Antibacterial effects of *Scrophularia striata* seed aqueous extract on *staphylococcus aureus*

Mona Zamanian-Azodi[1], Abdolreza Ardeshirylajimi [2], Nayebali Ahmadi[2], Mohamad Bagher Rezaee[3], Farid Azizi Jalilian [4,*], Reza Khodarahmi[5]

[1] Student Research Committee, Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Stem Cell Biology Department, Stem Cell Technology Research Center, Tehran, Iran
[3]Institute of Forest and Rangelands, Tehran, Iran
[4]Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran
[5]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

*Corresponding Author: email address: azizifarid@gmail.com (F A Jalilian)

ABSTRACT

Plant-based drugs are regarded promising recently. *Scrophularia* has been shown various biological activities such as antimicrobial, antitumor and anti-inflammatory properties. According to pervious researches, bacteria are becoming resistant to some kinds of antibiotics, so it is prominent to find more reliable sources against them. Here *Staphylococcus aureus* as a common type of Gram- positive bacteria has been chosen for this in vitro study. After culturing this bacterium, it was treated with various dosages of *Scrophularia striata* seed aqueous extract and tetracycline, and then its antibacterial effect was assessed by spectrophotometery method based on bacteria population alteration after 24h incubation. In order to investigate probable side effects of the seed extract its effect on human fibroblast cells has been studied. MTT assay was applied for cell survival determination of human fibroblast cells after 24h. Findings indicate that bacterial population has been declined between 1 to 20 μg mL^{-1} dosages of the extract, which 5 μg mL^{-1} is the most effective dosage without containing any cytotoxic effect on human fibroblast cells. In fact extract not only has no antiproliferation properties but also has evoked cell profileration, so it can be consider as a cell growth factor. Tetracycline, on the contrary, showed its potent impact merely in highest dosages with noticeable side effects. It can be concluded that, *Scrophularia striata* could be possibly a promising antibiotic which shows significant antibacterial properties, and with lesser side effects.

Keywords: *Scrophularia striata*; Antimicrobial effects; *Staphylococcus aureus*; Human fibroblast cell line

INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia[1]. In addition, they have been shown powerful antimicrobial properties[2]. *Scrophularia striata* is the member of flowering plants family called *Scrophulariaceae*. many *Scrophularia* plants have long been used in Asian countries as a medicinal herb for the treatment of diseases; it has been applied for treating various inflammatory diseases such as allergy, rheumatics and chronic inflammatory disorders[3, 4]. The plants are annual or perennial herbs with flowers with bilateral or rarely radial symmetry. Members of the *Scrophulariaceae* have a cosmopolitan distribution, most of them found in temperate areas, including tropical mountains. This family consists of about 3000 species and 220 genera [5-7]. Some species of the family have been used traditionally to treat eczema, wounds, goiter, ulcers, cancer and fistulae. Some of them are boiled in milk to prepare a poultice which is applied to the abdomen to reduce abdominal pain, whereas their aqueous extracts have been used as a bath to alleviate rheumatic pains. Biologically active compounds of numerous species of the *Scrophularia* have been identified; they have been known to be rich in iridoid glycosides, mainly aucubin and catalpol. Iridoids represent a large group of cyclopentan-[c]-pyran monoterpenoids occurring as constituents of sympetalous plants including ornamental as well as wild ones. Their

structures, properties and biosyntheses have been reviewed. They have shown multiple biological activities including antimicrobial, antitumoral, hemodynamic, choleretic, hepatoprotective and anti-inflammatory properties [1, 8, 9]. *Staphylococcus aureus* is a facultative anaerobic, gram-positive coccus and is the most common cause of staph infections. *Staphylococcus aureus* is a major human pathogen and also causes economically important infections in cows and sheep[10]. It is still one of the five most common causes of nosocomial infections. It is likely that 20% of the human population is long-term carriers of *S. aureus* [11, 12]. It can be found as a part of the normal skin flora and in anterior part of the nasal channel[13]. This bacterium can cause a range of illnesses from minor such as pimples, impetigo, boils (furuncles), cellulitisfolli culitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection. Bacteria are becoming resistant to common type of antibiotics; it has been reported in Japan, USA, Canada and Brazil [14-16]. Hence, the main objective of this study was to assess the possible antibacterial properties of *Scrophularia striata* on *Staphylococcus aureus*.

MATERIALS AND METHODS

Materials

Parts of *Scrophularia striata* were collected from Ilam province during the spring season. These aerial parts of *S. striata*, was exposed to sunlight, washed and put into plastic bags and immediately frozen at -20 °C. The plant material was then freeze-dried.

The cell culture medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were provided by Gibco BRL (Life Technologies, Paisley, Scotland). Fibroblast cell line was obtained from cell bank (Pastuer Institute,Tehran, Iran). 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), was obtained from Roche Diagnostics GmbH (Germany).

