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Bactericidal effects of 8,9-dihydroxy-1,5,6,10b-tetrahydropyrrolo[2,1-a]isoquinolin-3(2H)-one (Trolline) on selected entero-pathogenic bacteria

Sadiq Masud E.[*1] and Abdullahi Mikailu S[2]

[1]*Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria*
[2]*Nigerian Institute of Leather and Science Technology, Zaria, Nigeria*

QR Code

***Correspondence Info:**
Sadiq Masud E.
Department of Biochemistry,
Usmanu Danfodiyo University, Sokoto, Nigeria

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Abstract

The activity of the compound 8,9-dihydroxy-1,5,6,10b-tetrahydropyrrolo[2,1-a]isoquinolin-3(2H)-one (trolline) isolated from *Mirabilis jalapa* against selected enteropathogenic bacteria is being investigated. Clinical isolates of *Streptococcus feacalis, Shigella dysenteriae, Salmonella typhi, Bacillus cereus, Eschericia coli and Vibrio cholera* were screened for susceptibility to serial dilutions of an initial stock concentration of 100µg/ml (456.62µM) of the compound. Zones of inhibition on well labeled sub-cultured agar plates were measured in millimeters and recorded. The minimum inhibitory concentration (MIC) was determined using broth dilution methods while the least concentration that killed the bacterial cells was recorded as the minimum bactericidal concentration (MBC). *S. typhi, B. cereus, E. coli and S. dysenteriae* were susceptible to the test compound, with zones of inhibition measuring 32mm for *S. dysenteriae.* MIC of 57µM was observed for *S. typhi, B. cereus and S. dysenteriae* while bactericidal concentration (MBC) ranged between 114-228µM for all susceptible organisms tested. In conclusion, the *in-vitro* assessment of trolline shows the compound possess bioactive properties against diarrhea causing enteropathogenic bacteria.

Keywords: *Trolline, Mirabilis* jalapa, enteropathogenic bacteria, antibacterial.

1. Introduction

Enteric infections/diseases are usually associated with poor public sanitary condition which so far is a problem in developing countries. Failure to control the spread of enteric pathogens continues to worsen cases of diarrhoeal related diseases especially in regions of unrest, amongst displaced persons and urban conurbations. Studies have shown there is a risk of emergence of enteric pathogens with multidrug resistance as a result [1]. Development of resistance may be attributed to drug supply chain, misuse/abuse and exposures to the environment among others [2]. Since humans are primary hosts and reservoirs of these pathogens, then exporting "new virulent strains" may become inevitable [3].

Repeated bouts of diarrhoeal diseases in children prove to be life threatening and a major risk to proper growth and development in their formative years [4]. The absorptive function of the gastro-intestinal tract (GIT) is critical to optimal development of the brain and neuronal synapsis that determine human capacity. Thus a lasting impact is experienced in the growing child exposed to poor nutrient absorption, frequent diarrhoea and malnutrition [5]. It is estimated that six countries (including Nigeria) account for over 10,000,000 deaths in children under the age of five years from diarrhoeal related enteric infections, with malnutrition as underlying cause [6].

Host-pathogen Pathophysiological interactions in diarrheal diseases involve several processes associated with signaling pathways and transport of solutes across intestinal epithelial cell membranes. Pathologic mechanisms leading to disease states are diverse as the organisms themselves; some are directly linked to invasive capabilities of the organisms or their ability to produce enterotoxins [7]. While there are no intestinal histological changes in cholera

for example, *Salmonella spp* and *Shigella spp* infections result in extensive intestinal inflammatory changes leading to production and release of cytokines that affect intestinal epithelial functions which may lead to diarrhea [8].

