

Synthesis, Characterization and *In vitro* Antibacterial Activity of A Novel Strigolactones Analogues TIT3

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Abstract

Traditional antibiotic abuse caused increased the incidence of bacteria resistance to treatment. Strigolactones (SLs) are phytohormones that play a vital role in the plant growing root, shoot and flowering. We previously reported that, SLs analogues exert pro-apoptotic effects on HepG2 cell lines. The current study investigated the antimicrobial activity of selected SLs analogue TIT3 against different strains of bacteria including gram positive and gram negative bacteria: *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae* and *B. subtilis*. The results obtained were compared with 100 µg amoxicillin. Results obtained showed that, TIT3 inhibited the growth of *S. cereus* (50 µg), *Salmonella typhimurium* (20 µg), *Escherichia coli* (30µg), *Klebsiella pneumoniae* (50 µg) and *B. subtilis* (50 µg) with mean inhibition zones diameter being 12.1 mm , 13.2 mm, 12.5, 8.9 and 12.9 mm respectively. It was concluded that, TIT3 is promising effective antibacterial agents compared with amoxicillin for different strains of bacteria that resistant to different antibiotics.

Keywords: Strigolactons analogues, TIT3, antibacterial activity

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INTRODUCTION

Traditional antibiotic abuse caused increased the incidence of bacteria resistance to treatment. Bacteria that resist to major antibiotics have ability to grow and transform to dangerous species. Control of infections induced by multidrug-resistant species is a challenge obstacle for hospitals¹. In Clinics, patients suffered from these bacteria need more time and much cost for relief². Development of a novel antibacterial compounds with high efficacy from medicinal plants are suitable alternative sources. Microbial resistance to chemotherapeutic by antibiotics is considered as one of the most problems in medicine³. This resistance is an event of the adaption of pathogenic bacteria to antibiotics used in hospital^{4,5}. Some microbes have developed resistance to all types of antibiotics and these multidrug-resistant microbes are dangerous to invade different tissues⁶. In order to design a novel antibiotic with different mechanism of action, plants are considered as a main sources for new bioactive compounds with high efficacy. Phytochemical are vital sources of compounds against diverse organisms including fungi, yeasts and bacteria⁷. It exerts its activity by inhibiting cell membrane synthesis, modify bacterial permeability and sensitivity.

However, only 35 percent of the microbial infection have been treated using synthetic or derivatives products. This is due to resistant of pathogens for prolong use of antibiotics [8]. The resistance to antibiotics increased dramatically due to mutation of microbial genome. To decrease the microbial resistance to antibiotics is by explore a novel antibiotic resistance inhibitors produced from plant origin⁹. It was found that, plants possess ability to protect themselves against different pathogens by producing a variety of phytohormones. Medicinal plants are widely used as complementary medicine. Due to their safety to human phytomedicines are used as therapeutic index for the exploring novel drugs. It can be used as anticancer, antioxidants and anti-inflammatory¹⁰.

Strigolactones (SLs) are a novel class of phytohormones synthesized by different plant species¹⁰. The SLs structure consists fused-ring system connected via an enol ether bridge¹¹. Previous study reported that, SLs exert its effect

by regulating different parts in plant development including shoot and roots¹². It has been reported that, SLs analogs stimulate cell cycle phases arrest and programme cell death in different types of human cancer¹³. It was found that, growth inhibition of normal cells treated with SLs analogs. In other study, inhibition was observed in some cancer cells cell lines¹⁴.

Moreover, SLs showed as new anti-cancer agent in breast cancer¹⁵. SLs analogs showed down-regulation of proteins involved in cell cycle (cyclin)¹⁶. These facts proposed that SLs are potent and promising anti-cancer activity. SLs enhance induction of G2 arrest, stimulate apoptosis and suppress viability¹⁷. The bacteria is protected by thick cell wall that composed of layers of peptidoglycan, cross-linked covalently to form polymer of peptide-linked β -(1-4)-*N*-acetyl glucosamines. The mechanical strength of the cell wall is important for bacterium's ability to survive under vigorous environmental conditions as osmotic pressures. The degree of cross-linking can be correlated with the structural integrity of the cell. It was reported that, transglycosylase and transpeptidase enzymes, which add disaccharide pentapeptides contribute to peptidoglycan formation and cell wall integrity¹⁸. In order to contribute to explore a novel SLs analogues (TIT3) as antimicrobial activity against different pathogenic bacteria. This study investigated the antimicrobial activity of TIT3 against different species of bacteria as *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae* and *B. subtilis*.

