



International Journal of Pharmacology

ISSN 1811-7775

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Research Article

Phytochemical Analysis, Antibacterial Activity and Mode of Action of the Methanolic Extract of *Scutellaria barbata* Against Various Clinically Important Bacterial Pathogens

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Abstract

The objective of the current study was to evaluate the phytochemical composition and antibacterial effect of methanolic extract of *Scutellaria barbata* (*S. barbata*) against a panel of clinically important bacterial pathogens along with deciphering the mode of action of the extract. Liquid chromatography-mass spectrometry was performed to evaluate the phytochemical constituents of the bioactive extract. Disc diffusion assay and microdilution assay were used to test the antibacterial efficacy of the extract. Scanning Electron Microscopy (SEM) was used to evaluate the morphological changes induced by the extract. The 2', 7'-dichlorofluorescein diacetate (DCFDA) was used to detect the Reactive Oxygen Species (ROS) generation in these six bacterial strains induced by the methanol extract of *S. barbata*. The extract inhibited cell growth of all tested bacteria dose-dependently with *S. typhimurium* being the most susceptible pathogen. The percentage of bacterial cells killed within a 40-min contact time between the bacterial cells and the extract was around 24.7% which increased to 44.5% after 60-min time. The extract also induced potent ROS generation as well as intracellular protein leakage in all bacterial cultures with maximum peak in *S. typhimurium*. The SEM results revealed that the extract induces deleterious morphological changes in the bacterial cell membrane of *S. typhimurium* indicating that ROS generation can lead to membrane damage in bacterial cells. The present findings indicate that *S. barbata* has potential antibacterial effect against a variety of bacterial strains and the antibacterial effect is mediated via ROS generation, intracellular protein leakage and rupture of bacterial cell membrane.

Key words: *Scutellaria barbata*, *S. typhimurium*, antibacterial activity, reactive oxygen species, MIC, MBC

Received: September 22, 2015

Accepted: December 22, 2015

Published: January 15, 2016

Citation: Qi-Ling Tang, Ai-Rong Kang and Cong-Xiao Lu, 2016. Phytochemical analysis, antibacterial activity and mode of action of the methanolic extract of *Scutellaria barbata* against various clinically important bacterial pathogens. Int. J. Pharmacol., 12: 116-125.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infectious diseases caused due to bacteria and fungi are the main cause of mortality in tropical and subtropical countries. As per the World Health Organization (WHO) records over 50% deaths are due to infectious diseases in these parts (Kwete *et al.*, 2011). It is believed that due to the indiscriminate use of various antibiotic drugs particularly those of synthetic origin, the pathogenic microbes have developed multidrug resistance which creates serious health issues particularly in developing countries. Many times, this multidrug resistance can result in serious epidemic because no drug can have any effect on the microbes (Weiser *et al.*, 1968). This rapid development of multidrug resistant bacterial and fungal pathogens paves the way for scientists to develop and design novel, newer broad spectrum antimicrobial drugs with less chances of acquiring drug resistance. The less accessibility and high price of new generation antibiotics demands looking for the substances from alternative medicines with proved antimicrobial activity. A large number of medicinal plants have been reported to possess antimicrobial activity especially against drug resistant microbes (Balandrin *et al.*, 1985; Satish *et al.*, 1999; Jones, 1996). *Scutellaria barbata* D. Don (*S. barbata*) is broadly used in traditional Chinese medicine (named Ban-Zhi-Lian in Chinese), which mainly grows throughout Southern China. It contains a large number of alkaloids, flavones, steroids and polysaccharides (Qiao *et al.*, 2011; Liu *et al.*, 2011) due to which it has been found as a potential herb against numerous diseases. (Dai *et al.*, 2013). The biologically active components of *Scutellaria barbata* are *Scutellarin baicalin*, luteolin and apigenin. Studies have shown that *scutellarin* and *baicalin* were associated with antihypertrophic effects, antioxidant, antitumor, antiviral, inhibit the proteasome and anti-inflammatory activities (Wu *et al.*, 2013; Yang *et al.*, 2011). So, have tried to evaluate this herb against different microbial strains in order to determine its effects. It is estimated that plants have provided the models for 50% of Western drugs (Ncube *et al.*, 2008). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. The main bioactive chemical compounds present in medicinal plant extracts include phenolics and polyphenols, simple phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, coumarins, terpenoids, essential oils, lectins, polypeptides, alkaloids etc (Dorman and Deans, 2000).

The objective of the present research work was to evaluate the antibacterial activity of the methanolic extract of the root of *Scutellaria barbata* against five clinically important bacterial strains along with elucidating the mechanism of action of this extract by studying its effect on cell morphology of Gram negative bacteria *Salmonella typhimurium* using scanning electron microscopy. It is also demonstrated the effect of the extract on the generation of Reactive Oxygen Species (ROS) in *S. typhimurium*. Phytochemical analysis of the extract using Liquid Chromatography-Mass Spectrometry (LC-MS) was also done to identify bioactive chemical constituents of the extract.

