



## Antimicrobial activity of picolinic acid

Oladipo I. C<sup>1</sup>, Adeoye I.O<sup>2</sup> and Onawumi O.O.E<sup>2</sup>

<sup>1</sup>Department of Science Laboratory Technology, Ladoke Akintola University of Technology Ogbomoso Nigeria.

<sup>2</sup>Department of Science Pure and Applied Chemistry, Ladoke Akintola University of Technology Ogbomoso Nigeria.

### ARTICLE INFO

#### Article history:

Received: 4 March 2013;

Received in revised form:

17 April 2013;

Accepted: 6 May 2013;

#### Keywords

Antimicrobial,  
Picolinic Acid,  
*Proteus vulgaris*,  
*Bacillus subtilis*.

### ABSTRACT

The antibacterial properties of picolinic acid was tested against selected test organisms which includes *serratia marcescens*, *micrococcus luteus*, *proteus vulgaris*, *proteus mirabilis*, *bacillus cereus*, *bacillus subtilis*, *klebsiella pneumoniae*, *escherichia coli*, *shigella flexneri*, *lactococcus lactis*, *enterobacter cloacae*, *staphylococcus aureus*. The results obtained established that picolinic acid has antibacterial activities against *S. marcescens*, *K. pneumoniae*, *E. coli*, *S. flexneri*, *B. cereus*, *P. vulgaris*, *M. luteus* with minimum inhibitory concentration of 0.5mg/mL. It also had great antibacterial activity against *P. mirabilis* at minimum inhibitory concentration of 1.5mg/L. Picolinic acid evoked a strong antibacterial activities against *B. subtilis*, *S. aureus* and *L. lactis* at minimum inhibitory concentration of 2.0mg/mL and against *E. cloacae* at minimum inhibitory concentration of 1.0mg/mL. The diameter of zones of inhibition ranges between 7.0mm and 14.0mm.

© 2013 Elixir All rights reserved.

### Introduction

There has been considerable interest in the development of novel or adoption of new compounds as antimicrobial, antidepressant, antiviral, antimalarial, analgesic and antibacterial agents. Multiple antibacterial resistances of the bacterial pathogens are of great concern in drug production and also in production of preservatives in food industry. Antibacterial resistance is a serious problem in the treatment of human patients with infectious disease and also food contaminated with some bacterial pathogens like *s. aureus* has become resistant to many antibacterial through the acquisition of drug resistance genes via the role of plasmids in them. It has been discovered that picolinic acid and its derivatives are good food preservatives. Picolinic acid has been the subject of much interest to various researchers. This is mainly because of its wide ranging applications; it is a naturally occurring end product of L-tryptophan catabolism, a natural prime chelator. In the human body, it is synthesized in the liver, kidney, and other organs (Fernandez-Pol et al., 2001 Evans; Johnson, 1980). It also has noticeable antitumor activities in mice (Leuthauser et al., 1982). The effects of picolinic acid and derivatives on various microorganisms have been reported. Accordingly, *Escherichia coli* growth and *Bacillus subtilis* sporulation are transiently inhibited by picolinic acid because of its metal-chelating property (Collins et al., 1979; Fortnagel; Freese, 1968).

Borawska et al. (2008) reported that antimicrobial activity of picolinic acid (PA), sodium picolinate (PS), potassium picolinate (PP), benzoic acid (BA), sodium benzoate (BS) and potassium benzoate (BP) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* were measured using micro broth dilution method. Picolinic acid and sodium picolinate show a high antimicrobial activity. Recently, a comparative assessment of picolinic acid with common chemical preservatives in ginger fruit juice showed that it has a much better preservative power than most common chemical preservatives (Akinwande et al 2012).

Picolinic acid chelates metal ions, such as  $Zn^{2+}$  and  $Fe^{2+}$ , it is likely that its antimicrobial activity against *mycobacterium avium* complex organisms ( i.e a pulmonary infection caused by bacteria) is owing to its ability to chelate metal ions, especially Fe ions which are an essential nutrient for bacteria. Therefore, it appears that the activity of picolinic acid in inhibiting/killing extracellular *mycobacterium avium* complex is principally attributable to its iron-chelating function, which causes the depletion of nutrient iron essential for bacterial growth (Louis et al., 2001; Cai et al., 2006).

