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In vitro inhibitory potential of two synthesized fatty amides derivatives against some microbial pathogens

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Abstract

In the present study, two amide derivatives with long chains; N-cyclohexyloctamide 1 and N-Phenyldodecanamide (dodecanilide) 2 were synthesized. They were tested *in vitro* for a wide array of antimicrobial activities against; two Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), four Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enteric*), and three fungi (*Aspergillus carbonarius*, *Mucor ramannianus*, *Candida albicans*), to evaluate their potential use as antimicrobial agents. Zones of inhibition of these compounds were determined by disc diffusion assay. Results revealed high antimicrobial activities of the two amides. The new fatty N-cyclohexyloctamide 1 showed significant antimicrobial activity against *S. aureus* and *A. carbonarius*, and was more active against *C. albicans* than synthesized fatty anilide 2, as it caused higher zones of inhibition. On the other hand, the fatty anilide 2 demonstrated high antibacterial activity against *P. aeruginosa*, and was more active against *S. enteric* than substituted fatty amide 1. On the basis of these obtained results, it could be assumed that fatty amide derivatives may be used as components of disinfectants, and may be possible sources of new drugs effective against various infectious diseases.

Keywords: Amide derivatives, Synthesis, Antimicrobial activity, Disk diffusion assay

1. Introduction

Bacteria have been reported to cause a number of life-threatening infections all over the world (Berber *et al*., 2003). Large number of bacteria can cause infectious diseases or contaminate food, thus affecting their quality. It is therefore necessary to control these microbes. The use of antimicrobial substances included in disinfectants for example, can control microbial growth.

Zhang *et al*., (2010) defined the antimicrobial as a substance that reduce or completely block the growth and multiplication of microbes, and are helpful in the treatment of various infectious diseases.

Infectious diseases were the most significant causes of human death, accounting approximately for one-half of all deaths throughout the world (Gnanamani *et al*., 2003). Karaman *et al*., (2003) added that these numbers were still increasing due to the development of drug resistance in microorganisms, as a result of the indiscriminate use of antimicrobial drugs.

Thus, the search for new antimicrobial agents is an urgent need. With the advancement of Science and Technology, remarkable progress has been made in the field of medicine with the discovery of many efficient natural and synthetic drugs (Preeth *et al*., 2010).

Amide derivatives are important classes of organic compounds. They were associated with broad spectrum of biological activities including; anti-tuberculosis, anticonvulsant, analgesic, anti-inflammatory, insecticide, antifungal, and antitumor agents. They also showed anti-platelet activities. Pochampally *et al*., (2014) added that amides in conjugation with other aliphatic, aromatic and heterocyclic moieties; possessed various types of biological activities.

Anilides are important classes of amides. In the last decade, a special interest has been focused on anilides, because of their peculiar biological activities including; anti-bacterial, anti-fungicidal, anti-convulsant, anesthetic, and platelet aggregation (Narasimhan *et al*., 2007). Farshori *et al*., (2011) reported that several anilides have found wide applicability as bioactive species (i.e. antimicrobial, antioxidant and anti-atherosclerotic agents). Moreover, they were also involved as intermediate precursors in the synthesis of therapeutic agents (Malki *et al*., 2011; Malki *et al*., 2016a). For example, Cyanoacetanilides were important and versatile reagents; which have been specifically used for the synthesis of poly-functionalized heterocycles with diverse pharmacological activities (Farrag *et al*., 2015). In previous studies of Malki *et al*., (2016b); Malki *et al*., (2017a), they evaluated the *in vitro* antioxidant activities of some synthesized amide derivatives. Results revealed that the tested amides had variable and interesting antioxidant properties, and free radical scavenging activities; when compared to the standard antioxidants.

According to the wide variety of biological activities shown by amide derivatives, and in continuation of author's efforts in search for potent molecules exhibiting antimicrobial activities; the purpose of the current study was to evaluate the antimicrobial activities of two synthesized amides derivatives containing fatty chain alkyl, against diverse range of microorganisms including; Gram-positive, Gram-negative bacteria and fungi.

2. Material and methods

2.1. Chemicals

Chemicals used were of analytical grade purchased from Sigma-Aldrich, Merck, Prolabo and Biochem.

2.2. Synthesis

The two different amides derivatives with fatty chain alkyl, N-cyclohexyloctamide 1, and dodecanilide 2 have been synthesized according to procedures of Malki *et al*., (2011); Malki *et al*., (2016b).

Dodecanilide 1 was first prepared as follows; a mixture of aniline and dodecanoic acid was heated at 160-200°C for 3-4 h. Water from the reaction was continuously distilled off. Under similar operating conditions, the newly N-cyclohexyloctanamide 2 was prepared by reaction of an excess of cyclohexylamine with octanoic acid. The produced white solids were recrystallized in ethanol, and recovered as white crystals in quantitative yields. They were then characterized and their chemical structures were confirmed by spectroscopic analyses in reference to Malki *et al*., (2011); Malki *et al*., (2016b).