MATERIALS AND METHODS

Solvents: The HPLC-MS grade acetonitrile were purchased from Merck Co., (Darmstadt, GER). High-purity deionized water was obtained from a Milli-Q water purification system (Millipore Bedford, MA, USA). The HPLC-grade formic acid used for preparation of the mobile phase were from Anaoua chemical supply (Houston, USA), methanol used for plant extraction was from ANPEL Scientific Instrument Co. (Shanghai, CHN).

Plant material and preparation of the extract: The roots of *S. barbata* were collected during June-July 2014 from Jiuzhaigou, Chengdu, China. The plant material was identified by Prof. Xun-Xhao. The roots were thoroughly washed with tap water, shade dried and then chopped into small pieces. Methanol (95%) was used for hot extraction which was carried out for 4 h using a soxhlet extraction apparatus. The extract was then concentrated under reduced pressure in a rotary evaporator at 40°C and was then kept in a refrigerator at 4°C prior to use.

Liquid chromatography-tandem mass spectrometry (LC-ESI-MSMS)/HPLC analysis: The HPLC analysis was carried out by a Nexera HPLC system (Shimadzu, Japan) with a double-pump (LC-30AD), column oven (CTO 30A) and Autosampler (SIL-30AC). A Chromolith RP-18e column (4.6 mm ID, 50 mm length) (Merck) was used. The mobile phase was a gradient prepared from solvent A (0.1% aqueous formic acid) and solvent B (acetonitrile) and the conditions used for gradient elution were: 0-3 min, 5-18%B; 3-5 min, 18%B; 5-6 min, 18-45%B; 6-7 min, 45-100%B; 7-8 min, 100% B. The separation was conducted at 25 with a flow rate of 0.8 mL min⁻¹. The injection volume was 2 µL.

The LC-MS equipment (LC-MS QqQ-6410B Agilent Technologies) consisted of a chromatographic system (1260 In nity Agilent Technologies) coupled with an Agilent Triple

Quad mass spectrometer fitted with an ESI source. The MS conditions were the following: MS range 100-1200 Da, MSn spectra were obtained using both positive and negative modes, nebulizer gas 45 Psi, gas temperature 325°C, capillary voltage 4000 V.

Microorganism strains and culture media: *Streptococcus pneumoniae* (ATCC 2730), *Streptococcus pyogenes* (ATCC 12344), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 25619) and *Salmonella typhimurium* (ATCC 10535) were used in the current study. The bacterial strains were procured from the State Key Laboratory of Microbial Resources (SKLMR), the institute of microbiology, Chinese academy of Sciences, China. Bacterial strains were grown on nutrient agar plates at 37°C and maintained on nutrient agar slants. Cell suspension of microorganisms in 0.5% NaCl was adjusted at 0.5 Mcfarland to obtain approximately 10^5 CFU mL⁻¹.

Preliminary screening for antimicrobial activity: The antimicrobial test was performed by disc diffusion assay as per NCCLS, 1997 (NCCLS., 1997). The nutrient agar plates containing an inoculum size of 10^6 CFU mL⁻¹ on Saboraud glucose agar plates were used. Previously prepared extract impregnated disc (6 mm in diameter) at the concentrations of 200 µg mL⁻¹ for bacterial strains were placed aseptically on sensitivity plates with appropriate controls. Vancomycin (100 µg mL⁻¹) was used as standard antibacterial. Plates were incubated at 37°C for 30 h. Antibacterial activity was recorded by measuring the clean zone of growth inhibition on agar surface around the disc.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): The MIC and MBC tests were carried out by the broth microdilution method (Yu *et al.*, 2004). The extract was dissolved in sterilized physiological saline solution (0.9%) supplemented with Tween-80 (Sigma) at final concentration of 0.8% (v/v). Serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranging from 10-0.125%. Each dilution (100 µL) was dispensed into the wells, then inoculated with (100 µL) of the bacterial suspension. The final concentration of each strain was adjusted to 10^5 - 10^6 CFU mL⁻¹. The concentration of the extract at which the bacteria show no visible growth is defined as Minimum Inhibitory Concentration (MIC) while, as the extract concentration at which the bacteria are killed is defined as Minimum Bactericidal Concentration (MBC). All experiments were performed in triplicate.

Reactive Oxygen Species (ROS) generation by the extract:

The 2', 7'-dichlorofluorescein diacetate (DCFDA) was used to detect the Reactive Oxygen Species (ROS) generation in these six bacterial strains induced by the methanol extract of *S. barbata* (Kye *et al.*, 1999). The concentration of the extract used was 65 µg mL⁻¹ and the number of bacterial cells used was adjusted to 10^6 CFU mL⁻¹. After all cultures were incubated at 37°C for 4 h, they were centrifuged at 4°C for 20 min at 300×g and then each supernatant was treated with 50 µM DCFDA for 2 h. The ROS formed in the samples were detected using Fluorescence MultiDetection Reader (BIOTEK, USA) at 485 nm of fluorescence excitation wavelength and 528 nm of emission wavelength.

