Nickel (II) and Cobalt (II) Complexes of Some Amino Acid-based Surfactants with Antimicrobial Activity

DANA VARASTEANU, IRINA CHICAN*, SANDA MARIA DONCEA, IULIANA RAUT, MARIANA CALIN, LUIZA JECU
National Research & Development Institute for Chemistry and Petrochemistry ICECHIM-Bucharest, 202 Splaiul Independentei, postal code 060021, Romania

Nickel and cobalt complexes of lauroyl-glycine, lauroyl-glycylglycine and 1,12-dodecandioyl-diglycylglycine were synthesized. The FTIR spectroscopy confirmed the complex formation. The antimicrobial activity of synthesized nickel and cobalt complexes was investigated against several bacteria and fungi: Bacillus cereus, Pseudomonas aeruginosa, Candida albicans, and Aspergillus flavus. Generally, the nickel and cobalt complexes of the investigated surfactants showed no antimicrobial activity on Pseudomonas aeruginosa, a moderate one on Bacillus cereus and Aspergillus flavus. The most susceptible microorganism was Candida albicans, which is considered one of most common nosocomial infections. The results suggest the potential application of tested compounds as antimicrobial agents against targeted pathogens.

Keywords: amino acid-based surfactants, metal complex, antimicrobial activity

Metal and metal complex combinations with different ligands are of particular importance in medicine due to their anti-arthritic, antibacterial, antitumor, antidepressant and antihypertensive properties. Antimicrobial activity of complex combinations is explained by the theory of chelation. By forming chelates, the metal ion orbits overlap with the orbital atoms of the donor groups. As a result, delocalisation of ð electrons increases in the chelated ring, which increases the lipophilic character of the metal chelate and favours its penetration through the lipid membrane of the bacteria. At the same time, the binding sites of biologically active metals with the proteins of the microorganisms are blocked, thus reducing their structural stability. Metal complexes also cause changes in cellular respiration, blocking protein synthesis, thus preventing the development of microorganisms. Antimicrobial activity is influenced by the nature of the donor molecules in the ligand molecule but also by the nature of the metal ion in the complex combination [1]. The involvement of coordination compounds in these phenomena is determined by the possibility of interacting with microorganisms (bacteria and fungi) that cause it various infectious diseases. Metal ions can coordinate on some parts of the microorganism or complex combination can be accomplished via the ligand interactions through hydrogen bonds or weak physical links that can cause alterations that lead to the reduction or elimination of their ability to cause the disease.

Since Rosenberg's discovery of cisplatin (cis-diaminodichloroplatin (II)) an effective inorganic product in the treatment of various cancers, many products have been developed both in the field of anti-tumor products and those with antimicrobial properties [2, 3]. Products with antimicrobial properties have emerged as a necessity for the increased resistance of pathogenic microorganisms to current antibiotics. Transition metal complexes with various Schiff base derivatives have been studied for their antimicrobial and chemotherapeutic activity. Various studies show that the azomethine group> C = N- has a significant biological significance in obtaining these effects, but antimicrobial and chemotherapeutic activity may increase several times by complexation with metal ions [4-7].

Transition metal complexes (Co, Cu, Ni and Zn) with condensation products of diketones with amino acids [8], or with different amino acids: methionine, phenylalanine, valine, leucine, lysine, glycine [9-14] show antibacterial and antifungal activity tested against pathogenic strains such as *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Micrococcus luteus*, *Trichophyton logifusus*, *Candida albicans*, *Aspergillus flavus*, *Fusarium solani* .

The analysis of literature data has highlighted the fact that transition metal complexes of acylated amino acids were not used as antimicrobial agents, therefore we synthesized and characterized Ni and Co complexes of lauroyl-glycine, lauroyl-glycylglycine and 1,12-dodecandioyl-diglycylglycine and evaluated their antimicrobial activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus flavus* strains.

