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Full Length Research Paper

Antibacterial activities of nicotine and its zinc complex

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Nicotine, isolated from leaves of *Nicotiana tabacum* was complexed with zinc and studied for their antibacterial activities against ten different strains of Gram positive and Gram negative bacteria. Results showed that zinc (II) complex of nicotine is more active against different types of bacterial strains as compared to zinc metal salt used for complexation and nicotine alone.

Key words: Antibacterial activities, *Nicotiana tabacum*, nicotine, zinc (II) complex.

INTRODUCTION

Isolation and extraction of medicinal compounds from plant sources and their characterization have been a common practice since the recent past (Munir et al., 1994; Chohan et al., 2002). Many of these natural products have been reported without their biological properties. In some cases, their effective biological properties have been remained unknown for the long years. Natural products like Vinblastine and Vincristine were isolated in 1954 but their antitumor activities were discovered in 1980 (Johnson, 1994). The discovery of new natural products without accompanying biological data is not just more than pure phytochemistry. There is, no doubt, a real need of reliable, bioassays in general which can detect a broad spectrum of pharmacological activities in plants and metal complexes of their isolated components in particular (Munir et al., 1995).

Nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine is a colour-less, light pale yellow, hygroscopic oily liquid present in the leaves of *Nicotiana* tabacum (Figure 1). It is one of the highly toxic chemicals belonging to the tobacco alkaloids (Al-Tamrah, 1999). Since nicotine is the predominant component of cigarette smoking, this natural agent is thought to be responsible for the cigarettes' benefits to Alzheimer's disease (Shen et al., 2007).

Nicotine and zinc are closely related to a variety of brain pathologies, including schizophrenia, anxiety, major depression, Parkinson's and Alzheimer's diseases (Mocchegiani et al., 2005; Gotti et al., 2006; Levin et al., 2006; Takeda et al., 2007). It is widely accepted that metal chelators and antioxidants hold great potential to ameliorate these diseases (Shen et al., 2007). Nicotine, extracted from *N. tabacum,* is an important bioligand and has good chelating sites for coordination with numerous metals. From the literature concerning the complexes of Zn (II) with various nicotine derivatives, compounds with different compositions were synthesized and investigated (Ide et al., 2002; Bayari et al., 2003; Pasaoglu et al., 2006). They were mostly obtained as anhydrous compounds and for their preparation the Zn (II) chloride or iodide, as substrates, were used. The zinc (II) ion in these complexes is coordinated by two chloride or iodide ions and two pyridine ring N atoms (Ide et al., 2002; Bayari et al., 2003; Pasaoglu et al., 2006; Dziewulska-Kuaczkowska et al., 2009). But pure Zn (II) - nicotine complex without chloride ion bridging has not been reported so far.

Antimicrobial activities of plant ingredients and their metal complexes can be detected by observing the growth response of various micro-organisms placed in contact with

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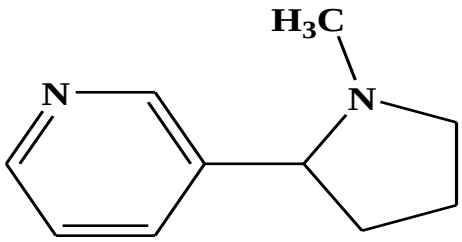
Table 1. Composition of Mueller-Hinton medium.

Materials	Ingredients (g/L)
Beef, Infusion	300.0
Casamino acids	17.5
Starch	1.5
Bacto Agar	17.0
Final pH at 25°C	7.3±0.1



3-(1-methylpyrrolidin-2-yl)pyridine

Figure 1. Nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine, extracted from leaves of *N. tabacum.*

them. Most of the methods for detecting such activities are based on the same principle and are not equally sensitive. The obtained results are profoundly influenced by the selected method and by the microorganisms being used for the required tests. It is clear that biological evaluation in general can be carried out much more efficiently on water soluble, nice crystalline compounds/complexes then on mixtures like plant extracts (Zaidi and Gul, 2005). However, to the best of authors knowledge, the antimicrobial activities of zinc (II) nicotine complexes have not been reported so far.

In our previous study, nicotine was isolated from the seeds of *N. tabacum* and its Zinc (II) complex was synthesized (Munir et al., 1994) and now in the present study, tested these materials for their anti-bacterial sensitivity against ten different micro-organisms.

MATERIALS AND METHODS

Anti-bacterial activity

The anti-microbial sensitivity tests of the title compounds were tested against ten different species of gram positive and gram negative bacteria including *Aeromonas sabriae*, *Salmonella typhii, Shigella boydii*, *Escherichia coli, Vibrio chlolerae, Pseudomonas pseudomallis, Pseudomonas aeroginosa, Bacillus subtilis, Staphylococcus aureus,* and *Streptococcus faecalis.* The compounds were used in two concentrations that is, 100 µg/100 µl (first dose level) and 200 µg/100 µl, the second dose level.

The anti-bacterial activities of nicotine and its zinc complex were determined by 'Agar Well diffusion Method' proposed by Akhtar et al. (1987). According to this method, the weighed components of the dehydrated medium were dissolved in distilled water and made the volume to one litre. Solution was heated till boiling for complete dissolution of the components. The medium was autoclaved at the pressure of 15 lbs/in^2 for 15 min while keeping the temperature of 121°C. The autoclaved medium was then poured in the sterile Petri plates and was allowed to solidify in the clean environment. Then these plates were incubated at 37°C for 24 h to check their sterility.

Preparation of stock solutions

Stock solutions of all the test samples in the concentration of 1 mg/ml were prepared in dimethylsulphoxide and then diluted to 100 µg and 200 µg/ml with the same solvent.

