d, 1H, *J* = 8.0, ArH), 6.521 (d, 1H, *J* = 15.6, C=CH), 4.600-4.554 (m, 1H, NCH), 4.085-4.032 (qd, 2H, *J* = 7.2, OCH_2), 3.801 (S, 3H, OCH_3), 3.065-2.929 (m, 2H, phCH_2), 1.135-1.099 (t, 3H, *J* = 7.2, CH_2CH_3). IR (KBr, ν_{max}, cm^{-1}): 3503 (HO-), 3365 (N-H), 3046 (Ar-H), 3027 (C=C-H), 1728 (C=O), 1654 (O=C-N), 1605 (C=C). MS (m/z, %): 369.0 ($M^{+\cdot}$, 43.0), 295.0 (80.0), 221.0 (100.0), 73 (95.3).

Caffeoyl amide of ethyl phenylalaninate (compound 20): Yield 90.6%, pale yellow solid, m.p. 167-168 ºC, ^{1}H NMR (400 MHz, DMSO-d_6): 9.430 (br s, 1H, OH), 9.208 (br s, 1H, OH), 8.414 (d, 1H, *J* = 7.6, NH), 7.288 (d, 1H, *J* = 15.6, phHC=C), 7.270-7.187 (m, 5H HC=C, ArH), 6.939 (S, 1H, ArH), 6.844 (d, 1H, *J* = 8.0, ArH), 6.748 (d, 1H, *J* = 8.0, ArH), 6.402 (d, 1H, *J* = 15.6, C=CH),4.580-4.524 (m, 1H, NCH), 4.089-4.070 (qd, 2H, *J* = 7.6, OCH_2), 3.073-2.924 (m, 2H, phCH_2), 1.128-1.093 (t, 3H, *J* = 7.6, CH_2CH_3). IR (KBr, ν_{max}, cm^{-1}): 3503 (HO-), 3365 (N-H), 3046 (Ar-H), 3027 (C=C-H), 1728 (C=O), 1654 (O=C-N), 1605 (C=C). MS (m/z, %): 355 ($M^{+\cdot}$, 81.2), 284.0 (28.3), 149.0 (65.7), 73 (100.0).

Cinnamoyl amide of ethyl methioninate (compound 21): Yield 84.7 %, white solid, m.p. 175-176 ºC. ^{1}H NMR (400 MHz, $CDCl_3$), 7.667 (d, 1H, *J* = 15.6, phCH=), 7.528-7.509 (m, 2H, Ar-H), 7.408-7.368 (m, 3H, Ar-H), 6.478 (d, 1H, *J* = 15.6, C=CH), 6.372 (br s, 1H, -NH), 4.881-4.832 (m, 1H, -NCH), 4.277-4.223 (qd, 2H, *J* = 7.2, -OCH_2), 2.630-2.504 (m, 2H, -SCH_2), 2.300-2.230 (m, 2H, -CH_2), 1.602 (S, 3H, -SCH_3), 1.333 (t, *J* = 7.2, 3H, -CH_2CH_3). MS (m/z, %): 308 ($M^{+\cdot}$, 100.0).

Feruloyl amide of ethyl valinate (compound 23): Yield 92.7 %, white solid, m.p. 79-80 ºC, ^{1}H NMR (600 MHz, $CDCl_3$), 7.573 (d, 1H, *J* = 15.6, phHC=C), 7.068 (d, 1H, *J* = 7.8, ArH), 7.009 (S, 1H, ArH), 6.915 (d, 1H, *J* = 7.8, ArH), 6.347 (d, 1H, *J* = 15.6, C=CH), 6.136 (br s, 1H, NH), 4.728-4.706 (m, 1H, NCH), 4.239 (qd, 2H, *J* = 6.0, OCH_2CH_3), 3.927 (S, 3H, OCH_3), 2.241-2.230 (m, 1H, $CHCH_3$), 1.316-1.292 (t, 3H, *J* = 4.2, OCH_2CH_3), 0.995-0.950 (m, 6H, $CHCH_3$). IR (KBr, ν_{max}, cm^{-1}): 3503 (HO-), 3365 (N-H), 3046 (Ar-H), 3027 (C=C-H), 1728 (C=O), 1654 (O=C-N), 1605 (C=C). MS (m/z, %): 322.0 ($M^{+\cdot}$, 100.0), 177(47.0).