The selection criterion for these strains was their pathogenic potential. *Pseudomonas aeruginosa* is a Gram-negative bacterium producing infections at humans, animals and plants. The strain causes nosocomial infections in immunocompromised individuals; this species infects the lung, urinary tract, burns and wounds. Biofilms of *P. aeruginosa* can cause bacteraemia in immuno-compromised and elderly people. Because it can cause nosocomial infections, it is considered a model body for the study of antibiotic-resistant bacteria [15]. *Bacillus cereus* is a strain of beta haemolytic Gram-positive bacterium. It is involved in a diarrhoeal type of food poisoning, caused by complex enterotoxins produced during vegetative growth of *B. cereus* in the small intestine [16]. *Candida albicans* is a species of yeast fungus that can grow in leaved and filamentous form, producing a series of infections. It can also produce systemic infections (fungi) in immunocompromised patients and may cause death [17]. It can form biofilms on the surface of medical instruments [18]. *Aspergillus flavus* is a species of filamentous fungus that is found in the environment and can be pathogenic to humans, being associated with lung aspergillosis [19] and sometimes with corneal, ear, and nasoorbital infections. Spores of this species are allergens. Many strains produce aflatoxin and toxic and carcinogenic compounds.

* email: organica@icechim.ro

Experimental

Synthesis of surfactants

In order to obtain products with antimicrobial properties, amino acid-based surfactants, namely lauroyl-glycine (LG), lauroyl-glycylglycine (LGG) and 1,12-dodecandioyl-diglycylglycine (L2GG), have been synthesized and characterized. The amino-acid based surfactants were synthesized according to Schotten-Baumann method. The synthesis of amino acid-based surfactants was described elsewhere [20, 21]. Products were neutralised with 25% NaOH solution in order to be in the deprotonated state, necessary in complexation step.

Synthesis of complexes

The synthesis of cobalt (II) and nickel (II) complexes of lauroyl-glycylglycine was described in a previous work [22]. Accordingly, for the synthesis of cobalt (II) and nickel (II) complexes of lauroyl-glycine and 1,12-dodecandioyl-diglycylglycine the same procedure was followed.

For the synthesis of lauroyl-glycine complexes with Ni and Co, 0.02 mole of sodium lauroyl glycinate were dissolved in 10 mL distilled water. To this solution was gradually added under stirring 0.01 moles of cobalt chloride, respectively nickel acetate tetrahydrate, dissolved in 10 mL of distilled water. The formed mixture was kept for 10 minutes under stirring at room temperature. The formed complexes were filtered off, washed with distilled water and dried in air at room temperature.

For the synthesis of 1,12-dodecandioyl-diglycylglycine complexes with Ni and Co, 0.01 mole of 1,12-dodecandioyl-diglycylglycine disodium salt were dissolved in 25 mL distilled water. To this solution was gradually added under stirring 0.01 moles of cobalt chloride, respectively nickel acetate tetrahydrate, dissolved in 10 mL of distilled water. The formed mixture was kept for 10 min under stirring at room temperature. The formed complexes were filtered off, washed with distilled water and dried in air at room temperature.

Infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra were registered with a Spectrum GX, Perkin Elmer instrument by transmission technique, in KBr pellet, for lauroyl-glycine, 1,12-dodecandioyl-diglycylglycine and the corresponding Co and Ni complexes. The lauroyl-glycylglycine and corresponding complexes were discussed elsewhere [22].

Antimicrobial activity

Antimicrobial activity of complexes was performed using the disc diffusion method. As culture media were used simple agar and solid Sabouraud medium, with following composition (g/L): peptone, 10 g; D+glucose, 40 g; agar, 15 g; distilled water, 1000 mL. Strains of *Bacillus cereus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus flavus* are from the Microbial Collection of the Biotechnology Department of ICECHIM. Solutions of 10^{-2} mol/L si 10^{-3} mol/L were made from Ni and Co complexes of lauroyl-glycine, lauroyl-glycylglycine and 1,12-dodecandioyl-diglycylglycine . The solubilising agent was isopropyl alcohol for lauroyl-glycine complexes and distilled water for lauroyl-glycylglycine and 1,12-dodecandioyl-diglycylglycine complexes.

Results and discussions

Amino acid-based surfactants are a relatively new class of ecological, biocompatible and biodegradable surfactants. Due to their safety profile, amino acid-based surfactants are recommended for biomedical applications. The variety of amino acids allows the synthesis of surfactants with tailored properties for specific biomedical applications. The surface active properties and self-assembly capacity are influenced by the nature of amino acid residue and the chirality. Cationic amino acid surfactants are known to have antimicrobial properties [23, 24], while anionic amino acid (N-acyl derivatives) do not show antimicrobial properties.

