British Journal of Pharmaceutical Research
4(4): 513-519, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial Screening of Some Synthesized Palmitoyl Amino Acids and Their Aromatic Analogues

Haruna Baba[1*], Cyril O. Usifoh[2] and Adebola Onanuga[3]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin city, Edo state, Nigeria.
[3]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author HB and COU designed and carried out the synthesis of the compounds and drafted the manuscript. Author AO carried out the antimicrobial screening. All authors read and approved the final manuscript.

Original Research Article

Received 20th July 2013
Accepted 22nd November 2013
Published 24th December 2013

ABSTRACT

Aims: The aims of the research were to synthesize, characterize some palmitoyl amino acids and their aromatic analogues and to screen the synthesized compounds for possible antibacterial activity
Study Design: Synthesis, characterization and antibacterial screening of palmitoyl amino acids and their aromatic analogues.
Place and Duration of Study: Department of Pharmaceutical and Medicinal Chemistry/ Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, between September 2012 and January 2013.
Methodology: Palmitoylchloride was condensed with the respective amino acids (glycine, β-alanine, γ-amino butyric acid) to form the corresponding palmitoyl amino acids. The opening of the isatoic anhydride ring with the three amino acids and subsequent condensation with palmitoyl chloride led to the formation of the amino acid benzamides. The antibacterial screening was carried out using agar well diffusion method.

**Corresponding author: Email: babharun@yahoo.co.uk;*

Results: All the synthesized compounds were obtained in good yield and high purity; they were unequivocally characterized by the combination IR, ^{1}H NMR, ^{13}C NMR and MS. The compounds were however found to possess no antibacterial activity against the tested microorganisms
Conclusion: This work has shown that both the straight chain and aromatic analogues of saturated long chain lipid amides are inactive against the tested strains of microorganism.

Keywords: Lipid amides; palmitoyl glycine; palmitoyl alanine; palmitoyl GABA; benzamides; antibacterial.

1. INTRODUCTION

Infectious disease is ranked among the leading cause of death even in the developed world [1]. Disease causing microbes are rapidly becoming more resistant to the best treatment available [2]. This has called for unrelenting effort towards the development of safe and efficacious medicines to combat the ever increasing problem of resistant organisms. Different groups of chemical agents are known to inhibit bacterial growth and quite a number of them are used clinically for the treatment of various forms of infections [3]. Fatty acids and their derivatives have also been investigated for antimicrobial activity and some of them have been found to show interesting activity against pathogenic organisms [4]. There have been increasing interest in this group of compounds because of their safety profile; in fact some of them are usually used as preservatives in food industry [4].

Fatty acids are characterized with a -COOH (carboxylic) group at one end and a methyl (CH_3) at the other [5]. The length of the carbon chain differs and normally ranges from 4-28. They are either saturated or unsaturated depending on whether there is a presence of double bond. Both the saturated and unsaturated are known to exhibit anti-microbial activity [6]. The exact mechanism of action of fatty acid is not quite clear but it seems the prime target of their antimicrobial activity is the cell membrane and the essential processes of the membrane activity [7]. The carboxylic functional group of the fatty acids can undergo chemical reaction to give rise to different types of compounds. Thus the activity of fatty acids can be modified at this point. The modification of the carboxylic group of palmitic acid was undertaken in order to obtain different lipid amides which were screened for antibacterial activity.

2. MATERIALS AND METHODS

2.1 Reagents, Materials and Equipment

The starting materials were purchased from commercial sources and used without further purification. Glycine, β-alanine, γ- aminobutyric acid (GABA), isatoic anhydride and palmitoyl chloride were obtained from Sigma Aldrich (Germany). Triethylamine (TEA) and dioxane were obtained from BDH (England). Muller- Hinton agar was obtained from Oxoids (U.K). The precoated thin layer chromatography (TLC), silica gel 60 F_{254} plates used to monitor the reaction was obtained from Merck (Darmstadt, Germany). Melting points were determined with an electrothermal melting point apparatus and were uncorrected. Infra red (IR) spectra were measured on a Buck scientific IR M500 instrument. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 200 (250MHz) and (63 MHz) respectively. Chemical shifts are reported in part per million (ppm) relative to

tetramethylsilane (TMS). Mass spectra (MS) were recorded on a Varian MAT 44S mass spectrometer operating at 70eV.