Antimicrobial activities

General: Both Gram positive (*Bacillus subtilis* MTCC 441)) and Gram negative (*Escherichia coli* ATCC 25922) bacterial strains were employed for antibacterial assays. The yeast *Saccharomyces cerevisiae* ATCC 2365 was employed for antifungal activity evaluation. These microbial were presented by College of Biology, Huazhong Agricultural University, Wuhan, China. Commercial benzoic acid, well-known food additive for its antimicrobial properties, was used as comparison reference in all the following antimicrobial activities of prepared compounds. All the data collected for each assay are the averages of three determinations of independent experiments. Cultural media for yeast used in this study was Malt Agar, for the bacteria Nutrient Agar (Shanghai reagent co. China).

Antimicrobial activities: The standard disks diffusion technique was employed to determine the biological activity against these three microorganisms[38-40]. In brief, mother cultures of each microorganism were set up 24 h before the assays to reach the stationary phase of growth. Petri dishes were seeded with these microorganisms to concentration of 10^6 cfu (colony forming units)/mL and 10^5 cfu/mL for bacteria and yeast, respectively. Then, sterile Whatmann filter paper discs (10 mm diameter) were impregnated with the tested compounds solutions (200, 400, 600 mg/L methanol solutions) and dried at room temperature to remove any residual solvent. These discs were placed on plates containing agar media seeded with the test organisms. The petri dishes were then incubated at 37 ºC for bacterial and 28 ºC for yeast for 24 h, respectively. The antimicrobial activities were evaluated by the inhibition zone diameter (IZD) (subtract the diameter of the filter paper disc). All experiments were carried out in triplicate.

Effect of pH on the antimicrobial activity: Test compounds (compound **4**) were tested at concentration of 200, 400, 600 mg/L. The growth of microbial was estimated by determining the absorbance at 560 nm quantitatively[41]. Microorganisms were cultivated in liquid media (bacterial-nutrient broth; yeast-malt extract broth) supplemented with test compounds at various pH. The pH of cultures was adjusted to 4.0, 5.0, 6.0, 7.0 and 7.5 by addition of different pH buffer solutions at a concentration of 1 % (w/v). About 0.2 mL microbial solution (10^4-10^5 CFU/mL) was added to every conical flask with 100 mL liquid media at different pH with tested compounds. The inoculated medium without test compound was used as control and the media with just tested compound was served as a blank. After shaking the plates for 15 s, the absorbance at 560 nm was determined after every 3 h during the incubation at 37 ºC for bacterial and 28 ºC for yeast for 24 h. All the analyses were performed in triplicate and the experimental data represent the average of them. The inhibitory index was calculated as following:

$$\text{Inhibition } (\%) = 100 - (\Delta A_t/\Delta A_c) \times 100$$

where, ΔA_t = absorbance of treated samples subtract blank sample, ΔA_c = absorbance of the control sample subtract blank sample.