Scanning Electron Microscopy (SEM) evaluation: The SEM was performed as reported already in the literature (Yenugu *et al.*, 2004; Agizzio *et al.*, 2006). *Salmonella typhimurium* was grown in MH agar at 37°C was put in a saline solution comprising of 0.2% tween-80. Four different concentrations of the extract (0, 5, 25 and 75 µg mL⁻¹) were prepared and added into this suspension and was incubated at room temperature. After 24 h, the bacterial cells were centrifuged at 10,000×g for 10 min. The bacterial cells were then washed with 0.1 mol L⁻¹ tris-acetate buffer (pH 7.1), fixed in tris-acetate buffer containing 1.5% glutaraldehyde and then freeze-dried. Each of the bacterial culture was observed by SEM (Hitachi, Japan) at magnifications of 10,000x. The bacterial cell suspension in saline with no extract treatment served as a negative control.

Determination of the rate of killing of the tested bacterium using the S.B extract: This assay determining the rate of killing of the *S. typhimurium* was evaluated as per an already reported method with minor modifications (Odenholt *et al.*, 2001). Tubes containing 10 mL of broth to which the extract had been added at concentrations of 1, 2 and 4 times the MIC were inoculated with a suspension of the test strain, giving a final bacterial count of approximately 5×10^6 CFU mL⁻¹. The tubes were thereafter incubated at 37°C and the killing rate was determined over a period of 2 h. A 1.5 mL volume of each suspension was withdrawn at time intervals and transferred to 5 mL of nutrient broth recovery medium containing 5% "Tween 80" to neutralize the effect of the antimicrobial compound carry-overs from the test organisms. The suspension was then successively diluted and plated for viable counts. The plates were later incubated at 37°C for 48 h. The control plates contained the test microorganisms without the

S.B extract. The remaining bacterial colonies were counted and compared with the counts of the culture control.

Determination of protein leakage in *S. typhimurium* bacterial cells:

Twelve hour old bacterial cells were washed twice with saline by centrifugation at 12,000 rpm for 15 min followed by re-suspension in physiological saline. The bacterial cells were then treated with varying concentrations of the *S. barbata* extract relative to MIC (1x MIC, 2x MIC and 4x MIC) for 2 h. Each bacterial suspension treated with extract was centrifuged at 10,000 rpm and the supernatant obtained was evaluated for protein estimation using the Bradford assay (Bradford, 1976). The concentration of protein was estimated from the established standard curve obtained using Bovine Serum Albumin (BSA).

Statistical analysis: All the experiments were done in triplicate and the results were expressed as Mean±SD. Two-way analysis ANOVA was used for comparisons. Statistical significance was calculated at $p < 0.05$.

RESULTS AND DISCUSSION

LC-ESI-MSMS analysis/HPLC analysis: The phytochemical analysis of the *S. barbata* root extract was carried out by LC-ESI-MS as well as HPLC-DAD techniques. The extract was run under both positive and negative ESI-MS conditions and it showed several major and minor ionic species. The total ion MS chromatogram is shown in Fig. 1, while as the identified compounds are shown in Fig. 2. Fragmentation of the major peaks was used for the identification of compounds. The identification of the chemical compounds was also carried out by comparing the molecular ion peaks along with the MS fragmentation pattern with those of the literature. The

five chemical constituents identified were (1) Scutellarin, (2) Luteolin, (3) Apigenin, (4) 4'-Hydroxywogonin, (5) Luteolin-7-O-glucuronide (Fig. 2). These phytochemicals have already been reported previously in this medicinal plant (Yao *et al.*, 2011).

Antibacterial activity of the *S. barbata* extract: The antibacterial activity of *S. barbata* extract was evaluated against a panel of six clinically important bacterial pathogens including *Streptococcus pneumoniae* (ATCC 2730), *Streptococcus pyogenes* (ATCC 12344), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* (ATCC 25619) and *Salmonella typhimurium* (ATCC 10535). Both the zones of inhibition as well as MIC/MBC values of the extract were calculated against these microbes using disc diffusion and microdilution assays respectively. The results of the disc diffusion assay (zones of inhibition) are shown in Fig. 3 while, as the results of the microdilution assay (MIC/MBC values) are shown in Table 1. As can be seen from Fig. 3, *Salmonella typhimurium* exhibited the maximum zone of inhibition at all extract concentrations and as such was most susceptible to the *S. barbata* extract treatment. *Pseudomonas aeruginosa* was the next most susceptible bacteria to the extract after *S. typhimurium* showing higher zones of inhibition at almost all extract doses. *Streptococcus pneumoniae*, *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus* bacterial strains were relatively less susceptible to the *S. barbata* extract. The MIC/MBC values almost also displayed the same trend (Table 1) with *S. typhimurium* bacterial strain showing the lowest MIC and MBC values indicating that the extract has potent antibacterial effect against this pathogen. MRSA showed the highest value of MIC/MBC indicating that the extract has lesser effect on this bacterial pathogen.

