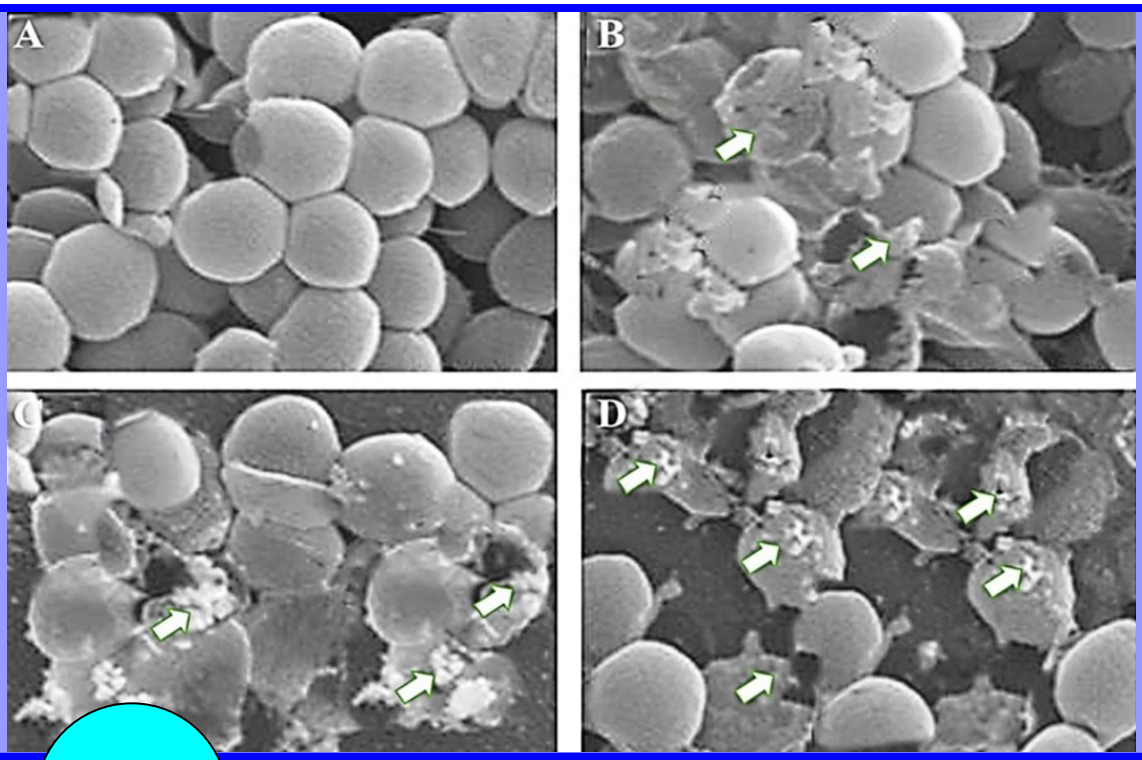

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Antibacterial activity of seneciolactone isolated from *Senecio scandens* against some common gastrointestinal tract disease causing bacterial pathogens

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Abstract

The objective of the present investigation was to study the antibacterial effect of seneciolactone isolated from the methanolic extract of *Senecio scandens* against five bacterial pathogens which are known to cause several gastrointestinal tract diseases. Disc diffusion assay and agar well diffusion assays were used to examine the antibacterial efficacy of this compound by measuring zones of inhibition and MIC/MBC values. Scanning electron microscopy (SEM) was involved to study the effect of this compound on cellular morphology of *Shigella dysenteriae*. Results revealed that seneciolactone exhibited moderate to potent antibacterial activity against different bacterial strains. Zones of inhibition and MIC/MBC values indicated that seneciolactone was most potent against *S. dysenteriae* followed by *Pseudomonas aeruginosa* and *E. coli*. SEM results indicated that seneciolactone induced potent damage to the cell membrane of the tested bacteria. As compared to the untreated control which exhibited normal cellular morphology, the seneciolactone treated bacterial cells revealed severe damage to the cellular membrane particularly at the higher doses.