RESULTS AND DISCUSSION

Antimicrobial activities of the tested compounds: The paper disc assay results are presented in Table-2 (the data for 600 mg/L were not provided in Table-2 for clarity). Based on inhibition zone diameter (IZD), compound **4**, **8**, **9**, **11**, **17** and **20** proved to be more effecttive against *Escherichia coli* than cinnamic acid at the concentration of 400 mg/L. Moreover, compound **4**, **6**, **7**, **9**, **14**, **16**, **17** showed higher antimicrobial activities to *Bacillus subtilis* than cinnamic acid at 400 mg/L. Therefore, compound **4**, **9**, **17** demonstrated higher activities on both *Escherichia coli* and *Bacillus subtilis* than cinnamic acid. On the other hand, all synthesized compounds were more active than cinnamic acid within the tested concentrations for yeast *Saccharomyces cerevisiae*, but showed different degrees of inhibition. As showed in Table-2, compound **4** showed to be the most effective among the synthesized compounds and the inhibition zone diameter was more than 8 mm against yeast Saccharomyces cerevisiae. The inhibition zone diameter, as expected, increased with increasing concentration in the tested concentration range. It is well known that *Bacillus subtilis* is Gram-positive bacteria and *Escherichia coli* is Gram-negative bacteria, *Saccharomyces cerevisiae* is yeast. So, it is obvious that cinnamoyl amides of amino acid ester had stronger effect against yeast than to bacteria. Compared to well-known benzoic acid, most of the compounds were less active in controlling the growth of the three microorganisms. However, benzoic acid is less effective than compound **4**, **9** on *Escherichia coli*, than compound **4**, **17** on *Bacillus subtilis* and than compound **4**, **6** on *Saccharomyces cerevisiae*. On the whole, compound **4** behavioured better than benzoic acid and was the most effective one among the all tested compounds for the three organisms.

Effect of pH on the antimicrobial activities: Figs. 1-3 showed the antimicrobial activities of benzoic acid and compound **4** on *Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae* after 24 h treatment at various pH at concentration of 200 mg/L (the data of them at different time at concentration of 400 and 600 mg/L on these three microorganism were not shown for clarity, but were provided in supporting information). Fig. 1 shows the antimicrobial activities of compound **4** and benzoic acid at pH of 4, 5, 6, 7, 7.5 after incubation of 24 h at concentration of 200 mg/mL against *Escherichia coli*. The inhibitory rates of benzoic acid diminished from 50.9 to 16.2 %; while compound **4** from 44.3 to 27.4 % when pH raised from 4.0 to 7.5 (as shown in Fig. 1). Increase in antimicrobial activity shown in Fig. 1 against *Escherichia coli* were comparable to *Bacillus subtilis* (Fig. 2) and *Saccharomyces cerevisiae* (Fig. 3). It is obvious that the antimicrobial activities of both benzoic acid and compound **4** were higher at lower pH. However, the activity of benzoic acid decreased more rapidly than compound **4** with the increase of pH. As we can see, at pH 4 benzoic acid were more active than compound **4**, while at pH 7.5 benzoic acid were much less active than compound **4**. On one hand, at lower pH, it is adverse for the viability of the microorganisms, so at pH 4 both compound **4** and benzoic acid were more active than at pH 7.5. On the other hand, the undissociated molecules at different pH values have great influence on the antimicrobial