MATERIALS AND METHODS

Synthesis of Strigolactones

The SLs (TIT3) was synthesized and identified by Prof. Tadao Asami (University of Tokyo, Japan). We previously mentioned the details of synthesis and published recently¹⁹. Working standard dilutions to 10 μ M was done using DMSO, the concentration of DMSO was maintained at 0.1- 0.5 %. The structure of tested SLs, as TIT3 with their molecular weights was shown in (Fig. 1) compared with amoxicillin as positive control (Fig. 1b).

Determination of MIC

Antimicrobial activity of the TIT3 at different concentrations were tested against

different bacterial strains including *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae* and *B. subtilis*. The bacteria samples were obtained from Microbiology Department, Faculty of Science, KAU . Growth standard media was used (Per one liter media, 1 g glucose, 15 g peptone, 6 g Sodium chloride) at pH 7.0. The antimicrobial activity of TIT3 were tested by using 10 µl of each type was added to sterile test tube contain 10 ml nutrient broth. Blank and negative control samples were done without microbe. After 24 h, growth was visually assessed by changes in turbidity and quantified by measuring O.D at 600 nm (Shimadzu spectrophotometer). Amoxicillin (100 µg/ml) was used as a positive control. The diameters of growth inhibition zones was measured in mm. The initial screen to determine MIC of TIT3 by adding different concentration (0 –100 µg). Each concentration was incubated an overnight with media. After 24 h, growth was visually assessed as mentioned above.

Bioassay the cytotoxicity of TIT3 by using brine shrimp lethality test

The toxicity of TIT3 was determined according to Michael *et al.*¹⁷ at different concentrations from (10- 1000 µg/mL in seawater containing 2% DMSO (v/v). Ten shrimps larvae was used in each test, three replica each test. A positive

control from potassium dichromate solution (LC₅₀ = 20-50 µg) was tested compared to blank test. The survivor count was recorded after 24 hours by using microscope and the mortality percent was calculated. Cytotoxicity was significant if the LC₅₀ < 200 µg/ml.

RESULTS

Bioassay lethality activity of the TIT3 was shown in Table 1. It was found that 30% mortality at 500 µg/ml concentration and its LC₅₀ was 400 µg/ml which was considered moderately toxic. The Standard potassium dichromate revealed LC₅₀ was 200 µg/ml. However DMSO showed no mortality.

Data obtained (Table 2) indicated that TIT3 had variable activity against different species. It was found that, the maximum activity against *S. aureus* at 50 µg/ml, *Salmonella typhimurium* at 20 µg /ml, *Escherichia coli* at 30 µg /ml and *Bacillus cereus* at 50 µg /ml.

We screened different concentrations of three SLs analogues TIT3, (0-100 µg). Results obtained in Fig.s (2-6) showed that, TIT3 at concentrations 50 and 20 µg showed the most effective inhibitory effect on *s.auresu* and *B. subtilis* respectively with inhibitory effect against *E. coli* >80% and 77% inhibition for *B. subtilis* respectively. It is clear that, above these concentrations showed decreased activity.

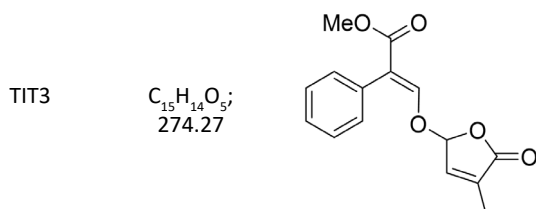


Fig. 1a. structure of TIT3 and its Molecular formula.