Extraction of plant components

The double distilled water (DW) was treated in a GFL system (1204, Germany). 8 mg and 10 mg of seeds were extracted with 80 mL and 100 mL of water at 65 °C for 1 h, respectively, and then filtered through filter paper. The filtrate was then divided into two parts. One part was filtered through a 0.2 μm Milipore membrane filter, and the other one was kept unfiltered for cell cycle growth procedure.

Human fibroblast cell lines studied in this research were obtained from National Cell Bank of Iran (NCBI) (Pastuer Institute, Tehran, Iran).Cells were cultured in the DMEM medium supplemented with FBS (10%, v/v), streptomycin (100 μg/ml), and penicillin (100 U/ml). Cultures were maintained at 37°C in 5% CO2 and 95% air. Cultures were examined regularly. Approximately 10^4 cells were seeded into 96-well cell culture plates containing 200 μl medium and incubated at 37 °C under 5% CO2 for 24 h for MTT assay purposes. Then, the various values of seed extract (0, 1, 3, 5, and 10) μg mL^{-1} were induced in triplicate to cells for 24h. Cell survival was assessed by Colorimetric MTT assay.

Antimicrobial and cytotoxic effects determinations

The bacterial culture of *Staphylococcus aureus* was used to evaluate the antibacterial properties of the both filtered and non-filtered extract of *Scrophularia striata* on *Staphylococcus aureus* as a gram-positive bacterium; spectrophotometery method was used for assessing the population alternations. Simultaneously tetracycline was used as a positive control. These bacteria were cultured in LB agar medium after dissolving in sterile distilled water. The plates were incubated at 37°C for 24h. Single colony from the plate was transferred into 4 mL fluid LB medium and incubated over night at 37°C and 200 rpm in shaking incubator. The cells were harvested by centrifugation at 3000 rpm for 15 min and 4°C. Subsequently, they were washed twice and re-suspended in Ringer solution to provide bacterial concentrations between 106–107 cfu/mL. The

medium was inoculated with the microorganism. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with various concentrations (1-20 µg/mL^{-1}) and blank. Moreover, tetracycline was used as a positive control at the similar concentrations. The dilution medium for the positive controls was sterile distilled water. The plaques were incubated at 37 °C for 24 h. The antimicrobial activities were calculated by spectrophotometery method. Besides, MTT assay was applied for testing fibroblast cell survival. The MTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye, MTT, to its insoluble formazan, giving a purple color[17, 18]. Cells in 96-well plates (5000 cells/well) were exposed to various concentrations of the aqueous extract substance (0 as control, 1, 3, 5and 10 µg/mL), then incubated at 37°C under 5% CO2 atmosphere for 3 h. The 30 µL MTT solution (5 mg/mL in phosphate buffered saline) was added and further incubated for 4 h at 37 °C. After aspirating the supernatant from the wells, 100 µL dimethyl sulfoxide (DMSO) were added to dissolve of formazan crystals. Finally, the absorbance of each well was observed at 570-630 nm using an ELISA plate reader.

RESULTS

Different dosages of this extract has been applied and treated on *S.areus* bacteria, and also on human fibroblast cell. It has significant antibacterial activities on *Staphylococcus aureus*, while showed growth stimulating effects on fibroblast cells.

In figure1, different Concentrations of non-filtered seed extract were used for assessing antimicrobial effect of the extract.

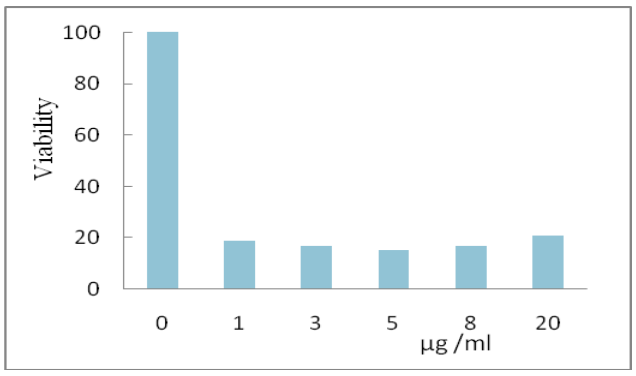

Figure 1: The comparative antibacterial effect of non-filtered seed extract (1, 3, 5, 8, and 20) µg /ml dosages on *S.areus*.

For more investigations, antibiotics effects of 1, 3, 5, 8, 15, 20 µg mL^{-1} of the filtered, non-filtered seed extract, and tetracycline on *S.areus* after 24h incubation has been studied (see figure2).