TABLE-2
ANTIMICROBIAL ACTIVITIES (INHIBITION ZONE DIAMETER, mm) OF TEST COMPOUNDS*

Compd.	*Escherichia coli* (mg/L)		*Bacillus subtilis* (mg/L)		*Saccharomyces cerevisiae* (mg/L)	
	200	400	200	400	200	400
1	2.1 ± 0.3	3.0 ± 0.3	0.5 ± 0.1	1.7 ± 0.2	2.0 ± 0.3	3.7 ± 0.2
2	1.5 ± 0.2	2.0 ± 0.3	1.0 ± 0.2	1.5 ± 0.4	3.0 ± 0.3	4.2 ± 0.2
3	0.8 ± 0.2	1.0 ± 0.1	1.5 ± 0.1	2.4 ± 0.3	3.5 ± 0.1	5.5 ± 0.4
4	5.5 ± 0.3	7.5 ± 0.4	14.2 ± 0.5	17.3 ± 0.7	8.2 ± 0.5	10.0 ± 0.3
5	1.5 ± 0.2	2.0 ± 0.3	1.7 ± 0.2	2.5 ± 0.4	1.5 ± 0.1	4.3 ± 0.3
6	0.5 ± 0.1	1.5 ± 0.2	2.0 ± 0.3	3.5 ± 0.2	6.0 ± 0.5	8.3 ± 0.4
7	1.6 ± 0.3	2.2 ± 0.2	2.5 ± 0.2	3.7 ± 0.3	5.8 ± 0.4	6.3 ± 0.5
8	3.5 ± 0.2	4.0 ± 0.2	0.6 ± 0.1	1.3 ± 0.2	1.7 ± 0.2	4.1 ± 0.3
9	5.0 ± 0.2	7.1 ± 0.5	2.0 ± 0.2	3.5 ± 0.3	3.5 ± 0.3	5.3 ± 0.3
10	0.7 ± 0.1	1.5 ± 0.2	0.5 ± 0.1	2.0 ± 0.2	5.0 ± 0.3	5.5 ± 0.4
11	2.5 ± 0.3	3.2 ± 0.2	0.7 ± 0.1	1.2 ± 0.1	3.5 ± 0.2	5.2 ± 0.3
12	1.7 ± 0.2	2.7 ± 0.2	1.0 ± 0.1	1.2 ± 0.2	3.0 ± 0.2	4.3 ± 0.1
13	1.7 ± 0.1	3.0 ± 0.3	0.8 ± 0.1	1.2 ± 0.1	4.5 ± 0.3	5.5 ± 0.3
14	0.6 ± 0.1	1.1 ± 0.2	3.0 ± 0.1	4.5 ± 0.2	4.3 ± 0.2	5.4 ± 0.3
15	2.0 ± 0.2	2.5 ± 0.2	0.5 ± 0.1	1.0 ± 0.1	2.5 ± 0.2	3.7 ± 0.3
16	0.7 ± 0.1	2.2 ± 0.1	2.5 ± 0.2	4.2 ± 0.3	3.5 ± 0.2	5.0 ± 0.4
17	1.5 ± 0.2	3.5 ± 0.3	3.0 ± 0.2	6.5 ± 0.4	3.4 ± 0.2	5.3 ± 0.2
18	1.0 ± 0.1	1.5 ± 0.2	0.7 ± 0.1	1.2 ± 0.1	4.0 ± 0.3	6.5 ± 0.4
19	1.5 ± 0.2	2.0 ± 0.2	0.5 ± 0.1	0.8 ± 0.2	1.5 ± 0.2	3.2 ± 0.3
20	3.6 ± 0.3	5.7 ± 0.4	1.2 ± 0.1	2.0 ± 0.2	1.7 ± 0.2	2.8 ± 0.2
21	0.5 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	4.5 ± 0.3	5.6 ± 0.2
22	0.7 ± 0.1	1.5 ± 0.2	0.6 ± 0.1	1.0 ± 0.1	1.7 ± 0.2	3.3 ± 0.1
23	1.5 ± 0.1	2.2 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	2.0 ± 0.2	3.3 ± 0.2
Cinnamic acid	1.2 ± 0.1	2.5 ± 0.2	1.2 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	1.8 ± 0.1
Benzoic acid	3.5 ± 0.2	6.7 ± 0.4	4.5 ± 0.2	5.8 ± 0.3	4.6 ± 0.2	6.9 ± 0.3

*Antimicrobial activities were evaluated by measuring the inhibition zone diameter (IZD) of the tested microorganism *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*. The concentrations were 200, 400, 600 mg/L for tested compounds, for clarity, the data for 600 mg/L was not provided. IZD value = mean ± SD.

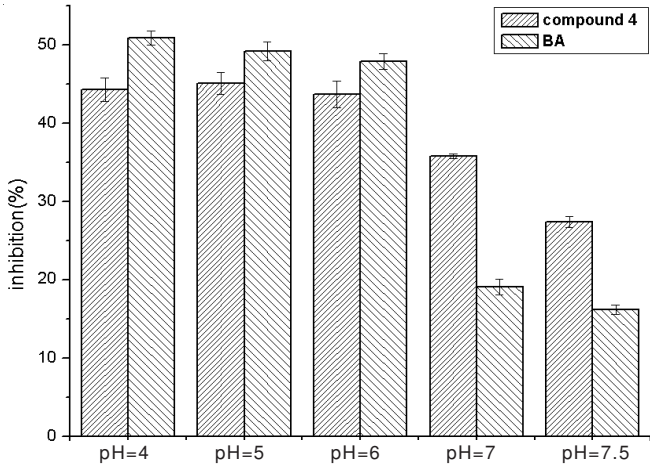


Fig. 1. Antimicrobial activities of compound **4** and benzoic acid at different pH values after 24 h incubation on *Escherichia coli* (BA is benzoic acid) the concentration of compound **4** and benzoic acid were 200 mg/mL