Introduction

The main cause of mortality in tropical and subtropical countries is the bacterial and fungal infections. The pathogenic microbes have developed multidrug resistance due to the excess use of various antibiotics including those of synthetic origin. This has led to the serious health problems especially in developing countries where these microbial infections are quite common. On many occasions, this drug resistance can result in epidemic as no drug can affect this life-threatening pathogen (Harrison and Svec, 1998; Rao, 1998). The pursuit for antimicrobial compounds has gained increasing significance in recent years, due to growing global concern about the startling increase in the rate of infection by antibiotic resistant microorganisms (Weisser et al., 1996). Nevertheless, there has also been a growing interest in the research for natural products from plants for the discovery of new antimicrobial drugs in the last 30-40 years (Satish et al., 1999; Balandrin et al., 1985). Plants are known to biosynthesize a wide-spectrum of bioactive secondary metabolites such as phenolic compounds, terpenoids, quinones, alkaloids, flavonoids etc with potent antimicrobial activities which certainly has shaped the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (Satish et al., 1999; Jones, 1996; Brito et al., 2015; Mota et al., 2015).

Many bacterial strains have been reported to cause serious gastrointestinal disorders including irritable bowel syndrome (IBS). IBS is a very common gastrointestinal disorder which involves changes in the gastrointestinal microbial population. Although IBS can

be treated with several antibiotics, but recently there has been an upsurge in the appearance of antibiotic resistant bacterial strains which are not susceptible to these commonly used antibiotics (Balandrin et al., 1985).

As such, there is an urgent need of alternative antibacterial agents which can target these drug resistant pathogenic microbes. Several plant based medicines have been reported to be effective against these gastrointestinal tract disease causing pathogenic microbes.

The objective of the present study was to investigate the antibacterial activity of seneciolactone isolated from the methanol extract of *Senecio scandens* against several gastrointestinal tract disease causing bacteria including *Escherichia coli, Salmonella typhimurium, S. typhi, Shigella dysenteriae. Pseudomonas aeruginosa*. We also investigated effects of this compound on bacterial cell morphology in *S. dysenteriae* using scanning electron microscopy.

Materials and Methods

Plant material, extraction and isolation

The aerial plant material of *S. scandens* was collected from Conghua, Guangdong Province (China) in August, 2014. The plant was identified and authenticated by a well-known taxonomist, Prof. Hua-Juan, School of Chinese Medicine, Hong Kong Baptist University. The voucher specimen (HKBU-B-2014-06-39 -042) was deposited at School of Chinese Medicine, Hong Kong Baptist University. The air dried, finely powdered aerial parts (4 Kg) were extracted for 48 hours with chloroform in a soxhlet apparatus to afford the extract, which was concentrated under reduced pressure. The chloroform extract (70 g) was loaded on silica gel (60-120 mesh, 200 g) column and eluted with an increasing gradient of petroleum ether and ethyl acetate. Fractions of 100 mL volume each were collected and pooled according to TLC analysis. The fraction (petroleum ether: ethyl acetate, 50:50) yielded seneciolactone as yellow colored crystalline solid, M.P. 80-82.5°C; UV λmax (methanol): 197 nm, 362 nm; IR: 3420 cm^{-1}, 1767 cm^{-1}, 1718 cm^{-1}, 1610 cm^{-1}, 1629 cm^{-1}, 1470 cm^{-1}.The molecular formula of seneciolactone was assigned as $C_{16}H_{12}O_6$, from HR-EI-MS spectrometry (m/z 300.0637, calcd 300.0634). The absorptions at 1767 cm^{-1} in the IR spectrum indicated the presence of a lactone structure. In the ^{13}C NMR spectrum, 16 carbon signals were observed as one methyl, one methylene, six methines and eight quaternaries. From ^{1}H NMR spectrum, we observed a typical ABX coupling system for a 1, 2, 4-trisubstituted aromatic ring [d7.08 (1H, d), 6.85 (1H, dd), 7.84 (1H, d)], an α, α-disubstituted furan ring substituted at positions of [7.35 (1H, d), 6.85 (1H, d)] and a methyl group [d2.25 (3H, s)], respectively.