Taking into consideration that transition metal complexes with different ligands possess antimicrobial activities, the aim of this work was to study the antimicrobial activity of Co and Ni complexes of glycine-based N-acylated surfactants.

Solubilisation of complexes of amino acid-based surfactants in distilled water can lead to colloidal dispersions. This is the case of Co and Ni complexes of lauroyl–glycylglycine, which forms colloidal dispersions in aqueous medium at 10^{-2} mol/L concentration. By diluting the complexes at 10^{-3} mol/L concentration the interaction between the molecules decreases, causing a decrease in viscosity. Co and Ni complexes of 1,12-dodecandioyl-diglycylglycine are more soluble in water, since bola-amphiphilic structure has two hydrophilic head groups, thus having a higher lipophilic-hidrophilic balance value. Co and Ni complexes of lauroyl-glycine are insoluble in water, but soluble in isopropyl alcohol. The complexes showed the characteristic colors, pink for Co complexes and pale green for Ni complexes, being in agreement with those obtained for similar coordination compounds.

*FTIR spectra c*onfirmed the complex formation. For Co and Ni complexes of lauroyl-glycylglycine FTIR spectra were analyzed in a previous work [22].

FTIR spectra of Co and Ni complexes of lauroyl-glycine and 1,12-dodecandioyl-diglycylglycine also confirmed the complex formation (fig. 1 and 2).

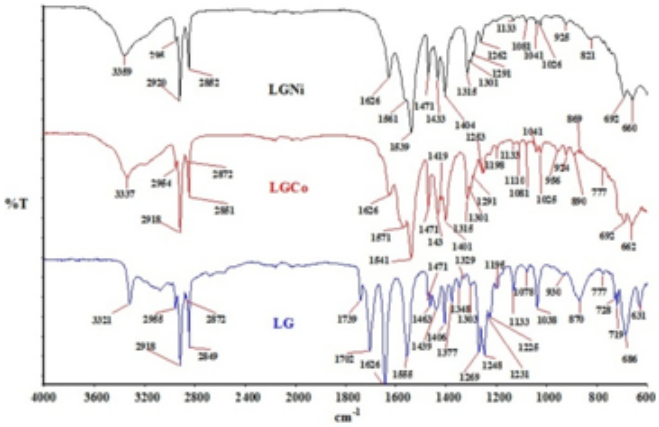

Fig. 1. FTIR spectra of lauroyl-glycine and its Co and Ni complexes

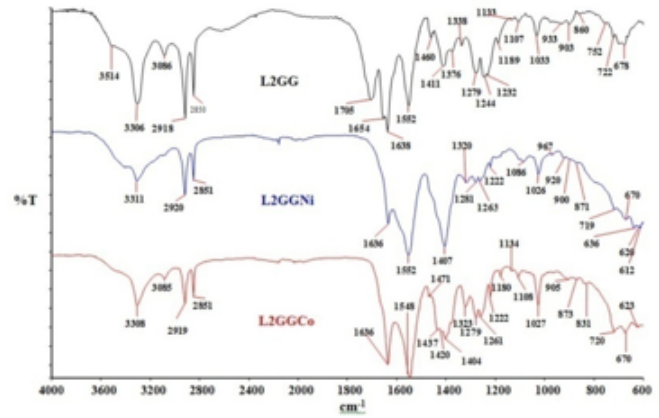

Fig. 2. FTIR spectra of 1,12-dodecanedioyl-diglycylglycine and its Co and Ni complexes

The complexation reaction of LG is demonstrated by the disappearance of the band from 1739 cm^{-1} specific for the C = O group and from 1702 cm^{-1} specific for the COO- group, by the vibrational attenuation for the -CH_2 chain groups and by shifted bands for CONH Amide I, for CONH Amide II and CH Amide III, as well as for C-N and C-C bands.

The difference between the Ni and Co complexes is well emphasized not only by the different colours of the two products but also by the FTIR spectra of the vibration bands and / or the appearance of new bands. The specific bands for the Ni complex are: -NH and / or -OH with Ni at 3359 cm^{-1}, CONH Amide II with Ni at 1561 cm^{-1} and C-C with Ni at 821 cm^{-1}. The specific bands for the Co complex are: -NH and / or -OH with Co at 3337 cm^{-1}, CONH Amide II with Co at 1571 cm^{-1}, -CH and / or N-H Amide III with Co at 1253 cm^{-1}, -CH_2, -CH_3 with Co at 1225 cm^{-1} and C-N with Co at 956 cm^{-1}. These absorption bands differentiate the two synthesized complexes.