2.2 Chemistry

2.2.1 Preparation of palmitoyl glycine

To a stirring mixture of glycine (0.546 g, 7.28 mmol.) in dichloromethane (10 mL) was added 1 mL triethylamime and palmitoyl chloride (2.2mL, 7.28 mmol.). At the end of the 4 h, the mixture was filtered and washed with 1N HCl (10 mL) and the combined organic filtrate was dried over anhydrous Na_2SO_4 and evaporated *in vacou*. The resulting product (white powder) was recrystallized from methanol/water (1:1). **Yield**: 1.03g (90%); **melting point:** 106 – 108ºC. **IR** (KBr) 3371 (NH), 2929 (CH), 2857 (CH), 1686 (C=O), 1629 (C=O), 1543, 1457, 1400, 1200 cm^{-1}; **^{1}H NMR** ($DMSO_{d6}$) δ: 0.86 (t, J = 6.0 Hz; 3H, CH_3), 1.22 (brs, 22H, $(CH_2)_{11}$) 1.45 (brs, 2H,CH_2) 1.55-1.64 (quint, J = 7 Hz 2H,CH_2) 2.01 (t, J = 7.5 Hz 2H,CH_2) 2.13-2.21 (quint, J = 4.75Hz 2H, CH_2) 3.0 (quint, J = 6.75 Hz 2H, CH_2) 7.74 (brt, 1H, NH) 11.97 (brs, 1H, COOH); **^{13}C NMR** ($DMSO_{d6}$) δ: 15, 22, 25.7,29.0, 29.1, 29.2, 29.22, 29.39, 29.49, 31.5, 31.74, 34.1, 35.84, 38.22, 172.48 (C=O), 174.62 (C=O); **MS** 313 (M^+, 12%), 256 (100), 214 (24), 185(17), 129(21), 116(26); **Elemental analysis**: $C_{18}H_{35}NO_3$ (313.488g); Found; C: 69.10, H:11.08, N :4.44, Calculated; C :68.97, H :11.25,N :4.47.

2.2.2 Preparation of palmitoyl alanine

To β-alanine (0.590g, 6.6 mmol.) in dry dichloromethane (10 mL) was added 1mL triethylamime (TEA) under stirring in an ice-cold chamber (0-5ºC). Palmitoylchloride was added dropwisely and finally washed down with another 10 mL dichloromethane. The ice removed and the resulting syrupy mixture was stirred for 2 h at room temperature. It was diluted with 20mL of dichloromethane and washed with 10mL of 1N HCl and water. The combined organic portion was dried over anhydrous Na_2SO_4, and solvent removed *in vacuo* to leave behind a whitish crude solid. The crude product was recrystallized from methanol/water (1:1). **Yield**: 1.10g (92%); melting point: 80 – 82ºC; **IR** 3301 (NH), 2923 (CH), 2853 (CH), 1694 (C=O), 1636 (C=O) 1544, 1463, 1377 cm^{-1} ; **^{1}H NMR** ($DMSO_{d6}$) δ: 0.86 (t, J = 6.0 Hz 3H, CH_3), 1.22 (brs 22H, $(CH_2)_{11}$), 1.43 – 1.46 (d, J = 6.8 Hz, 2H, CH_2) 1.97 - 2.03 (t, J = 7.5 Hz, 2H, CH_2), 2.13- 2.19 (t, J = 7.3 Hz, 2H, CH_2) 2.31 – 2.36 (t, J = 7.0 Hz, 2H, CH_2) 2.49 (d, J = 1.5 Hz, 2H, CH_2) 3.16 – 3.24 (quint, J = 7.3 Hz, 2H, CH_2) 7.8 (brs 1H, NH) 12.06 (brs, 1H, COOH).**^{13}C NMR** ($DMSO_{d6}$) 15, 22.53, 24.93, 25.69, 29.00, 29.08, 29.15, 29.19, 29.24, 29.38, 29.49, 31.74, 34.10, 34.39, 35.14, 35.72, 172.60 (C=O), 174.89 (C=O); **MS** 328 (M^+,14%), 256 (71), 213(20), 144(54), 131 (100), 90(45), 55(42), 43(54); **Elemental analysis**: $C_{20}H_{39}NO_3$ (327.515g) Found, C:70.10, H :11.20, N :4.02, Calculated, C :69.68, H :11.39, N :4.28.