Bacterial strains and culture media

E. coli, S. typhimurium, S. typhi, S. dysenteriae. P. aeruginosa were used in the present study. All these bacterial strains were obtained from the State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Shanghai, China. Bacterial strains were grown on nutrient agar plates at 37°C and maintained on nutrient agar slants. The cell suspension of microorganisms in 0.5% NaCl was adjusted to a 0.5 McFarland standard to obtain ~10^5 colony forming units (CFUs)/mL.

Determination of antibacterial activity by agar well diffusion assay

Agar well diffusion assay was performed to evaluate the antibacterial effect of seneciolactone. Briefly, the overnight bacterial cell cultures were added to 50 mL liquid nutrient agar (Sigma-Aldrich). After 30 min of solidification of the agar, different doses of the compound were added to separate wells on the plates and then incubated for 24 hours at 25°C. Subsequent to incubation, the antibacterial activity of the compound was evaluated by calculating the zone of inhibition expressed in millimeter. All experiments were done in triplicate. Vancomycin and streptomycin (20 µg/well each; Sigma-Aldrich) served as positive controls.

Evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC screening were performed using the broth microdilution method (Yu et al., 2004) as already reported. Seneciolactone was dissolved in physiological saline solution supplemented with Tween-80 at final concentration of 1.3%. Different concentrations of the compound were prepared in a 96-well microtiter plate. Each compound concentration was put into the wells containing 100 µL bacterial suspension and then inoculated. The final concentration of each bacterial strain was adjusted to 10^5-10^6 CFU/mL. The concentration of the compound at which the bacterial pathogens do not exhibit visible growth is described as MIC and the compound concentration at which the bacterial pathogens are killed is defined as MBC. Vancomycin and streptomycin (20 µg/well each; Sigma-Aldrich) served as positive controls.

Scanning electron microscopy (SEM) evaluation

Scanning electron microscopy was performed as described previously (Agizzio et al., 2006) with minor modification. In brief, *S. dysenteriae* was grown in LB broth to an exponential phase and centrifuged at 12,000 rpm for 10 min. The compound (0, 10, 25 and 50 µM, seneciolactone dissolved in DMSO) was mixed with an aliquot of bacterial solution (1 x 10^6 CFU/mL) having 5 mM phosphate buffer, pH 6.8. Then the bacterial culture was incubated at 37°C for 4 hours, followed by

washing and resuspending in 5 mM phosphate buffer and fixed overnight at 4°C with 1.5% gluteraldehyde. After this, the bacterial cells were washed three times with phosphate buffer and then treated with 0.8% osmium tetraoxide and then again washed with phosphate buffer followed by washing with distilled water. Subsequently, the bacterial cells were dehydrated with 50% acetone followed by 100% and mounted on cover slips to dry and then coated with gold using a sputter coater. Then the bacterial cells were examined using a scanning electron microscope (JEOL JSM 6060 LV, with the accelerating voltage of 5 kV). Microphotographs were taken at magnifications ranging from 100x to 5000x.

Statistical analysis

All experiments were performed in triplicate and the results are expressed as the mean ± standard deviation. Graphpad Prism version 5.01 (Graphpad Software, Inc., La Jolla, CA, USA) was used to perform statistical analyses and $p<0.05$ was considered to indicate a statistically significant difference.

Results

Antibacterial activity against various bacterial strains

The molecular structure of seneciolactone is shown in Figure 1. The antibacterial efficacy of seneciolactone isolated from *S. scandens* was evaluated against five gastrointestinal tract infection causing bacteria including *E. coli, S. typhimurium, S. typhi, S. dysenteriae and P. aeruginosa.* The antibacterial activity was expressed by measuring the zone of inhibition and MIC/MBC values against different bacteria. Figures 2 and 3 show the results of disc diffusion assay expressed as zones of inhibition while as Table I shows the MIC/MBC values of the compound against these tested bacteria. Seneciolactone exhibited a broad spectrum antibacterial effect against these five tested bacterial strains. *E. coli, S. typhimurium* and *S. typhi* were the lesser susceptible bacterial strains. *S. dysenteriae* at all the tested doses was the most susceptible bacterial strain with zone of inhibition almost equal to that of vancomycin. Vancomycin at a dose of 2.0 µM was used as a positive control.