The chemical structure of 1,12-dodecanedioyl diglycylglycine, synthesized from dodecanedioyl dichloride on which two dipeptide molecules are grafted, is similar to lauroyl glycine, both of which are amphoteric substances. Therefore, it can be concluded that the dodecanedioyl-diglycylglycine vibrational bands are of the same nature as those of the lauroyl-glycine.

The fact that the complexation reaction took place is highlighted by the disappearance of the 1705 cm^{-1} band, specific for COO- group, the attenuation of the vibrations for the -CH_2 chain groups and the shifted for the CONH Amide I, CONH Amide II and CH Amide III bands, and for CN and CC bands.

The difference between the Ni and Co complexes is well emphasized not only by the different colours of the two products but also by the FTIR spectra of the vibrational bands and / or the appearance of new bands. The specific bands for the Ni complex are: -NH and / or -OH with Ni at 3406 cm^{-1}, CONH Amide II with Ni at 1552 cm^{-1}, -CH_2 and / or -COC- with Ni at 1194 cm^{-1} respectively at 1086 cm^{-1} and C-C with Ni at 923 cm^{-1}, at 794 cm^{-1} and at 636 cm^{-1}. The specific bands for the Co complex are: -NH and / or -OH with Co at 3308 cm^{-1}, CONH Amide II with Co at 1548 cm^{-1}, -CH_2, -CH_3 with Co at 1471 cm^{-1}, at 1437 cm^{-1} and at 1420 cm^{-1}, CH_2 and / or -COC- with Co at 1134 cm^{-1}, and C-C with Co at 840 cm^{-1} and at 831 cm^{-1}. These absorption bands differentiate the two synthesized complexes.

Interpretation of the criteria for the diameter of inhibition zone is performed in accordance with the Performance standards for antimicrobial disk susceptibility tests; approved standard- ed. 11th; January 2012, M02-A11, vol. 32, no. 1, (Replaces M02-A10 Vol. 29 No. 1): diameter of inhibition zone ≥ 20 mm- susceptible; 15-19 mm - intermediate; ≤ 14 mm- resistant.

Antimicrobial activity was assessed by measuring the inhibition zone of the test microorganism. The results are presented in the tables 1, 2 and 3.

Evaluation of the antimicrobial activity of lauroyl-glycine cobalt and nickel complexes (table 1) led to the following conclusions:

- The bacterial strain of *Pseudomonas aeruginosa* was not sensitive at the action of tested complexes. Gram-negative bacteria, such as *Pseudomonas aeruginosa*, possess an outer membrane with polysaccharide structure, acting as a barrier against antibiotics [9, 25, 26];

- In the case of testing the activity of the compounds on the *Bacillus cereus* strain, high values of the diameter of the inhibition zone of 20 mm were obtained only for the cobalt complex at the concentration of 10^{-2} mol/L, double than the value recorded for the nickel compound at the same concentration. For the more diluted sample from

Complex/ Conc., mol/L	Diameter of inhibition zone, mm			
	Pseudomonas aeruginosa	*Bacillus cereus**	*Candida albicans*	*Aspergillus flavus*
LGNi 10^{-2}	0	10	23	21
LGNi 10^{-3}	0	10	22	10
LGCo 10^{-2}	0	20	28	15
LGCo 10^{-3}	0	10	25	8

**measured at 24 hours; after 48 hours inhibition zone was dimmed.*

Table1
INHIBITION OF PATHOGENS GROWTH BY Co AND Ni COMPLEXES OF LAUROYL-GLYCINE

Complex /Conc., mol/L	Diameter of inhibition zone, mm			
	Pseudomonas aeruginosa	*Bacillus cereus*	*Candida albicans*	*Aspergillus flavus*
LGGNi 10^{-2}	8	8	10	0
LGGNi 10^{-3}	0	10	0	0
LGGCo 10^{-2}	8	9	9	0
LGGCo 10^{-3}	0	11	0	0