2.2.3 Preparation of palmitoyl γ-aminobutyric acid

To a stirred solution of GABA (0.34g, 3.3 mmol.) in 1, 4-dioxan (15 mL) a solution of 1N NaOH (3.3 mL.3.3 mmol.) was added under ice and stirred for 15 minutes. The addition of palmitoyl chloride (3.3 mmol.) was followed by 1N NaOH (3.3 mL, 3.3 mmol) and mixture stirred at room temperature for 4 h. After acidification with 1N HCl to pH of 3, the solid was collected, washed with water thoroughly (pH 5-6). The final product was then recrystallized from methanol/water mixture (1:1). Yield: 1.14 g (92%); melting point: 110-112ºC; **IR** (KBr) 3286 (NH), 2929 (CH),2843 (CH), 1686 (C=O),1628 (C=O),1543, 1471,1414 cm^{-1}; **^{1}H NMR**

($DMSO_{d6}$) δ: 0.83 (t, J = 6.0 Hz, 3H,CH_3), 1.22 (brs, 22H, $(CH_2)_{11}$), 1.45(brs, 2H, CH_2), 1.55-1.64 (quint, J = 7.0 Hz, 2H, CH_2), 1.98 - 2.04 (t, J = 7.5 Hz, 2H, CH_2) 2.13- 2.21 (m, 2H, CH_2), 2.972- 3.05 (quint, J = 6.8 Hz, 2H, CH_2), 7.75 (brs, 1H, NH) 12.0 (brs, 1H, COOH); **^{13}C NMR** ($DMSO_{d6}$) 14.5, 23.0, 25.73, 28.99, 29.10, 29.15, 29.22, 29.39, 29.46, 29.48, 31.49,31.74,34.10, 35.84,38.23, 38.92, 172.55(C=O), 174.63 (C=O); **MS:** 341 (M^+12%), 256 (33), 213 (14), 158 (17), 145 (100), 86(21), 55(30), 43 (54). **Elemental analysis**: $C_{20}H_{39}NO_3$ (341.542 g) Found C: 70.10, H: 11.20, N: 4.02, Calculated C :70.33, H :11.51, N:4.10.

2.2.4 Preparation of *o*-palmitoylamino N-carboxymethylbenzamide

Isatoic anhydride (5 g, 30.67 mmol), glycine (2.3g, 30.67 mmol) and triethylamime (TEA) (4.25 ml, 30.67 mmol) was mixed in 50 mL of water and heated at 40-50ºC in an oil bath for 5 h; TLC analysis revealed the disappearance of starting material at the end of the reaction. The pH of the mixture was brought to (3-5) with 1N HCl. The mixture was then partitioned with ethyl acetate (3x50 mL), and the solvent removed after drying over anhydrous Na_2SO_4 to give a product which was not isolated. The product (*o*-amino carboxymethyl benzamide; 1.793 g, 9.24 mmol) from the above procedure was treated with palmitoylchloride (2.8 mL, 9.24 mmol) in 1, 4-dioxan in the presence of TEA. At the end of the reaction, the mixture was poured into water and exhaustively extracted with ethyl acetate (3x50 mL). The combined organic fraction was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to leave behind a crude product which was purified on column chromatography using dichloromethane and then dichloromethane/ethyl acetate (3:1). **Yield:** 2.84 g (71%), **Melting point:** 96-98ºC.**IR (KBr):** 3526 (OH), 3025 (C-H), 2917 (C-H), 2848(NH), 1700(C=O), 1643(C=O), 1586, 1514, 1471cm^{-1}. **^{1}H NMR (**$DMSO_{d6}$**)** δ: 0.80-0.83(t, J = 7.5 Hz, 3H, CH_3), 1.01-1.03 (d, J = 5.0 Hz, 2H, CH_2), 1.21 (brs, 22H, $(CH_2)_{11}$), 1.58 (brs, 2H, CH_2), 2.27-2.33 (t, J = 7.3 Hz, 2H, CH_2), 7.09 (s, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 7.67-7.70 (d, J = 7.0 Hz, 1H, Ar-H), 8.35-8.38 (d, J = 8.3 Hz, 1H, NH), 8.77 (s, 1H, NH), 11.23 (s,1H, COOH). **^{13}C NMR** ($DMSO_{d6}$)**:** 14.5, 22.3, 25.38, 28.93, 29.15, 29.32, 29.45, 29.47, 29.73, 31.56, 31.73, 34.10, 37.91, 120.79 (Ar –C), 121.30 (Ar – C), 122.80 (Ar –C), 128.44 (Ar-C), 132.14 (Ar-C), 139.41 (Ar-C), 168.76 (C=O), 171.43 (C=O), 174.90 (C=O). **MS:** 431.4 (M^+, 18%), 374.3(82), 309.2 (2), 293.2 (12), 255.3 (100).**Elemental analysis:** $C_{25}H_{40}N_2O_4$ (432.615 g) Found (C: 69.24, H: 9.14, N: 6.28), Calculated (C: 69.41, H: 9.32, N: 6.48).

