

# Evaluation of the in vitro antibacterial effect of the hydroalcoholic extract of *Scrophularia striata*

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**Abstract:** Increasing bacterial resistance to chemical antibiotics and their probabilistic side effects cause popularity of medicinal plants, so there is an instantaneous and steady need for novel antimicrobial compounds from plants. As we know, there is no documented proof on antibacterial activities of *Scrophularia striata* (SS) hydroalcoholic extract against *Pseudomonas aeruginosa* in west of Iran. As a screen test to discover antibacterial properties of the extract, agar disk and agar well diffusion methods were employed. Macrobroth tube test was performed to specify MIC. The results of agar disk and agar well diffusion tests indicated SS have prevented the growth of *P. aeruginosa*. Also, by increasing the concentration of SS, the inhibition zone increased ( $p \leq 0.001$ ). The MIC and MBC values was 0.031 and 0.062 g/ml for SS, respectively. Thus, the present research demonstrates the antibacterial effects of the medical plant on *P. aeruginosa*, suggesting to use as antibacterial supplement in the developing countries towards the development of new therapeutic agent.

**Keywords:** *Scrophularia striata*; Hydroalcoholic extract; Antibacterial effect.

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## 1 Introduction

Antibiotics are types of antibacterial drugs used in the treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria. Since the detection of these antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would cause to the presumptive eradication of infectious diseases. But overuse of antibiotics has become the main factor for the emergence and dissemination of multi-drug resistant strains of different groups of microorganisms [1]. The spread of drug resistant pathogens is one of the most serious threats to successful therapy of microbial diseases. The emergence of resistance of bacteria to antibiotics is a common phenomenon. Emergence of resistance often reflects evolutionary processes that take place during antibiotic therapy. Down the ages plants have evoked interest as sources of innate products. They have been screened for their potential uses as alternative remedies for the treatment of several infectious diseases [2]. Plant-derived products have a major

variety of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds. Some medicinal plants used in traditional Iranian medicine are efficient in treating diverse ailments caused by bacterial and oxidative stress [3]. A plant extract is a substance or an active with desirable properties that is removed from the tissue of a plant, to be used for a particular purpose. The antibacterial properties of extracts have been identified for many years, and their rudiment have found applications as naturally occurring antimicrobial agents in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food maintenance, etc. There are reports of the active principles of extracts from different plants with antifungal or antibacterial effect. The original benefit of plant extracts is that they do not increase the “antibiotic resistance”, an event usually encountered with the long-term use of synthetic antibiotics; because they have a significant role in the defense system of the plant to microbial diseases due to their intrinsic anti-oxidative and anti-microbial properties [4]. Herbal extracts have antimicrobial activity on a wide number of bacteria, and most of these compounds have phenolic groups in their structure. The compounds of plant extracts contain numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic antithrombotic and vasodilatory activities [5]. In Iranian medicine, plant extracts in the form of infusion, decoction, tincture or herbal extract are consumed by the population for the treatment of diseases including infectious diseases. In western states of Iran, a plant with the scientific name of *Scrophularia striata* has traditional medical usage. The genus *Scrophularia* of the family *Scrophulariaceae* comprises about 3000 species and 220 genera. Species of *Scrophularia* all share square stems, opposite leaves and open two-lipped flowers forming clusters at the end of their stems. The genus is concentrated in central Asia with only a few species in central Europe and North America [6]. SS have been used since ancient times in traditional medicines to treat eczema, wounds, goiter, ulcers, cancer and microbial disease. Different extracts of the plant are traditionally used in treating bacterial diseases. Probably, the antibacterial activities of the plant are related to its phenolic, flavonoid, and flavonol compounds. These components are adjoin to the bacterial outer membrane proteins, deactivate the matrix metalloproteinase and stop growth of bacteria or kill bacteria [7], [8], [9].

Based on knowledge of author, in comparison to many other pharmaceutical-industrial plants, there is a very little data about antibacterial effects of hydroalcoholic extract of SS collected from Ilam province, west of Iran. Hence, the aim of the current study was evaluation of antibacterial activity of the hydroalcoholic extract of plant against common pathogen (*P. aeruginosa*) with broth macro-dilution and agar disk and agar well diffusion methods.