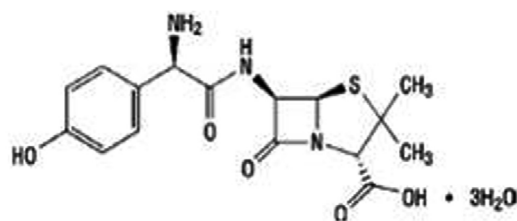


Fig. 1b. Amoxicillin structure ($C_{16}H_{19}N_3O_5S \cdot 3H_2O$)

Table 1. Bioassay of lethality test (mortality %)

compounds	Mortality % at different concentrations µg/ml.						
	10	30	100	200	400	500	
TIT3	1	90	6	16	18	30	Non toxic
Pot.dichromate	3	5.5	5	30	70	90	Toxic
DMSO	4	13.2	4.2	8.7	9	5.5	Non toxic

The values of triplicate experiments (mean ± SE), No of shrimp per test was 10, Scores for LC50, Highly toxic < 20 µg/ml, Toxic up to 200 µg/ml, Non toxic > 500 µg/ml.

DISCUSSION

The cytotoxicity of TIT3 was investigated on shrimp larvae. The data obtained in (Table 1) showed that by at very high concentration and exposure the toxicity increased. Recent published work by our research team Hasan et al.¹⁹ indicated that, TIT3 was effective on HepG2 cell line but more save of normal cell caused early apoptotic cell. For that, TIT3 is more save for eukaryotic cells. The MIC was similar to ampicilline for both Gram-positive and Gram-negative bacteria.

To examine the antimicrobial potential of the different concentrations of TIT3 . Overall, the results obtained revealed that TIT3 induced inhibitory effects against different species of bacteria with different efficacy. We found that by increasing the concentrations of TIT3, in making

it more efficient in inhibiting efficacy. This effect is most pronounced in the case of *S. aureus* at, where the higher concentration is clearly associated with higher inhibition. The antibacterial properties of the TIT3 may be due to interference with cell wall synthesis of bacteria, so inhibiting its growth. TIT3 showed a better antibacterial activity through inhibition of bacterial several enzymes, microbial adhesion, and cell envelope transport proteins²⁰. The antibacterial potential of TIT3 was found to be maximum against *Salmonella typhimurium* at low concentration. This antimicrobial susceptibility reported promising new vision for development and production of a novel agents against different pathogens. Thus MIC determined reflecting that TIT3 has antibacterial potential and that it gives real concentration required to inhibit bacterial

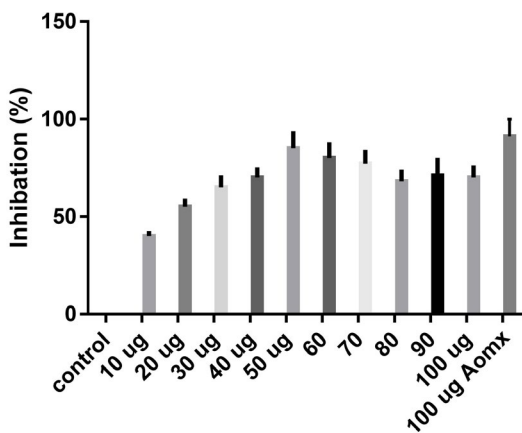


Fig. 2. Inhibition of *S.aureus* Concentrations of TIT3 (ug)

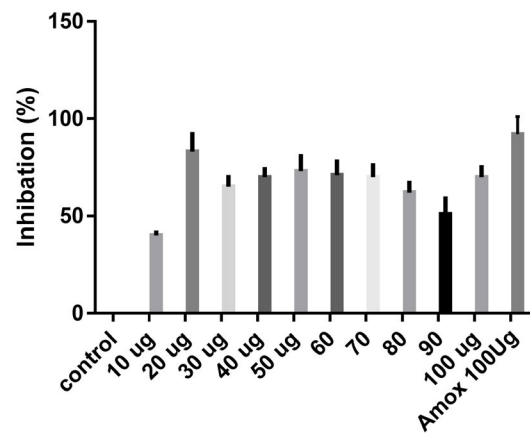


Fig. 3. Inhibition of *Salmonella typhimurium* Concentrations of TIT3 (ug)