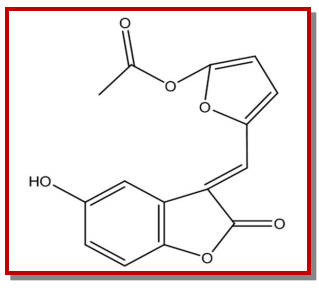

Figure 1: Chemical structure of seneciolactone isolated from *Senecio scandens*

Table I shows the MIC/MBC values of seneciolactone against these five tested bacteria at different doses. From the table also, it appeared that *S. dysenteriae* exhibited the lowest values of both MIC as well as MBC indicating that seneciolactone was most effective against this bacterial strain.

Morphological evaluation of the effect of seneciolactone on S. dysenteriae using SEM

Since *S. dysenteriae* was the most susceptible bacterial strain, we further examined the effect of seneciolactone on the cell morphology of this bacterial pathogen using scanning electron microscopy. SEM findings revealed that seneciolactone damages the cell membrane of *S. dysenteriae.* As compared to the untreated control which exhibited normal cellular morphology, the seneciolactone treated bacterial cultures (Figure 4) showed serious damage to the cellular membrane particularly at the higher doses. As the concentration of seneciolactone increased, the membrane damage also increased.

Discussion

In the present study, the antibacterial effect of seneciolactone isolated from the methanol extract of *S. scandens* was observed against a panel of clinically relevant gastrointestinal tract infection causing bacterial pathogens including *E. coli, S. typhimurium, S. typhi, S. dysenteriae* and *P. aeruginosa*. The results revealed that seneciolactone displayed a broad spectrum antibacterial activity with maximum effect on *S. dysenteriae* showing maximum zone of inhibition and lowest MIC/MBC values. Further, scanning electron microscopic examination revealed that seneciolactone induced cellular membrane damage in *S. dysenteriae*. As compared to the untreated pathogens which revealed normal cell morphology with no obvious damage, seneciolactone-treated bacterial cultures revealed lethal destruction of the cell membrane.

S. scandens locally known as "*Qianliguang*" in China, is one of the most popular species used as a Chinese medicinal herb. Various natural product compounds including pyrrolizidine alkaloids, alkaloids, phenolic acids, flavonoids, terpenes, essential oils, carotenoids and jacaranone glycosides have already been isolated from *S. scandens* (Bohlmann et al., 1977; Batra and Rajagopalan, 1977; Wang and Tu, 1980; Tian et al., 2006). Although seneciolactone has already been reported from this plant, but till date there are no reports on the antibacterial effect of seneciolactone against these gastrointestinal tract disease causing microbes. However, extracts of *S. scandens* have been shown to possess a wide range of pharmacological activities