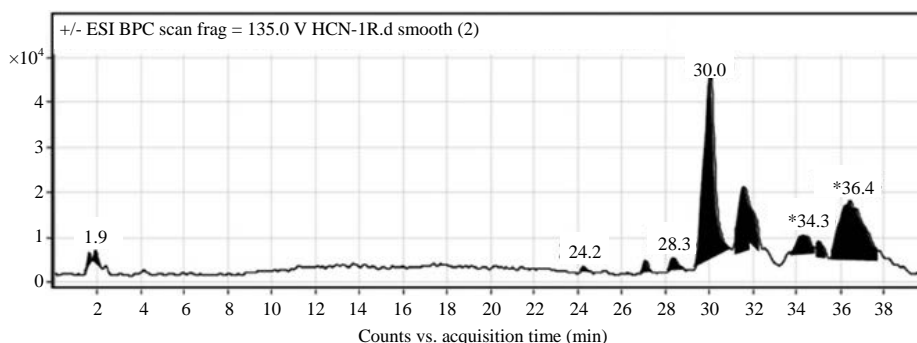


Fig. 1: LC-MS chromatogram of the methanol extract of *S. barbata*. The green numbers show peaks corresponding to compounds, (1) Scutellarin, (2) Luteolin, (3) Apigenin, (4) 4'-Hydroxywogonin and (5) Luteolin-7-O-glucuronide

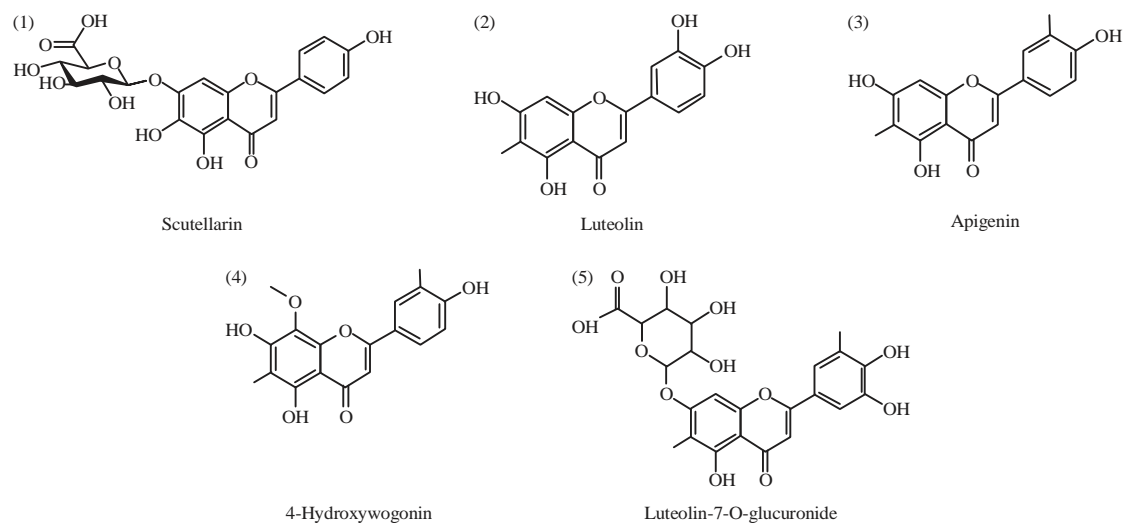


Fig. 2: Phytochemical constituents identified in the bioactive extract of *Scutellaria barbata* using HPLC/LC-MS analytical techniques

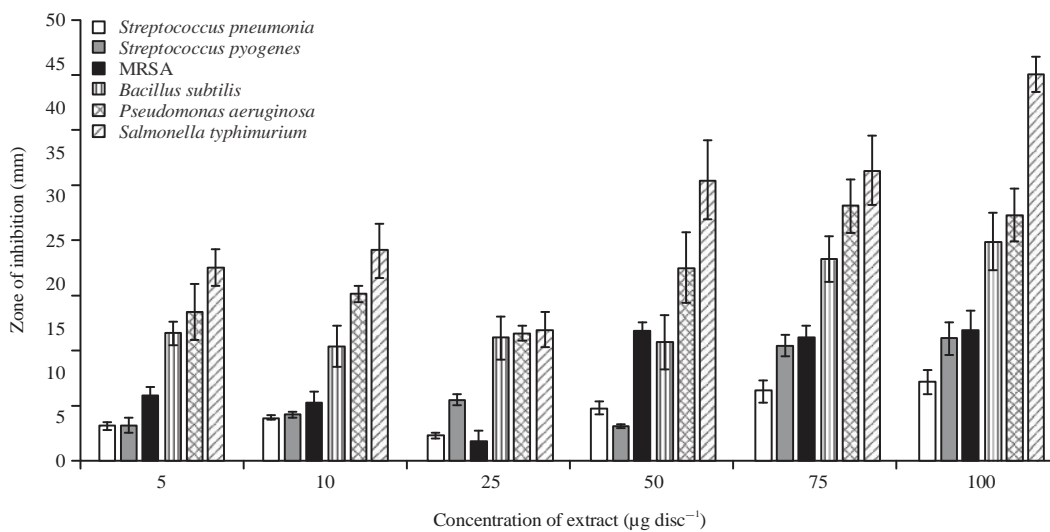


Fig. 3: Effect of the different concentrations of the *S. barbata* extract on the zones of inhibition (antibacterial susceptibility) of the various bacterial strains