## 2 Material and Methods

### 2.1 Plant sample collection

In this empirical-experimental study, medicine plant collected from Ilam. The sample was cleaned from any strange, plants, dust, or any other contaminants.

## 2.2 Preparation of hydroalcoholic extract

Successive solvent extraction was performed for SS. Plants were washed, air dried for 7-8 days, and ground into powder before they were placed into the flask of the Soxhlet apparatus for extraction using 70% ethanol with increasing order of polarity to extract the phytoconstituents separately at 20°C for 3-4 h (The ethanol used was HPLC grade obtained from Sigma-Aldrich, Germany). Whitman filter papers No.1 were then applied to filter the extract. After that, reduced pressure was applied to evaporate and dry the filtrates which were stored at -20°C in labeled, sterile, screw capped bottles.

## 2.3 Source of microorganisms

Bacterial specie namely *P. aeruginosa* (ATCC No. 1707) was procured from Veterinary school of Tehran University as lyophilized. Bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10<sup>8</sup> cfu/ml using Muller Hinton broth.

## 2.4 Culture media

Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

## 2.5 Evaluation of antimicrobial activities

Agar disk and agar well diffusion were used as screen tests to evaluate antibacterial property of SS based on standard protocol. The solution of the SS was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks were measured. Distillated water was used as negative control whereas kanamycin was used as positive control in case of *P. aeruginosa*. Minimum inhibitory concentration (MIC) means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them). The lowest dilution which no gross microbial growth has been seen indicates MIC. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and six dilutions before contents on agar plate. In this case, after incubation period, the lowest concentration which makes no growth indicates MBC. For

determination of MIC value, macrobroth dilution method was applied. Interpretation of the results was done due to national accepted letter [10].

## 2.6 Statistical Analysis

Antibacterial effect was determined by One way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at  $p \leq 0.05$ .

## 3 Results

### 3.1 Chemical composition

13 compounds such as Oleyl Alcohol (24.81 %), Di-n-octyl Phthalate (21.24 %), Bis(2-ethylhexyl)phthalate (14.91 %), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)ester (12.41 %), Hexadecanoic acid, methyl ester (5.10 %), 2,3,6-Trichlorobenzaldehyde (3.72 %), unknown (3.40 %), Phenol, 2,4-bis(1,1-dimethylethyl) (3.05%), 2-ethyl-butanal (2.54%), Octadecanoic acid, methyl ester (1.84%), 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester (1.79 %), 9-Octadecenoic acid (Z)-, methyl ester (1.77 %), Cyclohexene 1-methyl-4-(1-methylethylidene) (1.67 %), Phenol, 4-(3,4-dihydro-2,2,4-trimethyl-2H-1-benzopyran-4-yl) (1.65 %), representing 99.92% of the total hydroalcoholic extract composition of SS were identified using mass gas-chromatograph [11]. The most substance found in SS hydroalcoholic extract was Oleyl Alcohol. In contrast, Phenol, 4-(3,4-dihydro-2,2,4-trimethyl-2H-1-benzopyran-4-yl) was the least constituents discovered in the plant [11].

### 3.2 Agar disk diffusion test

About SS, the widest zone was seen in 0.25 g/ml concentration (The value of growth inhibition zone was 22 mm in this dilution). There was no inhibition zone in *P. aeruginosa* due to 0.007 g/ml concentration. Growth inhibition zones due to different dilutions are listed in table 1. No inhibition zone was observed due to distilled water.

Dilution(g/ml)	Inhibition zone in disk diffusion (mm)
<b>Microorganism</b>	<b><i>P. aeruginosa</i></b>
<b>Positive control</b>	25
<b>1/4 (0.25)</b>	22
<b>1/8 (0.125)</b>	11

<b>1/16 (0.062)</b>	9
<b>1/32 (0.031)</b>	8
<b>1/64 (0.015)</b>	8
<b>1/128 (0.007)</b>	0
<b>Negative control</b>	0

Table 1. The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of SS.

### 3.3 Agar well diffusion test

In regard to SS, the widest zone was seen in 0.25 g/ml concentration (The diameter of growth inhibition zone was 17 mm in this dilution). No inhibition zone was observed due to distilled water. The data are discoverable in table 2.