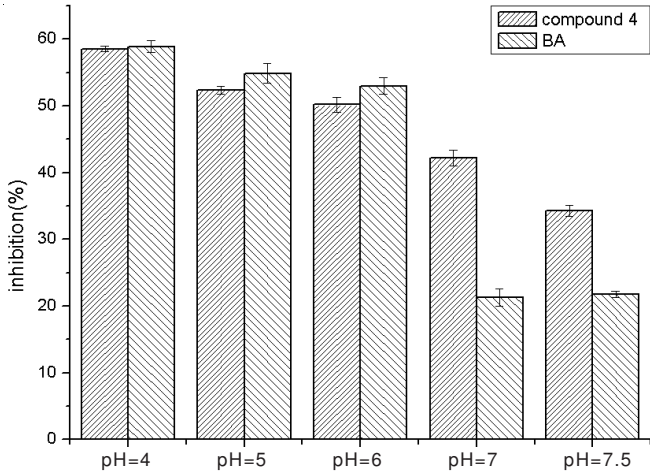


Fig. 2. Antimicrobial activities of compound **4** and benzoic acid at different pH values after 24 h incubation on *Bacillus subtilis* (BA is benzoic acid) the concentration of compound **4** and benzoic acid were 200 mg/mL

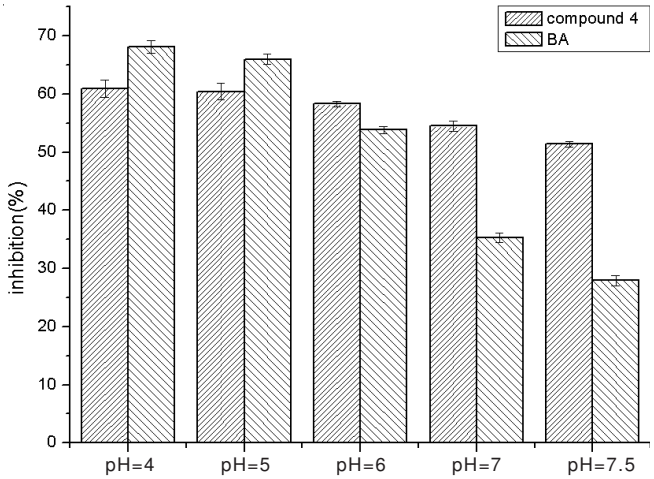


Fig. 3. Antimicrobial activities of compound **4** and benzoic acid at different pH values after 24 h incubation on *Saccharomyces cerevisiae* (BA is benzoic acid) the concentration of compound **4** and benzoic acid were 200 mg/mL

activity of benzoic acid. The undissociated form of acid is more hydrophobic than the dissociated one, making the undissociated acid more active[21,41]. The proportion of undissociated acid molecules is higher at lower pH and reduced at higher pH for benzoic acid, hence, the activities of benzoic acid decreased much more rapidly because it has a carboxylic acid group. For compound **4**, it has no free acid group, so pH can not make effect on its molecular existent form, so pH has less influence on its antimicrobial activities. In conclusion, compund **4** had a wider efficient pH region compared with benzoic acid.

Conclusion

Most of the cinnamoyl amide of amino acid derivatives showed good antimicrobial effects against the studied microorganisms (*Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae*) than cinnamic acid, especially, all of them were more active than cinnamic acid on yeast (*Saccharomyces cerevisiae*). Several compounds demonstrated better antimicrobial effects than benzoic acid; in particular compound **4** had higher activities in a broad pH value than benzoic acid. Moreover, the synthesized compounds are similar to natural equivalents, so as food antimicrobial additive, they are promising to be a good start point to further studies and These results gives us useful information for food preservaties design.

Supporting information description: All the known compounds are characterized by IR, MS, ^{1}HNMR, the confirmation data and the antimicrobial activities details are provided in supporting information.

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