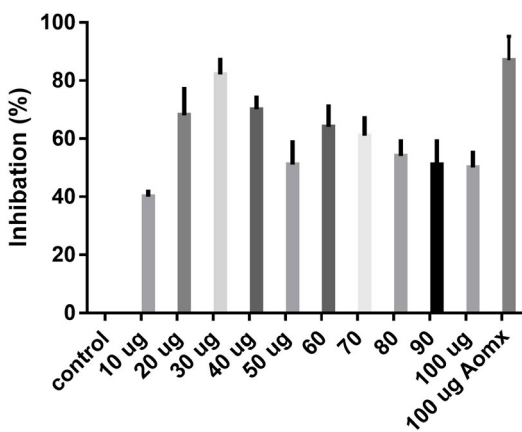


Fig. 4. Inhibition of *Escherichia coli* Concentrations of TIT3 (ug)

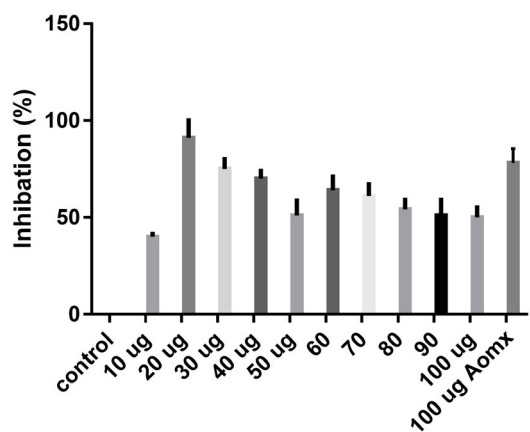
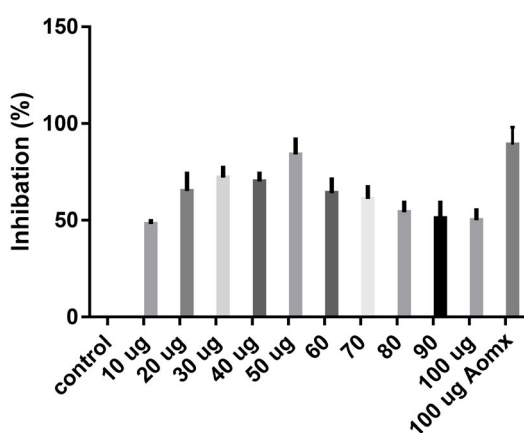


Fig. 5. Inhibition of *Klebsiella pneumoniae* Concentrations of TIT3 (ug)

Table 2. Inhibition activity of TIT3 at different concentration (mm)

Pathogen TIT3 (µg/ml)	<i>S. aureus</i>	<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus cereus</i>
0	---	--	--	---	----
10	3	--	--	5	7
20	4	13.2	4.2	8.7	9
30	8	10	12.5	5	7
40	10	6	7	3	-
50	12.1	5.5	6.6	8.9	12.6
60	8.5	7.6	7.3	6.6	7.6
70	9.1	7.3	8.2	6.2	9.2
80	8.3	6.9	7.1	5.8	7.8
90	7.9	8.1	7.5	3.9	6.4
100	8.9	5.2	4.1	6.3	7.2
Amoxicillin 100 µg/ml	11.9	14.5	13.6	10.5	14.2

**Fig. 6.** Inhibition of *Bacillus cereus* Concentrations of TIT3 (µg)

growth. In conclusion, the TIT3 was found to be more effective against *B. subtilis* and *S. cerevisiae*. The potent activity may be due to the penetrating power of TIT3 to bacterial cell.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors designed the experiments. EH

and SS performed the experiments. AA and SS analyzed the data. AA, EH and SS wrote the manuscript. All authors read and approved the manuscript.

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ETHICS STATEMENT

Not applicable.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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