Medicinal plants over the years have been recognized as major sources of natural product useful in the treatment of various diseases that are of infectious or non-infectious origin. The plant *Mirabilis jalapa* L. known to have medicinal properties is used in the treatment of various diseases including dysentery [9] and other diarrhoeal diseases [10,11]. Phytochemical analysis of the roots, leaves, stem and seeds reveal the plant to contain alkaloids, flavonoids, phenols, steroids, triterpenes, glycosides, amino acids, tannins and saponins [12]. Phytoconstituents of the various parts of the plant have been demonstrated to possess pharmacologic effects including antidiabetic [13], and anti-inflammatory [14]. Reports by several authors [15-17] have demonstrated bioactive potentials of methanol extracts of aerial parts of the plant on bacterial cells. The compound 8,9-dihydroxy-1,5,6,10b-tetrahydropyrrolo[2,1-a]isoquinolin-3(2H)-one (trolline) isolated from the aerial parts of *Mirabilis jalapa* [18] and *Portulaca oleracea* [19] had been shown to be moderately absorbed via the gastrointestinal tract as demonstrated using human Caco-2 cell monolayer models [20]. The present study investigates *in vitro* antibacterial effects of the compound trolline on clinical isolates of some diarrheal causing enterobacteria.

2. Materials and methods

Isolation of trolline from *M. jalapa* and spectroscopic characterization (data presented below) of the compound have been previously described [18]. Spectroscopic analyses and characterization were done at the International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan.

^{1}H NMR (600MHz, MeOD) δ 6.55-6.53 (s, 2H), 4.72 (dd *J*= 18.0, 7.8, 1H, 4.08 (ddd, *J*= 12.9, 6.0, 2.8, 1H), 3.04 (td, *J*= 12.0, 4.3, 1H), 2.76-2.70 (m, 1H), 2.66-2.60 (m, 2H), 2.56 (dd, *J*= 17.9, 7.9, 1H), 2.41-2.36 (m, 1H), 1.80-1.73 (m, 1H); **^{13}C NMR** (151MHz, MeOD DEPT) C 175.9, 145.6, 145.5, 129.8, 125.5; CH 116.2, 112.4, 58.3; CH_2 38.6, 32.7, 28.8, 28.8; **HREIMS** M* 219.0882 Molecular formula $C_{12}H_{13}O_3N$; Ring plus double bond (RDB) analysis =7; **FTIR (**3425, 2925, 1649, 1527, 1452cm^{-1})

2.1 Bacterial cell cultures

Clinical isolates of *Streptococcus feacalis, Shigella dysenteriae, Salmonella typhi, Bacillus cereus, Eschericia coli and Vibrio cholera* were obtained from the microbiology medical Department of Ahmadu Bello University Teaching Hospital, Shika, Zaria. The organisms were subcultured using sterile nutrient agar plates to obtain pure isolates of the microbial cells and preserved at 0^{o}C until needed.

2.2 Antimicrobial susceptibility screening

Exactly 1.0mg of trolline was weighed and dissolved in 10mls dimethylsulphoxide (DMSO) to obtain 100µg/ml stock solution. Two-fold dilutions were carried out using micropipettes to obtain working solutions of 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/ml. To well-labeled nutrient agar plates pre-seeded with selected microbes, 100µL of prepared working solutions was added to wells 6mm in diameter. The plates were then incubated at 37^{o}C for 24 hours. Zones of inhibition where no growth was observed was measured and recorded in millimeters using a transparent ruler and recorded. Similar preparations were made for positive control (ciprofloxacin and sparfloxacin 100µg/ml) and negative control containing only DMSO.

2.3 Determination of minimum inhibitory and bactericidal concentrations

Following methods described by EUCAST [21], prepared nutrient broth in test tubes was diluted until turbidity of solution compares, by visual inspection to the Mc-Farland turbidity standard No. 5, with an approximate microbial cell concentration of 1.5 x 10^{8} cfu/ml. to each prepared standard test solutions in test tubes, 100µL of organisms was introduced with gentle votexing for complete dispersal of cells. The cells were incubated at 37^{o}C for 24 hours. The least concentration where no change in turbidity was observed was recorded as the minimum inhibitory concentration (MIC). The minimum Bactericidal Concentration (MBC) was determined by subculturing the contents of each tube from the MIC experiments on prepared and well labeled sterile agar plates for 24 hours at 37^{o}C. The plate containing concentration of the compound observed to have no microbial growth suggest the cells were completely killed. This was recorded as the MBC.