Table 2
INHIBITION OF PATHOGENS GROWTH BY Co AND Ni COMPLEXES OF LAUROYL-GLYCYLGLYCINE

Complex /Conc., mol/L	Diameter of inhibition zone, mm			
	Pseudomonas aeruginosa	*Bacillus cereus*	*Candida albicans*	*Aspergillus flavus*
L2GGNi 10^{-2}	0	14	21	12
L2GGNi 10^{-3}	0	0	0	0
L2GGCo 10^{-2}	0	17	24	13
L2GGCo 10^{-3}	0	0	0	0

Table 3
INHIBITION OF PATHOGENS GROWTH BY Co AND Ni COMPLEXES OF 1,12-DODECANDIOYL-DIGLYCYLGLYCINE

both compounds, the inhibition zone values were similar, 10 mm. The moderate antibacterial activity of the tested compounds is not unexpected, as this bacterium forms spores very resistant to environmental conditions [9].

-The two metallic compounds were very active against *Candida albicans*, for which the diameter of the inhibition zone reached high values for both concentrations.

- *Aspergillus flavus* exhibited a relatively higher sensitivity for the nickel compound at 10^{-2} mol/L concentration, for which an inhibition area of 21 mm diameter was recorded, compared to the 15 mm diameter obtained with the cobalt compound at the same concentration.

As shown in table 2, LGGMe did not show antimicrobial activity against *Aspergillus flavus* fungus strain. In case of activity against the *Bacillus cereus* strain of the cobalt complex there were higher inhibition diameters at lower dilution, which can be explained by a better diffusion in the culture medium of the more dilute metal complex.

The tested metal complexes did not exhibit antibacterial activity against the *P. aeruginosa* strain (table 3). The most significant inhibition of the *B. cereus* strain exerted by the Nickel complex was obtained for 10^{-2} mol/L concentration, with a diameter of inhibition zone of 14 mm. At *C. albicans*, the higher inhibition of 21 mm was reached for the same concentration of complex. As regarding the activity of the cobalt compounds against *B. cereus*, the best inhibition of the microorganism was obtained in the case of L2GGNi 10^{-2} mol/L, where the inhibition diameter were was 17 mm. In the case of cobalt activity against the *C. albicans* strain, the best inhibition of 24 mm diameter was found for the same complex. *A. niger* strain was inhibited by Co and Ni complexes of 1,12-dodecandioyl-diglycylglycine, where the inhibition diameter were 13 and 12 mm, respectively.

Conclusions

Ni and Co complexes of some amino acid-based surfactants, namely lauroyl-glycine, lauroyl-glycylglycine and 1,12-dodecandioyl-diglycylglycine were synthesized and the FTIR spectra confirmed by structural evidence the complex formation.

The tested metal complexes did not exhibit antibacterial activity against the *P. aeruginosa* strain. In Gram-negative bacteria the presence of additional structures made up of polysaccharides protects the cell membrane from toxic molecules. Also, these types of bacteria produce extracellular polymeric compounds that reduce permeability of the cell membrane. In addition, electrostatic interactions between the bacterial cell and the tested metal compounds are poorer in Gram-negative bacteria compared to Gram-positive. In the case of testing the activity of the compounds on the *Bacillus cereus* strain, the studied complexes showed moderate activity, high values of the diameter of the inhibition zone of 20 mm were obtained only for the cobalt complex LGCo at the concentration of 10^{-2} mol/L. The synthesized complexes at the concentration of 10^{-2} mol/L were active especially against *C. albicans* and exhibited a moderate activity against *A. flavus*.

Synthesis, characterisation and evaluation of antimicrobial activity of cobalt and nickel complexes of lauroyl-glycine, lauroyl-glycylglycine and 1,12-dodecandioyl-diglycylglycine aimed to diversify the range of antimicrobial products. The results suggest their potential application as antifungal agents.