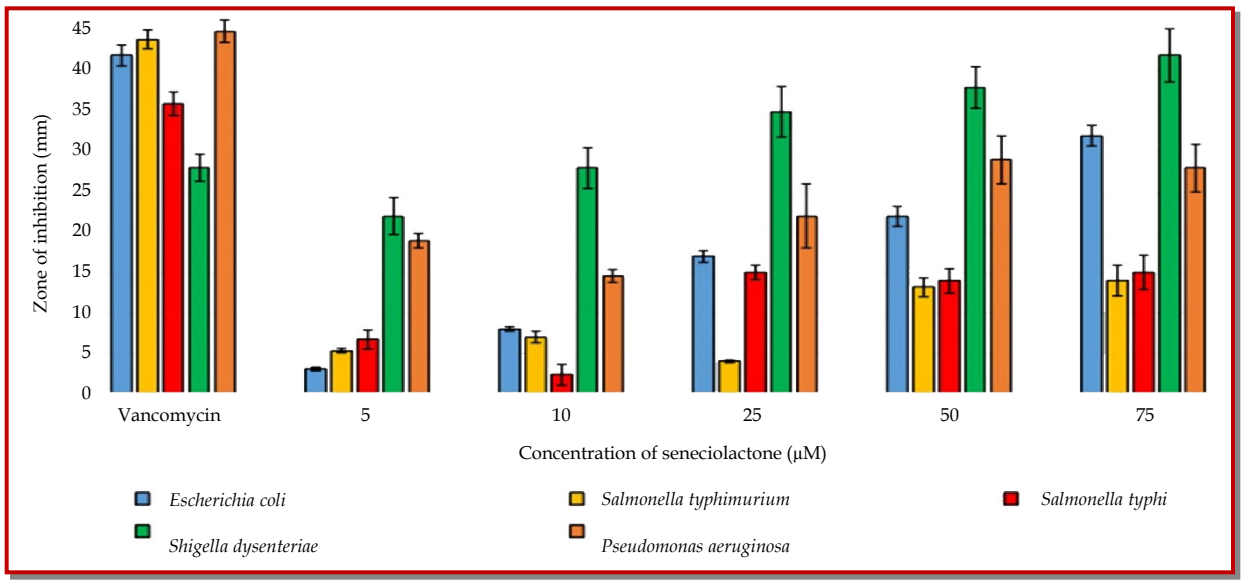

Figure 2: Seneciolactone shows a wide-range of antibacterial effect against the five tested bacterial strains. Effect of different concentrations of seneciolactone on the zones of inhibition (antibacterial effect) of the several tested bacterial strains. *Shigella dysenteriae* was the most susceptible pathogenic bacteria at all tested doses

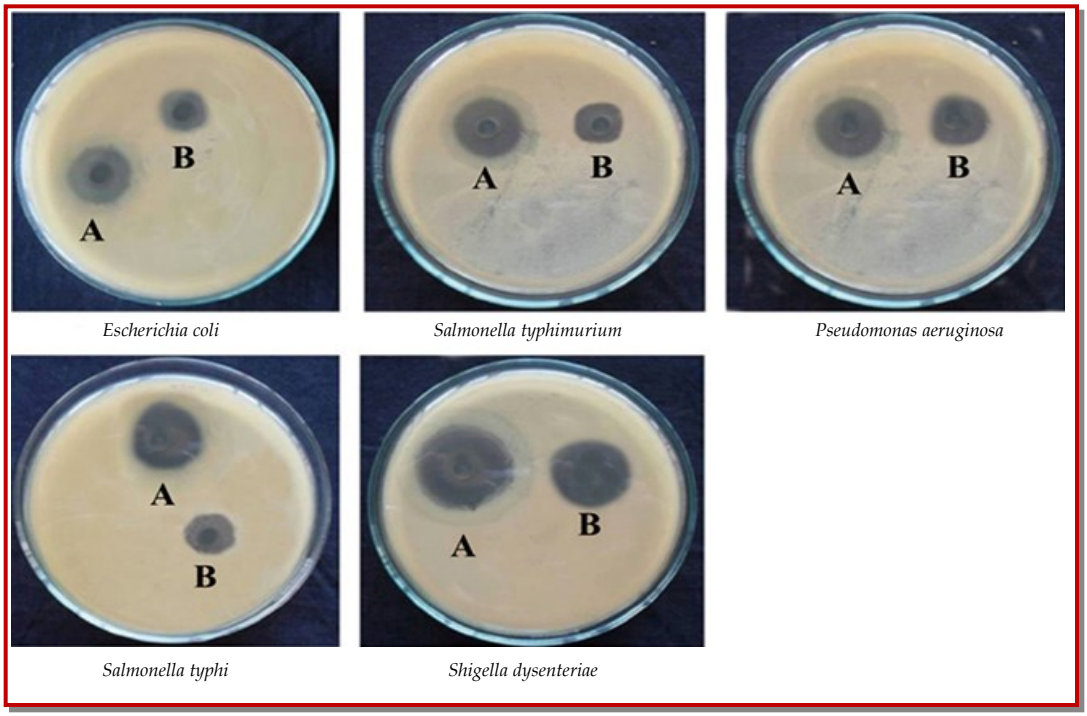

Figure 3: The evaluation of antibacterial effect of seneciolactone against five bacterial strains using disk diffusion assay. A and B represent zone of inhibition due to vancomycin and seneciolactone respectively

including anti-inflammatory, antimicrobial, hepatoprotective, antioxidant, antiviral, antitumor, mutagenic and toxicological activities (Wang et al., 2013).