<b>Dilution(g/ml)</b>	<b>Inhibition zone in well diffusion (mm)</b>
<b>Microorganism</b>	<b><i>P. aeruginosa</i></b>
<b>1/4 (0.25)</b>	17
<b>1/8 (0.125)</b>	11
<b>1/16 (0.062)</b>	10
<b>1/32 (0.031)</b>	9
<b>1/64 (0.015)</b>	8
<b>1/128 (0.007)</b>	8
<b>Negative control</b>	0

Table 2. The diameters of growth inhibition zones in agar well diffusion test in different dilutions of SS.

### 3.4 MIC and MBC determination

The values for MIC and MBC were acquired in 0.031 and 0.062 g/ml concentrations for *P. aeruginosa* in occasion of SS, respectively.

## 4 Discussion

Because of their security and low cost as well as their impact on a great number of bacteria, medicinal plants may have the potency to treat bacterial resistance to various types of antibiotics [12]. *P. aeruginosa* as a gram negative bacteria has been the main cause of serious illnesses recently. This bacterium is becoming resistance to certain type of antibiotics (such as kanamycin), so it has become a great concern for finding an eligible substitution (such as herbal remedies) for curing them. The antibacterial activities of plant extracts from a wide number of plants have been appraised and reviewed [13], [14], and the mechanisms that enable the natural components of herbs and spices to resist microbes have been considered [15]. The results indicated that these mechanisms vary greatly depending on the components of the plant [16], [17]. SS is the member of flowering plants family called Scrophulariaceae. Many SS plants have long been used in Asian countries as a medicinal plant for the treatment of diseases; it has been applied for treating different inflammatory and bacterial diseases [18], [19].

Fourteen components representing 99.92% of the total volatiles were identified for hydroalcoholic extract of SS. The main components were Oleyl alcohol (24.81%), Di-n-octyl phthalate (21.24%), Bis (2-ethylhexyl) phthalate (14.91 %), and 1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester (12.41 %) [11]. Among the compounds, Bis (2-ethylhexyl) phthalate has antibacterial activities. In a study indicated Bis (2-ethylhexyl) phthalate have strong effect against a number of Gram-positive bacteria; *Bacillus subtilis* with MIC 3.5 µg/ml, *Staphylococcus aureus* with MIC 1.47 µg/ml and *Streptococcus equosemens* with MIC 2.37 µg/ml but the inhibition of Gram negative bacteria was lower; *Escherichia coli* with MIC 5.4 µg/ml, *Pseudomonas aeruginosa* with MIC 6.2 µg/ml and *Closteridium perfringens* with MIC >50 µg/ml [20]. In previous study, showed the activity of Bis (2-ethylhexyl) phthalate against *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella typhimurium* and *Pseudomonas aurioginosa* [21]. In other study, revealed the antishigellosis activity of Bis (2-ethylhexyl) phthalate because it had better activity against *Shigella shiga*, *Shigella sonnei* and *Shigella dysenteriae* [22].

As the table showed, hydroalcoholic extract of SS have excluded the growth of *P. aeruginosa*. Also, by increasing the concentration of this extract, the inhibition zone augmented. The results defined that in tested bacterium, there was a considerable discrepancy in terms of sensitivity to SS. In disk diffusion test, no inhibitory effect of extract of the plant against the *P. aeruginosa* in 0.007 g/ml concentration. SS in 0.031 g/ml concentration has prevented from the growth of the bacterium and in 0.062 g/ml concentration has destroyed bacterium. Thus, the research represents the antibacterial effects of the medical herb on *P. aeruginosa*. There are correspondences between this result and the similar studies. Different studies have indicated that the many species of Scrophularia contains substances that have antimicrobial activities [23], [24], [25], [26]. Results obtained in pervious study show that SS extracts have selective antimicrobial activity on the basis of the cell-wall differences of bacterial microorganisms [27], [28]. In other study showed moderate antibacterial activity against *Actinomyces viscosus*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus fermentum*, *Lactobacillus casei* and *Eikenella corrodens* and indicated its

antibacterial activity can be attributed to the presence of phenolic acids, like other species of this genus [23]. The antimicrobial effect of the SS extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa* was studied and it was concluded that the obtained aqueous extract can be used as antiseptic product in treatment of external infections resulted from these two microorganisms [29].