Table 1: MIC and MBC values of the *S. barbata* extract as well as the standard antibacterial drug against a panel of bacterial strains

Microbes	<i>(Scutellaria barbata</i> extract) MIC/MBC (µg mL ⁻¹)		(Vancomycin) MIC/MBC (µg mL ⁻¹)	
<i>S. pneumoniae</i>	42	80	5	10
<i>S. pyogenes</i>	48	92	6	10
MRSA	55	96	4	10
<i>B. subtilis</i>	30	88	4	10
<i>P. aeruginosa</i>	33	90	5	12
<i>S. typhimurium</i>	20	32	4	8

Generation of ROS in bacterial cells treated with different doses of *S. barbata* extract: There is a direct relation between

Reactive Oxygen Species (ROS) generation and bacterial death. Various published reports demonstrated that

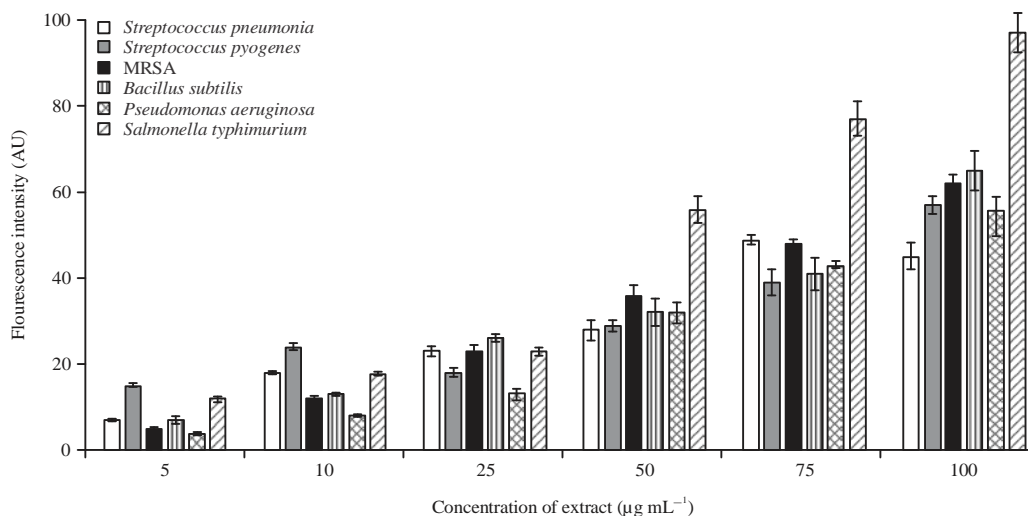


Fig. 4: Formation of ROS in *S. pneumonia*, *S. pyogenes*, MRSA, *B. subtilis*, *P. aeruginosa* and *S. typhimurium* bacterial cells exposed to different doses of the extract. Bacterial cultures were treated with the extract for 4 h

plant extracts could lead to the generation of free radicals within bacterial and fungal cells which finally result in deleterious bacterial membrane damage. In the current study, ROS was measured in six bacterial cultures using DCFDA. After 4 h incubation, elevated levels of ROS were detected and measured in the *S. barbata* extract treated bacterial group. As can be seen in Fig. 4, ROS generation was maximum in *S. typhimurium* at all extract concentrations. This result is consistent with the disc diffusion experiment as well as microdilution assay as both these experiments also showed that *S. typhimurium* is the most affected bacterial strain. The extract brings about generation of ROS in this bacteria more than other tested bacteria. The extract also induced variable ROS generation in other bacterial strains as can be seen in Fig. 4.

Morphological evaluation of *S. typhimurium* by scanning electron microscopy after extract treatment:

The SEM results indicated that the *S. barbata* extract targets cell membrane of *S. typhimurium* bacteria. As can be seen in Fig. 5a-d, *S. barbata* extract induced potential morphological changes in *S. typhimurium* bacteria. As compared to the negative control (Fig. 5a, untreated bacterial cells) which showed normal cellular morphology, the *S. barbata* extract treated bacteria (Fig. 5b-d) showed lot of cellular damage especially at 75 µg mL⁻¹ concentration of extract (D). The membrane damage followed dose-dependent relation with the extract. This membrane damage in *S. typhimurium* is

consistent with the Reactive Oxygen Species generation by the extract. The ROS generation can eventually lead to membrane damage which was demonstrated by SEM in this study.

