



**IN-VITRO EVALUATION OF ANTIBACTERIAL EFFECT OF AQUEOUS AND
METHANOLIC EXTRACTS OF *SCROPHULARIA KHORASSANICA* ON
*SALMONELLA TYPHI***

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ABSTRACT

Scrophulariakhorassanica (*S. khorassanica*), is a plant belonging to the *Scrophulariaceae* family that geographically grows wild only in Iran, Khorasan. Resources survey showed that there is no report of antibacterial activity of *Scrophulariakhorassanica*; therefore, this report can be considered as the first report on this subject. The aim of this study was to antimicrobial effect of aqueous and methanolic extracts of *Scrophulariakhorassanicain* "in-vitro" condition. The antimicrobial effect was studied on the growth of *Salmonella typhi* using micro-dilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Results of antibacterial analysis showed the aqueous extract (MIC=12.5 and MBC=12.5 mg/ml) was more effective than the methanol extract (MIC=25, MBC>50 mg/ml) on *Salmonella typhi* so forasmuch as aqueous extracts *Scrophulariakhorassanica* is a medicinal plant native in Iran (Sarakhs) may be used as an anti typhoid agent and natural antibacterial preservatives in food industry.

Keyword: *Salmonella typhi*, *Scrophulariakhorassanica*, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

INTRODUCTION

Typhoid fever is a global health problem with approximately 17 million cases and 600,000 deaths occurring annually. It is caused by *Salmonella typhi*, that a gram-negative pathogenic bacterium which is transmitted by ingestion of faecally-contaminated food such as poultry, meats, milk products, eggs, vegetables and water [1]. Typhoid fever can be prevented and can usually be treated with antibiotics [2]. Resistance of salmonellas is increasing against current antibiotics, as in some of the studies salmonella resistance against antibiotics has been reported as 95%, the increasing resistance to antibiotics in food borne Salmonella drive much of the current interest on plant antimicrobial molecules [3]. At the same time, increasing consumer demand for more natural products has led to the food industry to consider the incorporation of the natural preservative in a range of products [4,5]. Plants are complex chemical storehouses of undiscovered biodynamic compounds with unrealized potential for use in modern medicine, then finding a new antimicrobial agent, especially an herbal one, is important [6]. Different species of the genus *Scrophularia* have long been used in Asian countries in traditional medicine to

treat a wide diversity of disease such as 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 remove or reduce abdominal pain, whereas their aqueous extracts have been used as a bath to alleviate rheumatic pains [7]. The *Scrophulariaceae*, comprise approximately 5100 species belonging to 275 genera. They have shown a number of biological activities such as anticancer, antioxidative, antimicrobial, antiprotozoal, antifungal, antidiabetic, anti-inflammatory, choleric, hepatoprotective and neuroprotective activities [8,9]. *Scrophularia khorassanica* (*S. khorassanica*), is a plant belonging to the *Scrophulariaceae* family that geographically grows wild only in Eastern Iran, Khorassan (Mashhad), southwest of Sarakhs that perennial or biennial, totally pruinose, up to 40 cm tall. Stem indistinctly striate, simple or branched. Basal leaves in rosette, with up to 2 cm long petiole; lamina elliptic, irregularly dentate or parted; lower leaves opposite, shortly petiolate similar to basal; median leaves alternate, reduced, sessile [10]. Previous studies on the antimicrobial properties of the genus *Scrophularia*

showed a moderate to strong antimicrobial activity of this plant. Ardeshirylajimet *al* [11] and Abbasi *et al* [12] demonstrated the antibacterial effect of *Scrophularia striata* against Gram-negative and Gram positive bacteria. Havasian *et al.*, [13] and Ghasemipirbalouti *et al* [14] indicated the anti-candida activity of *Scrophularia striata*. This study aimed to evaluate the antimicrobial activity of aqueous and methanol extracts of *Scrophularia Khorassanica* on *Salmonella typhi*.

MATERIALS AND METHODS

Plant material

The aerial parts of the *S. khorassanica* were collected during the flowering stage of the plant, from sarakhs, Khorasan-Razavi province, Iran, in May 2014. The plant was identified by Mrs. Joharchi and voucher specimens (no. 45015) were deposited in the herbarium of Ferdowsi University, Mashhad, Iran. The plant material was dried at room temperature and used for all the extracts prepared.

Preparation of plant extracts

Maceration method was used for extraction. The air-dried plant materials were ground into fine powder in a grinder. A 50g Sample plant was soaked in 500 ml distilled water for aqueous extract and

100% methanol for methanol extract. Each solvent was replaced 3 times with fresh solvent and was allowed to remain in contact with the plant material for 24 h, the extracts were filtered with Whatman filter paper. After filtration, extracts were dried using a rotary evaporator at 50 °C for aqueous extracts and 35 °C for methanol extracts [15].

Bacterial strains and culture media

The test organisms used in this study included *Salmonella typhi* (PTCC 1609). Which were obtained Persian Type Culture Collection (PTCC), Iran.