2.2.5 Preparation of *o*-palmitoylamino N-carboxyethyl benzamide

Isatoic anhydride (5g, 30.67 mmol), β-alanine (2.73g, 30.67 mmol) and TEA (4.25mL,30.67mmol) was mixed in 50mL of water and stirred at 40-50ºC in an oil bath for 5 h. TLC showed completion of reaction. The mixture was acidified with 1N HCl (pH 3-5) and partitioned with ethyl acetate (3x50ml). The combined organic fraction after drying over anhydrous Na_2SO_4 was evaporated *in vacuo* and the final product (3.134g, 15.07 mmol) was treated with palmitoylchloride (4.56 mL, 15.07 mmol) in 1, 4-dioxan in the presence of TEA. At the end of the reaction, the mixture was poured into water and exhaustively extracted with ethyl acetate (3 x 50 mL). The combined organic fraction was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to leave behind a crude product which was purified on column chromatography (silica gel 70-230 mesh) eluting with dichloromethane and then dichloromethane/ ethylacetate (3:1). **Yield**: 6.56 g (97.47%) **Melting point**: 102-104ºC **IR (KBr)** 3528 (OH), 3028 (C-H), 2914 (C-H), 2843 (NH), 1700 (C=O), 1643 (C=O), 1586, 1514, 1471cm^{-1}. **^{1} H NMR (**$DMSO_{d6}$) δ: 0.80-0.83 (t, J = 6.8 Hz, 3H, CH_3), 1.02 (d, J = 6.3 Hz, 2H, CH_2), 1.21 (brs, 22H, $(CH_2)_{11}$), 1.58 (brs, 2H,CH_2), 2.30 (t, J = 7.3 Hz, 2H, CH_2), 7.09 (s, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 7.67-7.70 (d, J = 7.0 Hz, 1H, Ar-H), 8.35-8.39 (d, J = 8.25 Hz, 1H, NH), 8.77 (s, 1H, NH), 11.23 (s,1H, COOH). **^{13}C NMR (**$DMSO_{d6}$**):** 14.5, 22.3, 25.38,

25.91, 28.95, 29.14, 29.17, 29.33, 29.45, 29.47, 31.73, 33.95, 35.99, 37.88, 120.84 (Ar-C), 121.19 (Ar-C), 122.82 (Ar-C), 128.48 (Ar-C), 132.21 (Ar-C), 139.39 (Ar-C), 168.73 (C=O), 171.46 (C=O), 173.29 (C=O). **MS:** 446 (M^+ 6%), 430 (6), 358 (14), 256 (18), 250 (34), 222(21), 208(100), 146 (13), 120 (21), 43 (25).**Elemental analysis:** $C_{26}H_{42}N_2O_4$ (446.642g); Found C: 69.80, H: 9.32, N: 6.04, Calculated C: 69.92, H: 9.48, N: 6.27.