3. Results

The effects of 100µg/ml (456.62µM) concentration of trolline on test organisms are presented in Figure 1. Four organisms were susceptible: *B. cereus, E. coli, S. typhi* and *S. dysenteriae*, with *S. dysenteriae* being most susceptible having a zone of inhibition of 32mm as shown in Figure 2. *V. cholera* and *S. feacalis* were resistant at tested concentrations. The control drug sparfloxacin significantly inhibited the growth of all tested microbial cells.

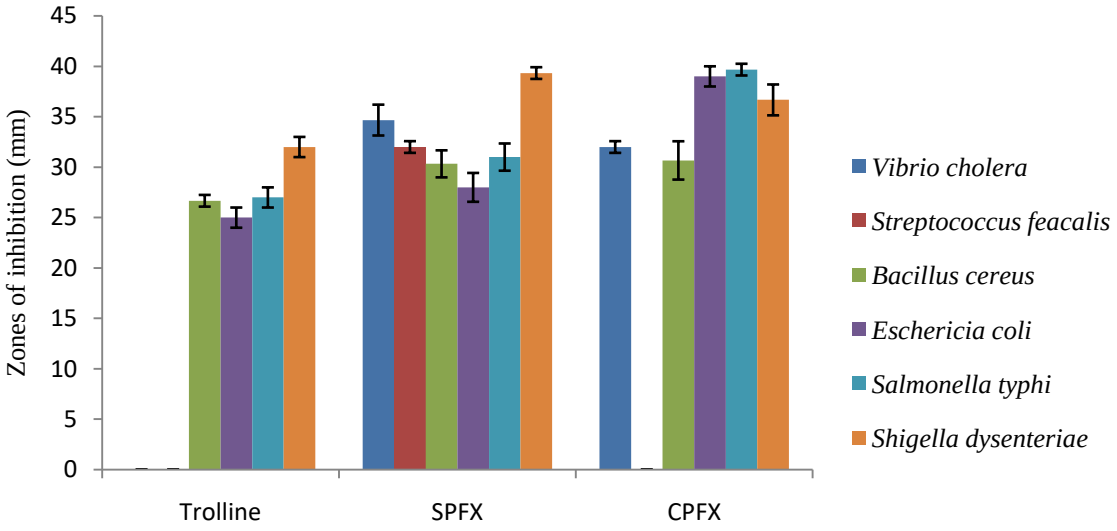

Figure 1: Zones of Inhibition of Trolline on Susceptible Organisms

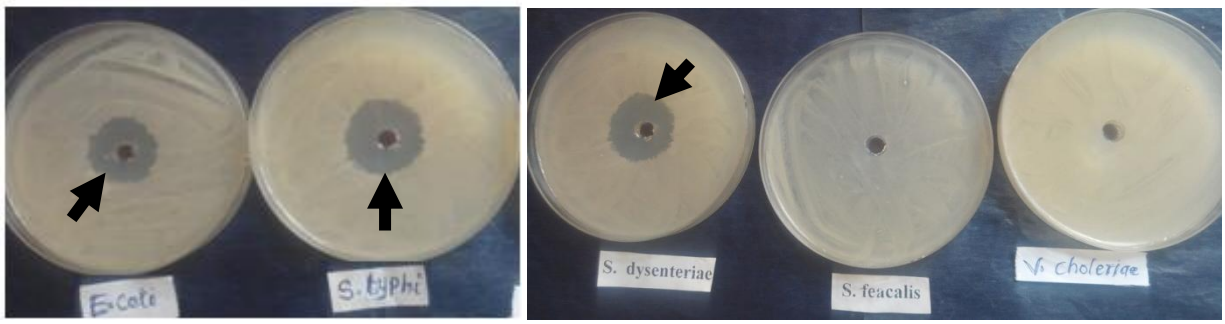

Figure 2: Insert arrows show clear zones of inhibition of trolline against test organisms.