Extent and rate of killing of *S. typhimurium* by the S.B extract:

The extent and rate of killing of the *S. typhimurium* by the S.B extract at a concentration of 1x MIC, 2x MIC and 4x MIC is shown in Fig. 6. The percentage of bacterial cells killed within a 40 min contact time between the bacterial cells and the extract was around 24.7% which increased to 44.5% after 60 min time. After 80 min, about 95% of the cells were killed. This behaviour was also seen when the concentration of the S.B extract was doubled, that is 2x MIC. The concentration of the extract was again increased to 4x MIC and the percentage of bacterial cells killed after 40 min contact time was 64.2%. Thus the S.B extract showed a dose-dependent and time-dependent bactericidal effects on *S. typhimurium* bacterial cells.

Scutellaria barbata extract induced protein leakage in *S. typhimurium* microbes:

Further, the bactericidal effects of the *S. barbata* extract were evaluated by observing its effect on protein leakage after bacterial cell membrane rupture. At a dose of 1x MIC and 40 min contact time of the bacterial cells with the extract, around 34.2 µg mL⁻¹ of protein leaked out of the *S. typhimurium* cells which increased to 38.1, 42.4, 56.2 and 58.1 µg mL⁻¹ after 60, 80, 100 and 120 min time periods respectively (Fig. 7). The same pattern of protein leakage was

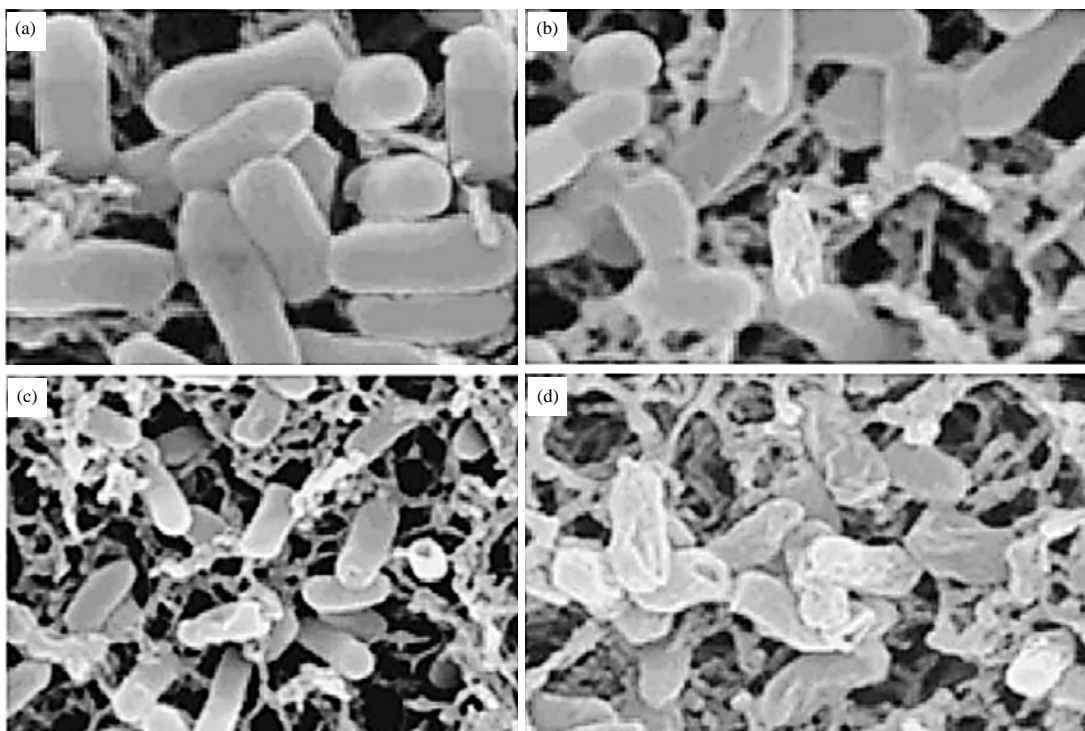


Fig. 5(a-d): Electron micrographs of *S. typhimurium* exposed to different concentrations of the extract. The images were taken at (a) Magnification of 10,000x and *S. typhimurium* 12 h in saline, (b-d) Scanning electron micrographs of *S. typhimurium* treated for 12 h with 5, 25 and 75 $\mu\text{g mL}^{-1}$ of the extract