2.2.6 Preparation of *o*-palmitoylamino N-carboxypropyl benzamide

Isatoic anhydride (5g, 30.67 mmol), GABA (3.16g, 30.67 mmol) and TEA (4.3 mL) were mixed and stirred in 50 mL of water for 5h at 40-50ºC. TLC showed completion of reaction. The mixture was acidified with 1N HCl to pH 3-5 and extracted with ethyl acetate (3x 50mL). The combined organic phase after drying over anhydrous Na_2SO_4 was evaporated *in vacuo*. The final product (1.643 g, 7.4 mmol) was treated with palmitoyl chloride (2.4 ml, 7.4 mmol) in1, 4-dioxan in the presence of TEA. At the end of the reaction, the mixture was poured into water and exhaustively extracted with ethyl acetate (3 x 50 mL). The combined organic fraction was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to leave behind a crude product, which was purified on column chromatography (silica gel 70-230 mesh) eluting with dichloromethane and dichloromethane/ ethyl acetate (3:1).**Yield**: 2.50 g (73.27%). **Melting point**: 98-100ºC **IR (KBr)** 3428 (OH), 3228 (NH), 2914 (CH), 2843 (CH), 1700 (C=O), 1643 (C=O), 1586, 1528 cm^{-1} **^{1}H NMR** ($DMSO_{d6}$) δ: 0.75-0.83 (t, J = 7.0 Hz, 3H, CH_3), 1.14- 1.21 (brs, 22H $(CH_2)_{11}$), 1.57 (brs, 2H, CH_2), 1.72 – 1.77 (t, J = 7.0 Hz, 2H, CH_2), 2.24 – 2.32 (m, 2H, CH_2) 7.07-7.13 (t, J = 7.8 Hz, 1H, Ar-H), 7.41-7.47 (t, J = 7.5 Hz, 1H, Ar-H), 7.67-7.71(d, J = 8.0 Hz, 1H, Ar-H), 8.72 (brt, 1H, N-H), 11.26 (s, 1H, N-H), 12.0 (brs, 1H,COOH). **^{13}C NMR** ($DMSO_{d6}$)**:** 14.5, 22.3, 25.38, 28.93, 29.15, 29.32, 29.45, 29.47, 29.73, 31.56, 31.73, 34.10, 37.91, 120.79 (Ar –C), 121.30 (Ar – C), 122.80 (Ar –C), 128.44 (Ar-C), 132.14 (Ar-C), 139.41 (Ar-C), 168.76 (C=O), 171.43 (C=O), 174.90 (C=O). **MS:** 461 (M^+), 264 (10%), 256 (18), 223 (18), 222 (100), 185 (6), 174 (9), 161 (16), 146 (18), 120 (25), 104 (14). **Elemental analysis:** $C_{27}H_{44}N_2O_4$ (460.669); Found C: 70.24, H: 9.42, N: 6.02, Calculated C: 70.59, H: 9.63, N: 6.08.

2.3 Agar Diffusion Method (Zone of Inhibition Measurement)

Four clinical bacterial isolates (*Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*) and one typed culture of *E. coli* were obtained from the microbial bank of Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria for the antimicrobial screening of the synthesized compounds. The bacterial isolates were standardized using colony suspension method and the strain's suspension was matched with 0.5 McFarland standards to give a resultant concentration of 1.5 x 10^8 cfu/ml. The antibiotic susceptibility testing was determined using the modified Kirby-Bauer diffusion technique by swabbing the Mueller–Hinton agar (MHA) (Oxoids U.K) plates with the resultant saline suspension of each strain and four wells were made in the agar with the aid of cork borer (No. 4, the diameter of the borer is 6mm). The wells were sealed at the bottom with molten sterilized agar. Then 0.1ml, 0.2ml, 0.3ml, 0.4ml solutions of the synthesized compounds representing 100µg, 200µg, 300µg and 400µg respectively were aseptically dispensed into the labeled wells while antibiotic disc (ciprofloxacin 5µg) used as control were placed on the agar aseptically. The plates were then incubated at 37ºC for 24 hours. The zone diameters of inhibition produced by each concentration of the compounds and that of the antibiotic discs were measured and recorded [8].

3. RESULTS AND DISCUSSION

The synthesized compounds were obtained in good yield (70-94%) and high purity as demonstrated by the elemental analysis. The compounds were unequivocally characterized using the combination of infra red (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). The result of the antibacterial screening is as shown in Table 1. None of the synthesized compound was found to exhibit appreciable antibacterial activity.