Bacteriostatic and bactericidal potency of trolline (Figure 3) showed a minimum inhibitory concentration (MIC) of 57µM against *B. cereus*, *S. typhi* and *S. dysenteriae* while growth of *E. coli* was inhibited at 114µM. The least minimum bactericidal concentration (MBC) recorded was observed for *S. dysenteriae*, with MBC values of 114µM. The MBC observed for the other susceptible cells was 228µM.

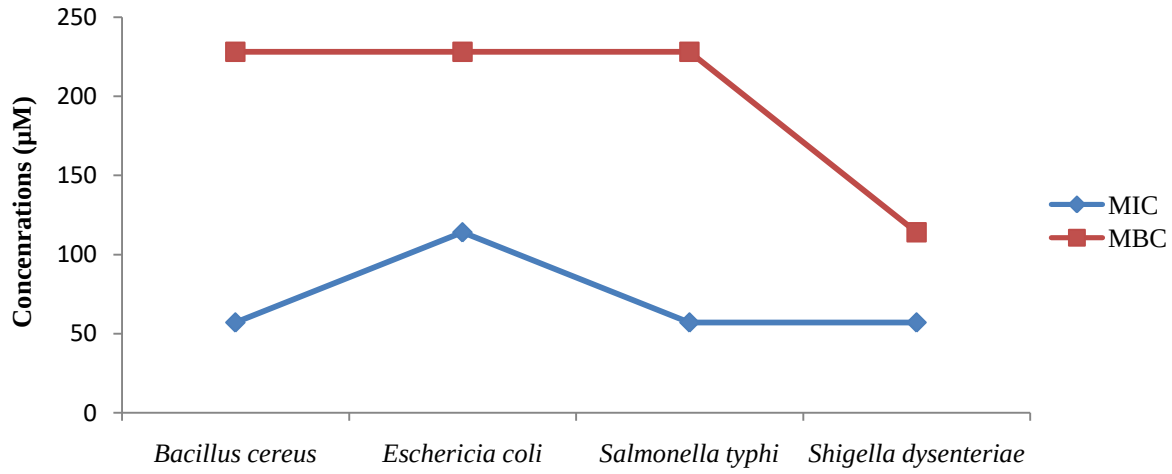

Figure 3: Minimum Inhibition and Bactericidal Concentrations of Trolline on Susceptible Organisms

4. Discussion

The compound trolline demonstrated as a bacteriocide against pathogenic enterobacteria specifically against *S. dysenteriae* reveals the importance of the medicinal plant *Mirabilis jalapa* as an antibacterial and its use in treatment of diarrhoeal diseases [17,18,22,23]. Similar findings reported by Wang et al [24] Also showed trolline to have antibacterial activity against *Staphyloccocus aureus, Staphylococcus pneumonia* and *Klebsiella pneumonia*. Diarrheal diseases resulting from infectious enterobacteria not only compromise well being but also nutritional status especially in children less than five years. The increasing threats of drug resistant organisms put further constraints on health care services thus necessitating the urgent need to augment existing medicines with new drug candidates. Synthetic pyrrolo[2, 1-a] isoquinoline based compounds are reported to have hypotensive, antitumor [25] and antioxidant activities [26]. The bioactivity associated with the pyrrolo[2, 1-a] isoquinoline core interestingly is the subject of several techniques for synthesis of novel heterocyclic analogs [27-29]. Studies on the pharmacokinetics and mechanism of bacterial growth inhibition may unlock the potential of this compound as a prospective candidate either as a drug component or nutraceutic for preventive intervention or low-cost administration in disease prone areas.

5. Conclusion

The compound 8,9-dihydroxy-1,5,6,10b-tetrahydropyrrolo[2,1-a]isoquinolin-3(2H)-one (trolline) a phytoconstituent of the aerial parts of *Mirabilis jalapa* has antibacterial potential especially against *S. typhi, B. cereus* and *S. dysenteriae* as suggested by findings of this research. The search for bioactive natural products would continue to furnish novel carbon skeletons that may be useful in the fight against infectious organisms and other forms of diseases.

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