Measurement of antibacterial activity

One loop, full of 24 h old bacterial culture containing approximately 104 to 106 CFU, was spread on the surface of Mueller-Hinton agar plates. The composition of Mueller-Hinton agar medium is given in Table 1. Wells were dug in the medium with the help of sterile metallic borer. The marked area was filled with diluted solutions of the test samples, metal salts and solvent dimethylsulphoxide. These plates were incubated at 37°C for 24 h. At the end of the incubation period, the inhibition zones were measured to the nearest millimeters. Anti-bacterial activity was indicated by a clear zone encircling the marked area. Beyond the marked area, there was a homogenous confluent lawn of bacterial growth.

Comparison with standard antibiotics

The anti-bacterial activity of nicotine and its complex was compared with three standard antibiotics, namely, Gentamicin, Tetracycline and Tobramycin. This was done by agar disc diffusion method (Zaidi and Gul, 2005). In this method, the oxoid multidisc was used in the study. The trypticase soy agar was seeded with over-night culture of the test organisms. An excess of inoculums was removed. The multodisc was aseptically placed over the agar and incubated at 37°C for 24 h. The zone of inhibition was measured to the nearest millimeter.

RESULTS AND DISCUSSIONS

Nicotine anion is useful moieties in forming an extended structure because of its unsymmetrical divergent ligand properties with a nitrogen atom. Structure of synthesized zinc (II) – nicotine complex is given in Figure 2. Here only antimicrobial properties are discussed with reference to the standard antibiotics used.

The anti-microbial sensitivity tests of the presently studied compounds were carried out against the Gram negative and Gram positive organisms. The compounds were used in two concentrations that is, 100 µg/100 µl and 200 µg/100 µl, (first dose level and the second dose level). Results of antibacterial activities of zinc (II) chloride, nicotine and the zinc (II) - nicotine complex for the concentrations of first and second dose level are given in Table 2.

These results indicate that nicotine is inactive at first

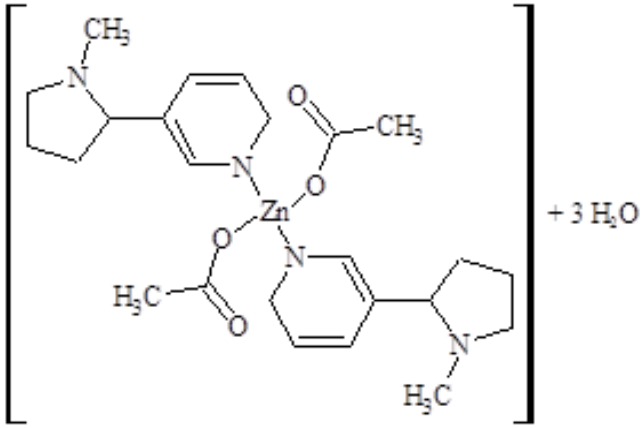


Figure 2. Structure of Zinc(II)–Nicotine complex.

Table 2. Antibacterial activities of standard antibiotics, zinc(II) chloride, nicotine and the zinc(II)-nicotine complex at concentration of first and second dose levels

Compound used	Conc. (µg/100 µg)	Gram Negative Organism							Gram Positive Organism		
		A. sabriae	*S. typhii*	*S. boydii*	*E. coli*	*V. chlolerae*	*P. pseudomallis*	*P. aeroginosa*	*B. subtilis*	*S. aureus*	*S. faecalis*
		Zone of Inhibition (mm)									
Gentamicin	100	20	25	25	40	35	35	36	_	31	_
	200	40	42	39	85	80	85	71	_	40	_
Tetracycline	100	_	_	15	10	51	_	_	45	_	_
	200	_	_	35	27	90	_	_	67	_	_
Tobramycin	100	25	27	31	35	31	34	31	30	30	32
	200	51	60	61	55	50	85	90	65	69	71
Zinc (II) chloride	100	8	–	–	8	6	6	8	–	8	6
	200	10	6	6	10	8	8	10	6	9	8
Nicotine	100	–	–	–	–	–	–	–	–	–	–
	200	–	–	–	14	–	–	14	–	–	14
Zinc(II)– Nicotine complex	100	14	–	14	17	–	16	–	–	15	–
	200	17	–	18	18	17	18	14	17	16	–

dose level, and is effective antibiotic at the second dose level against *E. coli* and *P. aeroginosa* (Gram negative organism) and *S. faecalis* (Gram positive organism) with an inhibition zone of 14 mm. Photos of all inhibition zone are not shown here.

Zinc-nicotine complex inhibited only five bacterial species at first concentration, however at second dose level; it inhibited the growth of eight test bacterial species. In the case of zinc (II) chloride, the zone of inhibition ranged 6 to 18 mm at first dose level and 7 to 20 mm at second dose level.

Compared to nicotine alone; the zinc (II) complex of nicotine is able to inhibit almost all the studied gram positive and gram negative organisms at the higher dose level. These results are also well comparable with the three reference antibiotics that is, Gentamicin, Tetracycline and Tobramycin. Therefore, we comment that this complex is broad spectrum anti-microbial agent active against the variety of gram positive and gram negative bacterial species.

Further research is underway on the antibacterial mechanism of this zinc-nicotine complex that either this is cell wall inhibitors or bactericidal or bacteriostatic. However, some researchers (Munir et al., 1994, 1995; Chohan et al., 2002) studied considerable changes in the bacterial cell membranes upon metal ion treatment, which might be one of the cause or consequence of cell death.

Conclusion

Zinc is relatively abundant element in biological organisms, plays an essential role in the large number of enzymatic reactions. Having the broad spectrum anti-microbial activities, zinc and its nicotine compounds may be used as a therapeutic agent and anti-sickness agent playing a role in the prevention of pain crisis in sickle-cell disease and in the treatment of various sicknesses.

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