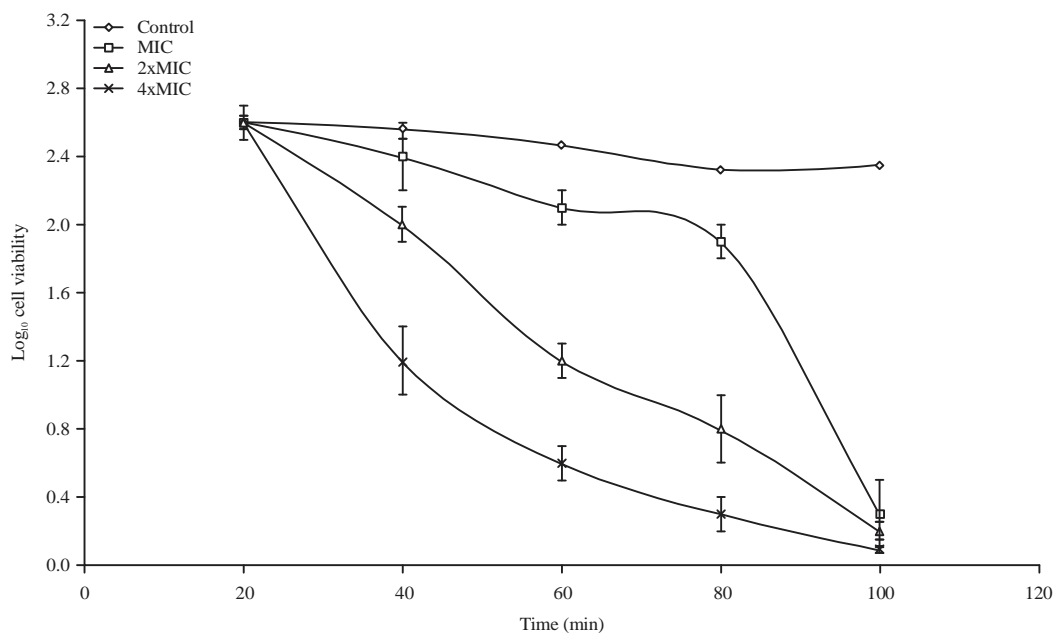


Fig. 6: The extent and the rate of killing of *S. typhimurium* by the S.B extract at concentrations of 1x MIC, 2x MIC, 4x MIC and the control. Each point represents the \log_{10} of the mean survival of bacterial cells at a particular time interval in the presence of the extract

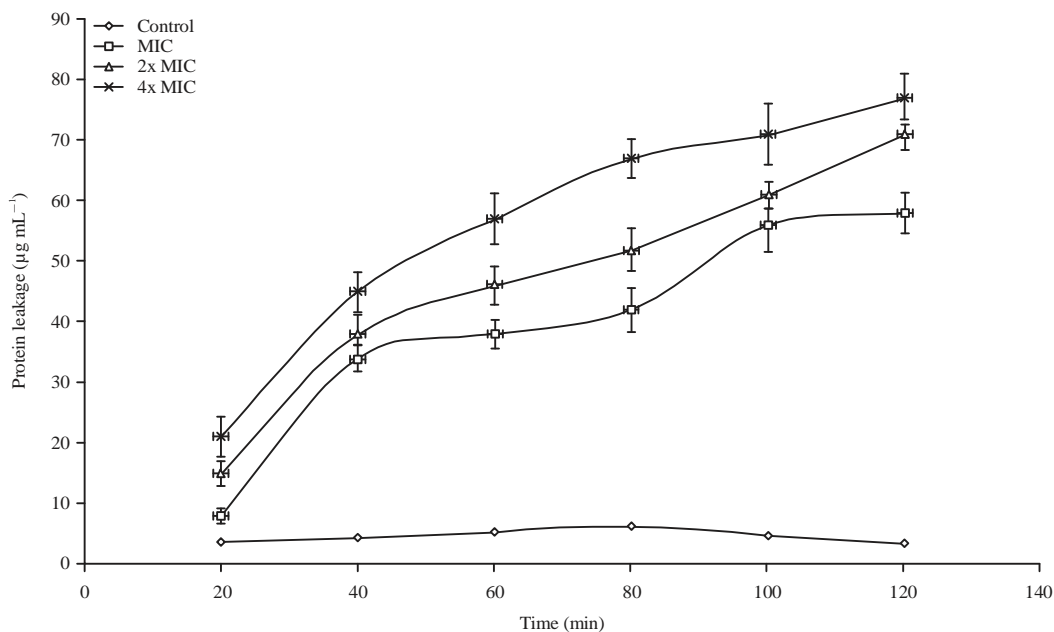


Fig. 7: The effect of *S. barbata* extract on protein leakage from *S. typhimurium* bacterial cells at concentrations of 1x MIC, 2x MIC, 4x MIC and control. Each point characterizes the amount of protein leaked ($\mu\text{g mL}^{-1}$) from the bacterial cells at a specific time interval in the presence of the extract

seen when the *S. barbata* extract concentration was increased to 2x MIC and then 4x MIC. The protein leakage was much significant at 4x MIC concentration having values of 45.1, 57.3, 67.5, 71.3 and 77.3 $\mu\text{g mL}^{-1}$ after 40, 60, 80, 100 and 120 min contact of the bacterial cells with the extract, respectively.