In addition, picolinic acid is a potent co-stimulus in the induction of macrophage - or neutrophil-mediated microbicidal activity against *Candida albicans* (Abe et al., 2004; Mucci et al., 2003; Applberg, 2000).

This study is aimed at evaluating the antibacterial properties of picolinic acid against selected bacterial pathogen and to determine their minimum inhibitory concentration against the selected bacterial pathogens.

### Material and methods

The picolinic acid was collected at The department of Pure And Applied Chemistry Ladoke Akintola University of Technology Ogbomoso, Nigeria.

Also the test organism was collected from The Department of Microbiology University of Ibadan, Nigeria. The test organism includes: *Serratia marcescens*, *Micrococcus luteus*, *Proteus vulgaris*, *Proteus mirabilis*, *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Shigella flexneri*, *Lactococcus lactis*, *Enterobacter cloacae*, *Staphylococcus aureus*.

Agar plate dilution test was used to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent.

### Preparation of Picolinic Acid Solution

Solutions of picolinic acid were distilled water and perforated filter paper soaked in it.

Dilutions of picolinic acid were prepared in sterile distilled water at ten times concentration.

### Preparation of Plates

500ml Muller hilton agar of each flask was sterilized in a pressure pot and allowed to cool in water bath at 50°C. Appropriate volume of picolinic acid was added to each flask at 10 times concentration, mixed thoroughly and Mueller hitton agar media was immediately poured on to a plate. The picolinic acid was serially diluted and the soaked perforated filter disc was added to the according to Washington and Wood (1995). The concentration of picolinic acid in different plates was 0.5mg/mL, 1.0mg/mL, 1.5mg/mL, 2.0mg/mL and 2.5mg/mL.

Inoculum for the MIC test was prepared by taking colonies from an overnight culture and inoculated into broth. The broth culture was incubated at 35° for 3 hours until it reaches the desired turbidity. This was then swabbed into the prepared plate and the picolinic acid soaked paper disc was introduced into sterile plate and incubated for 24 hours.

The picolinic acid was serially diluted and perforated filter disc were soaked in each concentration of picolinic acid according to the modified methods of Washington and wood (1995). The concentration of picolinic acid in different plates was 0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml and 2.5mg/ml.

Antibacterial susceptibility test of the isolated organism was done by disc diffusion using the Kirby-Bauer technique (Bauer *et al.*, 1966) and as per recommendation of NCCLS (NCCLS 1997). Panels of picolinic acid were used. All tests were performed on Mueller Hilton agar. The surface was lightly and uniformly inoculated by cotton swab. Prior to inoculation, the swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5Mc farland standard. The swab stick was then took out and squeezed on the wall of the test tube to discard extra suspension

The picolinic acid soaked paper disc was placed on agar surface of the plates already swabbed with different selected bacteria. Inoculated agar plates were allowed to stand until the soaked paper disc were completely absorbed and after then it was incubated at 35°C for overnight. On the next day, plates were read by taking measurement of zones of inhibition. Result were recorded and graded as resistant (R) and sensitivity (S) according to the reference zone of inhibition of particular picolinic acid.

### Results and Discussion

Antibacterial activity of picolinic acid against selected bacteria pathogen are shown in Table 4.1 The results of the Minimum Inhibitory Concentration of Picolinic acid are shown in Table 4.2 There were variations in the results of the antibacterial activity of the picolinic acid against selected bacterial pathogens. Picolinic acid has antibacterial activity against *Serratia marscesces*, *Klebsiella pneumonia*, *Escherichia coli*, *Shigella flexneri*, *Bacillus cereus*, *Proteus vulgaris* and *Micrococcus luteus* with minimum inhibitory concentration of 0.5mg/mL as shown in Table 4.2.

It also had antibacterial activity against *Enterobacter cloacae* with minimum inhibitory concentration of 1.0mg/mL as shown in Table 4.2. The diameter of zones of inhibition was 7.0mm (Table 4.1).