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References

1. RIZZOTTO, M., Metal complexes as antimicrobial agents. In: A Search for Antibacterial Agents. InTech, 2012
2. CHANG, E. L., SIMMERS, C., KNIGHT, D. A., Pharmaceuticals, vol. **3**, no. 6, 2010, p. 1711-1728
3. SADLER, P. J., GUO, Z., Pure &Appl. Chem., vol. **70**, no. 4, 1998, p. 863-871
4. JOSEYPHUS, R. S., NAIR, M. S., Arabian J. Chem., vol. **3**, no. 4, 2010, p. 195–204
5. REISS, A., FLOREA, S., CAPROIU, T., STANICA, N., Turk. J. Chem., vol. **33**, no. 6, 2009, p. 775-783
6. CIOLAN, F., PATRON, L., MURESEANU, M., ROTARU, P., GEORGESCU, I., Rev. Chim. (Bucharest), **63**, no. 1, 2012, p. 34-39
7. KRIZA, A., IGNAT, I. U., STANICA, N., DRAGHICI, C. O., Rev. Chim. (Bucharest), vol. **62**, no. 7, 2011, p. 696-701
8. CHOHAN, Z., ARIF, M., AKHTAR, M. A., SUPURAN, C. T., Bioinorg. Chem. Appl., vol. **2006**, 2006, p. 1-13
9. STANILA, A., BRAICU, C., STANILA, S., POP, R. M., Not. Bot. Horti. Agrobo., vol. **39**, no. 2, 2011, p. 124-129
10. AL-JEBOORI, F. H. , AL-SHIMIESAWI, T. A. , JASSIM, O. M., J. Chem. Pharm. Res., vol. **5**, no. 10, 2013, p. 172-176
11. INDIRA DEVI, G., SMITHA, P., Int. Res. J. Biological Sci, vol. **2**, no. 6, 2013, p. 16-21
12. AIYELABOLA, T., OJO, I., ADEBAJO, A., OGUNLUSI, G., OYETUNJI, O., AKINKUNMI, E., ADEOYE, A., Adv. Biol. Chem., vol. **2**, no. 3, 2012, p. 268-273
13.SAHA, S., DHANASEKARAN, D., CHANDRALEKA, S., PANNEERSELVAM, A., Adv. Biol. Chem., vol. **4**, no. 4, 2010, p. 224-229
14. MARCU, A., STANILA, A., RUSU, D. , RUSU, M. , COZAR, O., DAVID, L., JOAM, vol. **9**, no. 3, 2007, p. 741-746
15. YAYAN, J., GHEBREMEDHIN, B., RASCHE, K., PLoS one, vol. **10**, no. 10, 2015, e0139836
16. GRANUM, P. E., LUND, T., FEMS microbiology letters, vol. **157**, no. 2, 1997, p. 223-228
17. GROHSKOPF, L. A., ANDRIOLE, V. T., YJBM, vol. **69**, no. 6, 1996, p. 505-515
18. TSUI, C., KONG, E. F., JABRA-RIZK, M. A., FEMS Pathogens and Disease, vol. **74**, no. 4, 2016, ftw018
19. MAHGOUB, E. S., EL HASSAN, A. M., Thorax, vol. **27**, no. 1, 1972, p. 33-37
20. VARASTEANU, D., PISCUREANU, A., CHICAN, I.E., COROBEA, M.C., U.P.B. Sci. Bull., Series B, vol. **73**, no. 3, 2011, p. 147-154
21. CHICAN, I.E., VARASTEANU, D.S., OPROIU, L. C., DONCEA, S.M., Proceeding of International Symposium SIMI The Environment and the Industry, 2016, p. 271-278
22. VARASTEANU, D., COROBEA, M.C., GHIUREA, M., POP, S., CHICAN, I., FLOREA, D., PISCUREANU, A., CALINESCU, I., OAM-RC, vol. **7**no. 11-12, 2013, p. 991-996
23. PEREZ, L., PINAZO, A., GARCIA, M. T., LOZANO, M., MANRESA, A., ANGELET, M., PILAR VINARDELL, M., MITJANS, M., PONS, R., INFANTE, M. R., Eur. J. Med. Chem., vol. **44**, no. 5, 2009, p. 1884-1892
24. *** US Patent US 5780658, (1998)
25. KABBANI, A. T., HAMMUD, H. H., GHANNOUM, A. M., Chem. Pharm. Bull., vol. **55**, no. 3, 2007, p. 446-450
26. FAUNDEZ, G., TRONCOSO, M., NAVARRETE, P., FIGUEROA, G., BMC Microbiol., vol. **4**, no. 1, 19, (2004)

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