DISCUSSION

There are approximately 20,000 plant species being used as ethno-medicines all over the world. However there has been an enormous increase in the multidrug resistant strains of clinically relevant bacterial pathogens which is posing a great challenge to the scientific community. This increase in multidrug resistance is believed to arise due to the indiscriminate use of antibiotics (Srividya *et al.*, 2010; Martin and Yost, 2011; Bibi *et al.*, 2011). Among the available treatments maximum drugs used to treat infectious diseases happen to be secondary metabolites isolated from natural resources. Importantly, these therapeutic secondary metabolites also work as prototypes or lead compounds for the derivation of novel antibiotics. One important feature of plant natural compounds is their hydrophobic nature which render them capable of partitioning the lipid bilayer of the membrane and thus cause disruption of the cell membrane and make them permeable making it vulnerable to the compound effects. Many plant derived compounds including

phenols, quinones, terpenoids, flavones have been reported to possess antibacterial activity. Flavonoids and phenols exhibit effective antibacterial activity owing to their ability to form complexes with extra cellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria (Kuethe, 2010; Cox *et al.*, 2000; Carson *et al.*, 2002; Cowan, 1999). More specifically flavonoids have been extensively studied and most of the studies have established their role as potential anti-microbial agents (Daglia, 2012; Cushnie and Lamb, 2005; Zhang *et al.*, 2011).

Scutellaria barbata D. Don (Lamiaceae) is a perennial herb which is distributed throughout southern China. This herb is known in traditional Chinese Medicine as Ban-Zhi-Lian. It has been explored for the possible treatment of inflammation, tumor and gynecological diseases in China (Dai *et al.*, 2008). Phytochemically, *S. barbata* is known to contain alkaloids, polyphenols and flavones (Jiangsu New Medical College, 1977; Qian, 1987). *Scutellaria barbata* is widely used as an antitumor agent in China. Ethanol extract of *S. barbata* has been reported to induce apoptosis in the lung cancer cells (A549) while as methylene chloride fraction of the plant has been shown to induce apoptosis in human leukemia cells through the mitochondrial signaling pathway (Yin *et al.*, 2004; Cha *et al.*, 2004). Flavonoids (apigenin and luteolin) were isolated from a 50% ethanolic extract of the plant as active constituents against methicillin-resistant *Staphylococcus*

aureus. The E-1-(40-Hydroxyphenyl)-but-1-en-3-one was isolated from methanol extract of the plant and displayed strong cytotoxicity in K562 human leukaemia cell lines (Sato *et al.*, 2000; Ducki *et al.*, 1996).

S. barbata also contains essential oil with hexahydrofarnesylacetone, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, menthol and 1-octen-3-ol as main chemical constituents. The essential oil has also been reported to possess antibacterial activity against a wide-spectrum of bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *methicillin-sensitive and methicillin-resistant Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus heamolyticus*, *Staphylococcus simulans*, *Enterococcus faecalis* among others (Yu *et al.*, 2004). Keeping in consideration the broad spectrum effects of *S. barbata*, we have evaluated the antibacterial activity and mode of action of the methanolic extract of the root of *S. barbata* along with phytochemical analysis of the extract to identify main chemical constituents. Antibacterial efficacy of the extract was measured by studying the zone of inhibition and MIC/MBC values of the extract against different bacterial strains using Disc diffusion and microdilution assays. Scanning Electron Microscopy (SEM) was employed to evaluate the morphological changes induced by the extract in cellular membrane of the bacteria. The extract inhibited growth inhibition of all tested bacteria with *S. typhimurium* being the most susceptible pathogen towards the extract followed by *P. aeruginosa*. The extract showed dose-dependent growth inhibitory effects. In addition it was found to induce potent ROS generation in all bacterial cultures with maximum peak in *S. typhimurium*. The SEM results revealed that the methanolic extract of *S. barbata* induces deleterious morphological changes in the bacterial cell membrane of *S. typhimurium* indicating that ROS generation can lead to membrane damage in bacterial cells. The percentage of bacterial cells killed within a 40 min contact time between the bacterial cells and the extract was around 24.7% which increased to 44.5% after 60 min time. After 80 min, about 95% of the cells were killed. This behaviour was evident when the extract concentrations was doubled, that is 2x MIC. Further the intracellular protein leakage was found among the the *S. typhimurium* bacterial cells when exposed to different doses of the extract indicating bacterial cell wall rupture by the extract and the biocidal effects of the extract on the cells, as evident by the killing rate of test results. Based on the previous studied on *S. barbata* regarding the potential of this herb as anti-inflammatory, anti-tumor, diuretic and gynecological diseases, it is evident that the herb can a potential lead against different bacterial strains that are clinically important.

CONCLUSION

In conclusion, this study demonstrates that the *Scutellaria barbata* methanol extract is a potent antimicrobial agent capable of inhibiting the growth of various clinically important microbes. The present study demonstrates that the antibacterial effect of the extract is mediated through membrane damage induced due to ROS generation by the extract followed by the leakage of protein molecules. The extract could be used as a safe and natural alternative to antibiotics with less incidence of showing multidrug resistance.

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