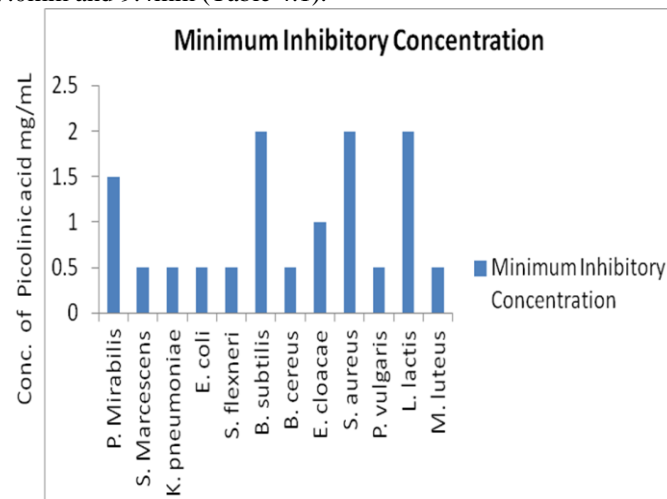
Picolinic acid had antibacterial activity against *Serratia marscesces*, *Klebsiella pneumonia*, *Escherichia coli*, *Shigella flexneri*, *Bacillus cereus*, *Proteus vulgaris* and *Micrococcus luteus* with minimum inhibitory concentration of 0.5mg/mL as shown in Table 4.2.

It also had antibacterial activity against *Enterobacter cloacae* with minimum inhibitory concentration of 1.0mg/mL

as shown in Table 4.2. The diameter of zones of inhibition was 7.0mm (Table 4.1).

Picolinic acid had antibacterial activity against *Proteus mirabilis* with minimum inhibitory concentration of 1.5mg/mL (Table 4.2). The diameter of zones of inhibition was 8.0mm as shown in Table 4.1.

Furthermore, picolinic acid has antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Lactococcus lactis* with minimum inhibitory concentration of 2.0mg/mL as shown in Table 4.2. The diameter of zones of inhibition ranges between 7.0mm and 9.4mm (Table 4.1).



### Conclusion

Picolinic acid in this study had a broad spectrum of activity against selected test bacteria and could be used as antibacterial agent in food industry.

In lieu of this, a similar result was gotten on the antibacterial effect using a micro broth dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) and applied by other authors as well Espinel–Ingroff *et al.* (1995), Speciale *et al.* (2002) and Woods *et al.* (1995)

In this present study it was found that picolinic acid showed broad spectrum of antibacterial activity just like the alkaline picolines (koczoń *et al.*, 2005). This finding also suggest possible usefulness of picolinic acid as a food preservative due to its non-toxic nature. Evans and Johnson (2001) reported that picolinic acid can be found in man so ingesting a food containing picolinic acid as its preservative will not cause harm to man.

### References

- Abe S., HU, W., Ishibashi H., Hasumi K., Yamaguchi H., Augmented inhibition of *Candida albicans* growth by murine neutrophils in the presence of a tryptophan metabolite, picolinic acid. *Journal of Infection and Chemotherapy*, 10,( 3),181-184,
- Akinwande, B. A., Adeoye, I. O. and Anjorin, I. B.(2012) Comparative assessment of picolinic acid with common chemical preservatives in ginger juice during *African Journal of Pure and Applied Chemistry* Vol. 6(13), pp. 179-183
- Bauer AW, Kirby WM, Sherris JC, Turck M. 1966 Antibiotic susceptibility testing by a standardized single disk method *Am J Clin Pathol.* 45(4):493-6
- Borawska MH, Czechowska SK, Markiewicz, R, Palka, J, RenataŚwisłocka R, Lewandowski W (2008). Antimicrobial activity and cytotoxicity of picolinic acids and selected picolines as new potential food preservatives. *Pol. J. Food Nutr. Sci.* 58(4):415-418

**Table 4.1: Antibacterial activity of picolinic acid against selected bacteria pathogen**

TEST ORGANISMS	0.5mg/mL	Conc. 1.0mg/mL	1.5mg/mL	2.0mg/mL	2.5mg/mL
<i>Proteus mirabilis</i>	R	R	8.0	9.5	10.5
<i>Serratia marcescens</i>	8.0	9.5	10.5	11.0	14.0
<i>Klebsiella pneumonia</i>	7.0	7.5	8.5	9.0	9.5
<i>Escherichia coli</i>	7.0	7.5	7.8	8.0	8.5
<i>Shigella flexneri</i>	7.0	7.4	7.8	8.0	8.6
<i>Bacillus subtilis</i>	R	R	R	7.5	8.5
<i>Bacillus cereus</i>	7.0	7.2	7.4	7.8	8.0
<i>Enterobacter cloacae</i>	R	7.0	7.4	7.8	8.2
<i>Staphylococcus aureus</i>	R	R	R	7.0	7.5
<i>Proteus vulgaris</i>	7.0	7.4	8.0	10.0	11.0
<i>Lactococcus lactis</i>	R	R	R	8.5	9.4
<i>Micrococcus luteus</i>	7.0	7.5	8.0	8.5	9.0