It is important to mention here that most of the anti-infectious drugs happen to be derived from natural sources including plants and microbes. Many of these secondary metabolites also function as models for the production of effective and novel antibiotics. The hydrophobic nature of plant based natural products is their hydrophobic nature which makes them able to partition the lipid bilayer of the cell membrane and therefore cause damage of the cell membrane and render them permeable to the compound effects. Many plant derived compounds including phenols, quinones, terpenoids, flavones have been reported to possess antibacterial activity. (Djouossi et al., 2015; Cabral et al., 2015).

Table I

MIC and MBC values of seneciolactone and the standard antibacterial drug (vancomycin) against a panel of five bacterial strains

Microbes	Seneciolactone (µM)		Vancomycin (µM)	
	MIC	MBC	MIC	MBC
Escherichia coli	47.2	72	0.3	0.9
Salmonella typhimurium	44.3	67	0.3	0.9
Salmonella typhi	49.6	74.4	0.5	0.9
Shigella dysenteriae	26.7	50.3	0.7	1.0
Pseudomonas aeruginosa	46.3	74.2	0.4	0.9

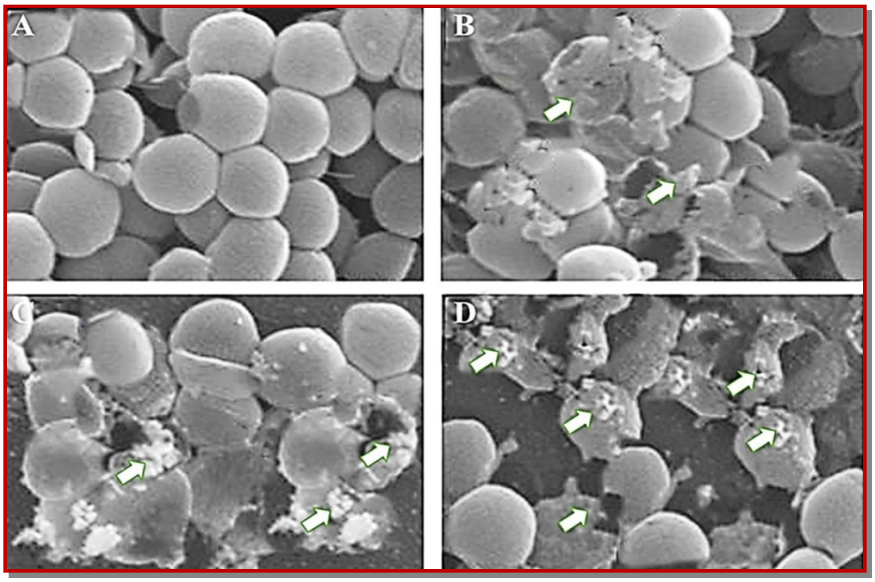

Figure 4: Effect of different doses of seneciolactone on the cell membrane integrity of *Shigella dysenteriae* using scanning electron microscopy. As compared to the untreated control (A), 10 µM (B), 25 µM (C) and 50 µM seneciolactone-treated bacterial cultures revealed significant damage to the cellular membrane (white arrows)

Conclusion

Seneciolactone shows antibacterial activity against five common bacteria which are known to cause several gastrointestinal tract disorders including IBS. The molecule showed medium to potent antibacterial activity against different bacteria. Further, the antibacterial effect of this compound was mediated through the destruction of cell membrane in *S. dysenteriae*.

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Conflict of Interest

Authors declare no competing interest

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