2.3. *In vitro* antimicrobial activity

The synthesized amides 1, 2 were screened for *in vitro* antimicrobial activities by the paper disk-

diffusion assay according to Balouiri *et al*., (2016). The tested microorganisms were obtained from Laboratory of Biology of Microbial Systems (LBSM), Ecole Normale Supérieure, Kouba, Algiers, Algeria. They included two Gram-positive bacteria (*S. aureus* CIP 7625, *B. subtilis* ATCC 6633), four Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. enterica*, *K. pneumonia*, and three fungi; *M. ramannianus* NRRL 1829, *C. albicans* IPA 200, *A. carbonarius* M 333). The assays were carried out on ISP-2 (International Streptomyces Project) medium (Shirling and Gottlieb, 1966).

Briefly, both amides 1 and 2 were dissolved in methanol (1 mg/mL). Sterile 6-mm diameter filter paper disks were impregnated with solution of the compound to be tested (20, 60 µg/disk), then these paper disks were placed aseptically on the surface of ISP-2 medium, seeded separately with each indicator microorganism. For positive control, Gentamicin (10 µg/disk) was used to determine the sensitivity of Gram-positive and Gram-negative bacteria, while Fluconazole and amphotericin B (25 µg/disk) were used for yeast and fungal species, respectively. Petri plates were left at 4°C for 2 h to allow diffusion of the amides in the medium, and then incubated at 37°C for 24 h for bacteria, and at 30°C 96 h for yeasts and fungi. Positive antimicrobial activity was observed as a clear zone around the disk indicating inhibition of microbial growth (Gülçin *et al*., 2008). The antimicrobial activity was evaluated by measuring the diameters of inhibition zones in mm. All assays were performed in triplicates, in three independent experimental sets. Data obtained was presented as mean of diameters of inhibition zones of different concentrations of each amide against each tested bacteria and fungi.

2.4. Statistical analysis

Data were analyzed with the statistical software statistica. Values were expressed as means ± standard deviations (SD). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

Fatty amides 1, 2 were synthesized in satisfactory yields about 70, 73 %; respectively, and were then characterized as N-cyclohexyloctanamide 1, N-Phenyldodecanamide 2. Their structures were confirmed using common spectroscopic analyses in reference to Malki *et al*., (2011); Malki *et al*., (2016b); Malki *et al*., (2017b), as clear in Fig. (1).

3.1. *In vitro* antimicrobial potency

Both amides 1 and 2 were screened for their *in vitro* antibacterial and antifungal activities against different bacterial and fungal species, and results were represented in Table 1.

Results observed in Table 1 demonstrated that both fatty amides showed inhibitory effects against the Gram-negative bacterium *S. enteric*, and that fatty anilide 2 was more active than fatty amide 1. On the other hand, only anilide 2 exhibited potent inhibitory activity against Gram-negative bacterium *P. aeruginosa*; however, at the concentration of (60 µg/disk) none of the two amides manifested antibacterial activities against either *E. coli*, *K. pneumonia*, or against *B. subtilis*. Both fatty amides showed antifungal activities against *C. albicans*, where amide 1 showed the highest activity.

Only amide 1 demonstrated the ability to inhibit *A. carbonarius* growth, whereas the two fatty amides were inactive against *M. ramannianus*, which proved thus to be resistant to both amide derivatives. It should also be noted that the various inhibition assays were carried out at a charge of disk about 20 and 60 µg/disk. Maximum inhibitions were practically achieved at charge of 60 µg/disk for all the tested strains.

N-cyclohexyloctanamide 1 N-Phenyldodecanamide 2

Fig.1. Chemical structure of the synthesized amide derivatives 1, 2

Table 1: Zones of inhibition (mm) of the tested amides 1, 2 against some microbial pathogens

Compound tested (60 µg/disk)	G + bacteria		G- bacteria				Fungi		
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*S. enterica*	*A. carbonarius*	*M. ramannianus*	*C. albicans*
1	20 ± 1^{b}	-	-	-	-	13 ± 0.5^{a}	18 ± 0.7^{b}	-	17 ± 1^{b}
2	-	-	47 ± 1.5^{c}	-	-	$16\pm0.7^{a,b}$	-	-	14 ± 0.5^{a}

-Results demonstrated were averages of 3 replicate plates. -: No inhibition observed. Different letters indicated significantly different activities ($P<0.05$)

These findings implied the selective antimicrobial activities of both amide derivatives, and the positive impact of fatty acid chain substituted within their structure.

Conclusion

In this study, results demonstrated the potential antimicrobial properties of the tested amides. Both fatty amides showed interesting antibacterial activities against *S. enteric*, and antifungal activities against *C. albicans.*

High antifungal activity against *A. carbonarius* was recorded specifically for the amide 1; whereas, the fatty anilide 2 demonstrated high antibacterial activity against *P. aeruginosa*. The antimicrobial potency increased with increasing the corresponding concentration of the amides, and was directly related to the nature of the substituents on the amide group.

Thus both active compounds recorded in this study could be useful for developing new antimicrobial drugs, and may be used in the future for treatment of many diseases.

Conflict of interests

The authors declare that there was no conflict of interests.

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