Table 1. Result of the antibacterial screening

Compound/Concent-ration (mcg/ml)	Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	Escherichia coli(ATCC)
Palmitoyl glycine					
400	NI	NI	NI	NI	NI
300	NI	NI	NI	NI	NI
200	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
Palmitoyl alanine					
400	NI	NI	NI	NI	NI
300	NI	NI	NI	NI	NI
200	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
Palmitoyl GABA					
400	NI	NI	NI	NI	NI
300	NI	NI	NI	NI	NI
200	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
o-Palmitoylamino N-carboxymethyl-benzamide					
400	NI	NI	NI	NI	NI
300	NI	NI	NI	NI	NI
200	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
o-Palmitoylamino N-carboxyethyl-benzamide					
400	NI	NI	NI	NI	NI
300	NI	NI	NI	NI	NI
200	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
o-Palmitoylamino N-carboxypropyl-benzamide					
400	NI	NI	NI	NI	NI
300	NI	NI	NI	NI	NI
200	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
Ciprofloxacin (5mcg)	21	21	25	15	16

NI = No inhibition, the zone of inhibition is in mm.

The syntheses of palmitoyl glycine, palmitoyl alanine and palmitoyl GABA were accomplished by direct condensation of the amino acids with palmitoyl chloride. While that of *o*-Palmitoylamino N-carboxymethyl benzamide, *o*-palmitoylamino N-carboxyethyl benzamide and *o*-palmitoylamino N-carboxypropyl benzamide were carried out by first treating the respective amino acids with isatoic anhydride and subsequently condensing the resulting product with palmitoyl chloride. The compounds were unequivocally characterized using the combination of ^{1}H and ^{13}C NMR (nuclear magnetic resonance) and mass spectrometry. The result of the antimicrobial activity is shown in Table 1. Contrary to earlier report [9], the result

showed that the six synthesized compounds have no antibacterial activity against the tested strains of the microorganisms. It could be inferred from this result that the presence of the free carboxylic functional group is necessary for anti-microbial activity. Also the introduction of the aromatic group in some of the compounds increased the bulkiness of the compounds making it difficult for the compounds to cross the bacterial cell wall. The minimum inhibitory concentration was not determined since there was no zone of inhibition.

4. CONCLUSION

This work has shown that both the straight chain and aromatic analogues of saturated long chain lipid amides are inactive against the tested strains of microorganism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cohen ML. Changing patterns of infectious disease. Nature. 2000;406:762-767.
2. Levy SB. The challenge of antibiotic resistance. Scientific America. 1998;1-7.
3. Grinten E, Pikkemaat MG, Brandhof E, Stroomberg GJ, Kraak MHS. Comparing the sensitivity of algal, cyanobacterial and bacterial bioassays to different groups of antibiotics. Chemosphere. 2010;80(1):1-6
4. Altieri C, Bevilacqua A, Cardillo D, Sinigaglia M. Antifungal activity of fatty acids and their monoglycerides against *Fusarium* spp. in a laboratory medium. Int J Food Sci Technol. 2009;44:242-245.
5. Liu S, Weibin R, Jing L, Hua X, Jingan W, Yubao G, Jingguo W. Biological control of phytopathogenic fungi by fatty acids. Mycopathologia. 2008;166:93-102.
6. Sylvain LS, Lucia VM, Elisabetta G. Effect of α-linolenic, capric and lauric acid on the fatty acid biosynthesis in *Staphylococcus aureus*. Int. J. Food Microbiol. 2009;129:288-294.
7. Wojtczak L, Więckowski MR .The mechanisms 1 of fatty acid-induced proton permeability of the inner mitochondrial membrane. J Bioenerg Biomembr. 1999;31:447-455.
8. Clinical and Laboratory Standard Institute (CLSI) .Performance standards for Antimicrobial Susceptibility Testing Eighteenth informational supplement M100-S18. 2008;28(1):34-52
9. Ouattara B, Simard RE, Holley RA, Piette GJP, Be´gin A. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 1997;37:155–162.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=374&id=14&aid=2835