## 5 Conclusion

The results indicated hydroalcoholic extract of SS have antibacterial activity. In addition to, the phytochemical compounds responsible for the antibacterial activity of SS can be subjected to isolation of the therapeutic antimicrobials. Our results defend the use of the plant in traditional medicine and offer that SS possess compounds with good antibacterial properties. It can be used as antibacterial supplement in the developing countries towards the development of new remedial agent. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of the plant as an antibacterial agent in topical or oral applications.

## Acknowledgment (Funding/Support)

We, the authors wish to thank Medical Sciences University of Kermanshah, Iran for the financial support of this work.

## Authors' Contribution

The core idea of this work came from Mohammad Mahdi Zangeneh and Akram Zangeneh, also the experiments, evaluation and Statistical Analysis of antimicrobial activities done by Mohammad Mahdi Zangeneh, Reza Tahvilian, Fariba Najafi, Akram Zangeneh, Narges Souri, Marjan Moeini Arya, and Saba Zhaleh.

## References

- [1] Luria SE, Delbrück M. 1943. Mutations of Bacteria from Virus Sensitivity to Virus Resistance. *Genetics*. 28 (6): 491–511.
- [2] Hemalatha M, Arirudran B, Thenmozhi A, Mahadeva Rao US. 2011. Antimicrobial Effect of Separate Extract of Acetone, Ethyl Acetate, Methanol and Aqueous from Leaf of Milkweed (*Calotropis gigantea* L.). *Asian Journal of Pharmaceutical Research*. 1(4): 102-107.



- [3] Rangarajan N, Sathiyamoorthy M. 2016. Phytochemical Screening and Antioxidant Studies in the Pulp Extracts of *Cucurbita maxima*. *South Asian Journal of Engineering and Technology*. 2(24):131-140.
- [4] Patil SB, Lende MY, Thakur VS, Naikwade NS, Magdum CS, Chavan GM. 2012. Protective effect from UV rays by Medicinal flowers. *Asian J. Res. Pharm.* 2(1): 24-25.
- [5] Samydurai P, M. Saradha. 2016. Effects of Various Solvent on the Extraction of Antimicrobial, Antioxidant Phenolics from the Stem Bark of *Decalepis hamiltonii* Wight and Arn. *Asian J. Res. Pharm.* 6(2): 129-134.
- [6] Richman AD, Broothaerts W and Kohn JR. 1997. Self-incompatibility rnaes from three plant families homology or convergence? *Am. J. Botany*. 84: 912-917.
- [7] Bahrami A, Valadi A. 2010. Effect of *Scrophularia* ethanolic leaves extracts on *Staphylococcus aureus*. *Int J Pharm.* 6: 393-396.
- [8] Hajiaghaee R, Monsef-Esfahani HR, Khorramizadeh MR, Saadat F, Shahverdi AR, Attar F. 2007. Inhibitory effect of aerial parts of *Scrophularia striata* on matrix metalloproteinases expression. *Phytother Res.* 21: 1127.
- [9] Mahboubi M, Kazempour N, Nazar ARB. 2013. Total phenolic, total flavonoids, antioxidant and antimicrobial activities of *Scrophularia striata* Boiss extracts. *Jundishapur J Natural Pharmaceut Products*. 8: 15.
- [10] Clinical and laboratory standards institute (CLSI). 2006. M7-A7, 26(2).
- [11] Jafari AK, Shohrati M, Mahmoudi R, Haj Hoseini R, Nosratpour S, Pajohi-Alamoti, Latifi AM. 2014. Chemical composition and biological activities of *Scrophularia striata* extracts. *MINERVA BIOTEC*. 26(3): 183-189
- [12] Charde RM, Charde MS, Fulzele SV, Satturwar PM, Kasture AV, Joshi SB. 2011. Evaluation of Ethanolic Extract of *Moringa Oleifera* for Wound Healing, Anti-inflammatory and Antioxidant Activities on Rats. *Research Journal of Pharmacy and Technology*. 2(4): 254-258.
- [13] Abhijeet P, Jui V, Jagtap, Polshettiwar SA, Kuchekar BS. 2011. Formulation and Evaluation of Antibacterial and Antifungal Activity of Herbal Gel Containing *Aloe vera*, *Azadirachta indica* and *Lycopersicon esculentum* Seed Extract. *Research Journal of Pharmacy and Technology*. 4(4): 552-554.
- [14] Suresh M, Rath PK, Panneerselvam A, Dhanasekaran D, Thajuddin N. 2010. Anti-Mycobacterial Effect of Leaf Extract of *Centella asiatica* (Mackinlayaceae). *Research Journal of Pharmacy and Technology*. 3(3): 872-876.