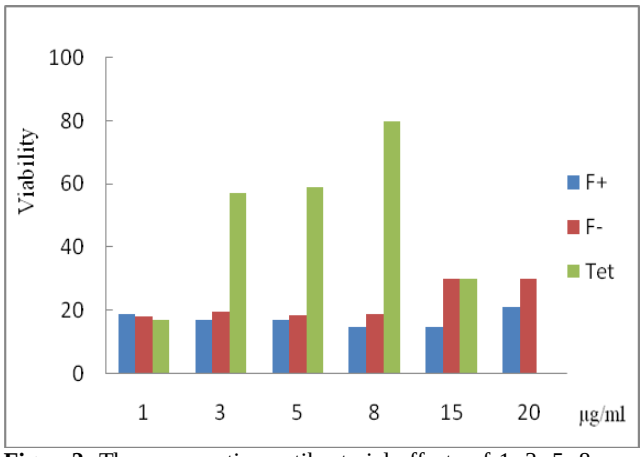

Figure2: The comparative antibacterial effects of 1, 3, 5, 8, 15, 20 µg mL^{-1} of the filtered (blue), non-filtered (red) seed extract, and tetracycline (green) on *S.areus* after 24h incubation. Statistical analyses indicate that all applied concentrations of both filtered and non-filtered aqueous extract of seed have antibacterial activity. (*p* Value< 0.001)

For assessing possible cytotoxic effect of the extract, its effect on human fibroblast cells has been studied.

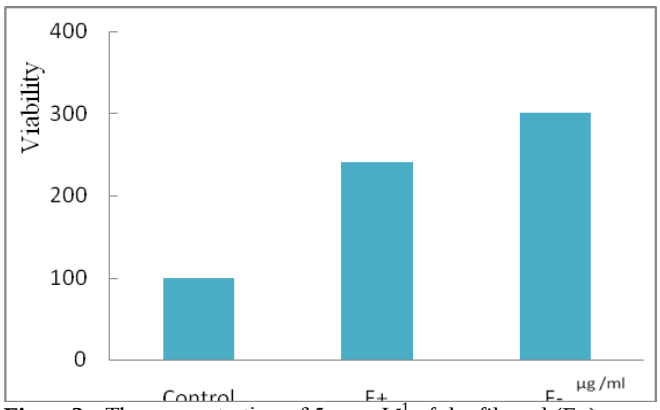

Figure3: The concentration of 5 µg mL^{-1} of the filtered (F+) non-filtered (F-) seed extract on human fibroblast cells after 24h incubation. Statistical analysis shows meaningful difference (p Value<0.001)

DISCUSSION

In recent years, tendency of using natural sources as alternative medicine has been raised[19]. Plants are candidate source of

potentially valuable structures for achieving effective chemotherapeutic agents[20]. *Scrophularia* as a member of *Scrophulariaceae* has been found to possess antibacterial, antiprotozoal, antitumor, anti-inflammatory, and diuretic activities and have been used in the treatment of mental, nervous and gastrointestinal conditions[21-23]. *Staphylococcus aureus* as a Gram positive bacterium has been the main cause of serious illnesses recently. It has been reported that this bacterium is becoming resistance to certain type of antibiotics[15], so it has became a great concern for finding an eligible substitution for curing them. The first step towards this goal is the *in vitro* antibacterial activities method. As we represented in figure1, the *Scrophularia* aqueous seed extract (non-filtered) in the low dosages effect remarkably on bacteria survival, especially in 5 μg mL^{-1} has its most effective impact. It is reported that 2μ filter in some cases can be used as a separator for estimating agent dimensions[8]. Therefore, here filtered and non-filtered extracts are examined. Figure 2 reveals a comparison between filtered, non-filtered extract, and tetracycline antibacterial properties. Tetracycline as a common type of antibiotics has been used as a positive control. It is clear that as the dosage of tetracycline increases the viability of bacteria declines dramatically, while the fact is totally different in *Scrophularia striata* extract activity. Filtered and non-filtered seed extract of this plant approximately have similar effects on *Staphylococcus aureus* in low dosages, whereas tetracycline (as a potent antibiotic) considerable effect is in the high dosages. Tetracycline inhibits bacterium growth perfectly in 20 μg mL^{-1} but in low dosages except for 1 μg mL^{-1} cannot compete with the extract. In other words, extract effects in a steady manner, but the drug shows fluctuations in various dosages. In many studies [5, 8, 24] fibroblast cells are used as probe for measuring side effects of the treatment. Here, thus, it has been used as a criterion for assessing possible side effects of the extract. As it is shown in figure 3, the both filtered and non-filtered aqueous seed extract does not contain any antiproliferation effects since the viability has been increased in the concentration of 5 μg mL^{-1} after 24h incubation. As it can be concluded from figure3, extract evoked cell growth, so it can be consider as cell growth factor[25]. In summary, both filtered and non-filtered extract of the seed has been shown effective on viability of the bacteria; particularly it is considerable in low dosages, which can be possibly benefits its low side effects when come to the fact that this low dosages are the dosages that not only does not contain any antiproliferation effects on fibroblast cells, but also show significant cell growth activity.

CONCLUSION

In conclusion, *Scrophularia striata* aqueous seed extract has significant antibacterial effects on a typical type of Gram positive bacteria. As long as the aqueous seed extract contains cell growth inducing properties on fibroblast cells, no side effects can be estimated. On the other hand, tetracycline showed its potent effect only in higher dosages comparing with the extract.

For the purpose that *Scrophularia striata* seed aqueous extract can be utilize as a promising and potent antibiotic in the future, more information of this plant is crucial to be achieved by in vivo studies, and also evaluating other types of bacteria treating with this extract.

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