**Table 4.2: Minimum Inhibitory Concentration of Picolinic acid**

TEST ORGANISMS	MINIMUM INHIBITORY CONCENTRATION
<i>Proteus mirabilis</i>	1.5
<i>Serratia marcescens</i>	0.5
<i>Klebsiella pneumonia</i>	0.5
<i>Escherichia coli</i>	0.5
<i>Shigella flexneri</i>	0.5
<i>Bacillus subtilis</i>	2.0
<i>Bacillus cereus</i>	0.5
<i>Enterobacter cloacae</i>	1.0
<i>Staphylococcus aureus</i>	2.0
<i>Proteus vulgaris</i>	0.5
<i>Lactococcus lactis</i>	2.0
<i>Micrococcus luteus</i>	0.5

Cai, S.; Sato, K.; Shimizu, T.; Yamabe, S.; Hiraki, M.; Sano, C.; Tomioka, H. 2006 Antimicrobial activity of picolinic acid against extracellular and intracellular *Mycobacterium avium* complex and its combined activity with clarithromycin, rifampicin and fluoroquinolones. *The Journal of Antimicrobial Chemotherapy*, 57, 1, 85-93,

Collins J. J., Adler C. R., Fernandez-Pol J.A., Court D., Johnson G. S., (1979) Transient growth inhibition of *Escherichia coli* K-12 by iron chelators: *in vivo* inhibition of ribonucleic acid synthesis. *Journal of Bacteriology*, 138(3), p. 923-932,

Collins, J. J.; Adler, C. R.; Fernandez-pol, J.A.; Court, D.; Johnson, G. S 1979 Transient growth inhibition of *Escherichia coli* K-12 by iron chelators: *in vivo* inhibition of ribonucleic acid synthesis. *Journal of Bacteriology*, 138, 3, 923-932,

Espinel-Ingroff, A., A. Fothergill, J. Peter, M.G. Rinaldi and T.J. Walsh, 2002. Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for *Aspergillus* spp.: NCCLS collaborative study. *J. Clin. Microbiol.*, 40: 3204-3208.

Evans G.W., Johnson P.E., (1980). Characterization and quantitation of a zinc-binding ligand in human milk. *Pediatr. Res.* 14:876-880.

Fernandez-Pol J.A., Hamilton P.D., Klos D.J., (2001). Essential viral and cellular zinc and iron containing metalloproteins as targets for novel antiviral and anticancer agents: implications for

prevention and therapy of viral diseases and cancer. *Anticancer Res.* 21:931-58.

Fortnagel, P.; Freese, E. 1968 Inhibition of aconitase by chelation of transition metals causing inhibition of sporulation in *Bacillus subtilis*. *The Journal of Biological Chemistry*, v. 243, n. 20, p. 5289-5295,

Leuthauser, S. W.; Oberley, L. W.; Oberley, T.D. 1982. Antitumor activity of picolinic acid in CBA/J mice. *Journal of the National Cancer Institute*, 68 (1)123-126,

NCCLS (1997). Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved

Standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, PA

Pais, T. F.; Appelberg, R. 2000 Macrophage control of mycobacterial growth induced by picolinic acid is dependent on host cell apoptosis. *Journal of Immunology*, 164, 1, 389-397.

Speciale A., Musumeci R., Blandino G., Milazzo I., Caccamo F., Nicoletti G., Minimal inhibitory concentrations and time-kill determination of moxifloxacin against aerobic and anaerobic isolates. *Int. J. Antimicrob. Agents.*, 2002, 19, 111-118.

Woods G.L., Washington J.A., Antibacterial susceptibility tests: dilution and disk diffusion methods. 1995, in: *Manual of Clinical Microbiology* (ed. P.R. Murray). ASM Press, Washington, 1334-1336.