- [15] Venkatachalam T, Kishor Kumar V, Satheesh Kumar P, Kalaiselvi P, Chitra M, Senthil Kumar N. 2009. In-vitro Anti Oxidant and Antimicrobial Activities of Ethyl Acetate Extract of *Evodia lunu-Ankenda* (Gaertn) Merr. Bark. *Research J. Pharmacognosy and Phytochemistry*. 1(3): 201-203.
- [16] Jafferri SAH, Sandhya S, Vinod KR, Narender Prasad D, Venkataramana K. 2010. Evaluation of Anti Bacterial and Anti Platelet Activity of Ethanolic Leaf Extract of *Physalis angulata*. *Research J. Pharmacognosy and Phytochemistry*. 2(1): 67-69.
- [17] Ladda PL, Naikwade NS, Magdum CS. 2010. Antimycobacterial and Antimicrobial Activity of Leaf Extracts of *Vitex negundo* Linn. *Research J. Pharmacognosy and Phytochemistry*. 2(2): 166-168.
- [18] Azadmehr A, Afshari A, Baradaran B, Hajiaghaee R, Rezazadeh S, Monsef-Esfahani H. 2009. Suppression of nitric oxide production in activated murine peritoneal macrophages in vitro and ex vivo by *Scrophularia striata* ethanolic extract. *Journal of Ethnopharmacology*. 124: 166-169.
- [19] YC KSaK. 2000. Neuroprotective phenylpropanoid esters of rhamnose isolated from roots of *Scrophularia buergeriana*. *Phytochemistry*. 54: 503-509.
- [20] Mohammed H. El-Sayed. 2012. Di-(2-ethylhexyl) Phthalate, a Major Bioactive Metabolite with Antimicrobial and Cytotoxic Activity Isolated from the Culture Filtrate of Newly Isolated Soil *Streptomyces* (*Streptomyces mirabilis* Strain NSQu-25). *World Applied Sciences Journal*. 20 (9): 1202-1212.
- [21] Sastry, V. M. V. S. and Rao, G. R. K. 1995. Dioctyl phthalate and antibacterial compound from the marine brown alga-*Sargassum wightii*. *J. Appl. Phycol*. 7:185-186.
- [22] Rowshanul H. M, Rezaul KM. 2009. Antimicrobial and Cytotoxic Activity of Di-(2-ethylhexyl) Phthalate and Anhydrosophoradiol-3-acetate Isolated from *Calotropis gigantea* (Linn.) Flower. *Mycobiology*. 37(1): 31-36.
- [23] Vahabi S, Najafi E, Alizadeh S. 2011. In vitro antimicrobial effects of some herbal essences against oral pathogens. *Journal of Medicinal Plants Research*. 5: 4870-4878.
- [24] Stavri M, Mathew KT, Gibbons S. 2006. Antimicrobial constituents of *Scrophularia deserti*. *Phytochem*. 67: 1530-1533.
- [25] Fernandez MA, Garcia MD, Saenz MT. 1996. Antibacterial activity of the phenolic acids fractions of *Scrophularia frutescens* and *Scrophularia sambucifolia*. *J Ethnopharmacol*. 53:11-14.
- [26] Bahrami AM, Valadi A. 2010. Effects of *Scrophularia striata* ethanolic leaves extracts on *Staphylococcus aureus*. *Int J Phamacol*. 6:431-434.

[27] Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F. 1999. In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. *Lett Appl Microbiol.* 29:130-135.

[28] Karaman I, Sahin F, Gulluce M, Ogutcu H, Sengul M, Adiguzel. 2003. Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. *J Ethnopharmacol.* 85:231-235.

[29] Abbasi N, Azizi Jalilian F, Abdi M, Saifmanesh M. 2007. A comparative study of the antimicrobial effect of *Scrophularia striata* Boiss: extract and selective antibiotics against Staphylococcus aureus and Pseudomonas aeruginosa. *J Med Plants.* 1(6):10-18.

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