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

The stocked pathogens, which were stored in a -70 °C refrigerator, were thawed at room temperature and after cultivation on Muller Hinton agar (Merck, Germany) and were incubated at 37 °C for 24 hours. In order to prepare the McFarland standard 0.5 ml of 0.048 MBaCl₂ (Merck, Germany) was added to 99.5 ml of 0.18 M H₂SO₄. The observance of the standard was measured in a spectrophotometer to ensure that it was in the acceptable range (0.08-0.13 at a wavelength of 625 NM) [16].

Determination of MIC was carried out using the micro-dilution method. One hundred µl

of the culture medium with 100 µl of each extract concentration was inoculated with 20 µl of the microbial suspension in a sterile 96-well microtitre plate. The first well was filled by aqueous and methanolic extracts, respectively at 25 and 50mg/ml concentration. Then the concentration was half folded for the next well to the last. Two wells of each row were designated to be as positive (without the extract) and negative (without the organism) controls. After an overnight incubation at 37°C, 20 µl of 2,3,5 triphenyltetrazolium chloride (TTC) (5 mg/ml) was added to each well as a colorimetric indicator of bacterial growth and incubated for 60 min at 37°C. The MIC was determined as the lowest concentration of the extracts that showed no color change.

The MBC were determined, 10 µl from each well (without any red dye production) was sub-cultured on the Muller Hinton agar plates and incubated at 37°C for 24 h. The lowest concentration without any bacterial growth represented MBC.

RESULTS

The antimicrobial effect of extracts on *Salmonella typhi* is shown in **Table 1**. Results obtained from the microdilution method, showed the aqueous extract (MIC=12.5 and MBC=12.5 mg/ml) was more effective than the methanol extract (MIC=25, MBC >50 mg/ml) on *Salmonella typhi*. In **Figure 1** showed the effect of aqueous extract on *Salmonella typhi*.

Table 1: Minimum inhibitory concentration (mg/ml) and minimum bactericidal concentration (mg/ml) of extracts of *S. khorassanica* on *Salmonella typhi*

Microorganism	Aqueous extract		Methanol extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>Salmonella typhi</i>	12.5	12.5	25	>50

Table 2: Effect of different plants on *Salmonella typhi* in other studies

Plants	Type of preparation	Main finding	References
<i>Ocimum Gratissimum</i> , <i>Vernonia amygdalina</i> , <i>Aframomum melegueta</i>	Aqueous, ethanol and methanol extracts	Ethanol and methanolic extracts of three plants profoundly inhibited the growth of <i>S. typhi</i>	[18]
<i>Schrebera swietenoides</i> , <i>Wrightia tomentosa</i>	Methanol extract	The methanol extract of both plant showed a MIC value of 4 mg/ml for <i>S. typhi</i> .	[19]
<i>Eucalyptus</i>	Ethanol, aqueous extracts	The extract of eucalyptus from root, leaf and stem had exhibited activity against <i>S. typhi</i> .	[20]
<i>Phyllanthus amarus</i> , <i>parquetin nigrescens</i>	Ethanol, aqueous extracts	Ethanol extract of <i>Phyllanthus amarus</i> had the strongest activity against <i>Salmonella typhi</i> with 8 mm zone.	[21]
<i>Entada Africa</i> & <i>Mimosa</i>	Ethanol	Ethanol extracts and other	

<i>pigra L</i>	extract	solvent fractions from this plants inhibitory effect on <i>S. typhi</i> .	[22]
<i>Sennasiamae</i>	Ethanol, acetone, aqueous extracts	The ethanol extracts showed the highest activity, followed by acetone extracts , while the aqueous extracts showed the lowest activity.	[23]



Figure 1: Effect of aqueous extract on *Salmonella typhi*

DISCUSSION

The antimicrobial effects of *S. khorasanica* had not been found, whereas the extracts of other species of *Scrophularia* exhibited antibacterial and anti-inflammatory effects. Mahboubiet al [17] indicated higher activity of ethanol extract *Scrophularia strata* antimicrobial activity than the other extracts (aqueous, methanol) against *Salmonella typhi* (MIC=6.4), and aqueous extract showed lower antimicrobial activity, and the positive relation between the total phenolic content and its antimicrobial activity and according **Table 2** methanol and ethanol extracts of other plants to have higher antibacterial activity against *Salmonella typhi* too, But in our research

the aqueous extract of *S. khorasanica* has higher antimicrobial activity than that of methanol extract. Fernandez *et al.*, [24] reported activity of the phenolic acid fractions of *Scrophularia frutescens* and *Scrophularia sambucifolia* against *Salmonella typhi*, showed the results *S. frutescens* (MIC=3.5 mg/ml) and *S. sambucifolia* (MIC=4.5 mg/ml) and *S. frutescens* demonstrated a more pronounced activity than *S. sambucifolia*. The species most rich in phenolic acid, were also found to be the most active species in the antibacterial assay. The highest proportion of the phenolic compounds detected in *S. frutescens* could explain the more potent activity of this species. Medicinal herbs are rich in phenolic compounds and are widely

used to increase the shelf life of food and in traditional medicine to treat many diseases are used [25]. So, perhaps we can say that the important antimicrobial compound in *S. khorassanicus* was carvacrol and thymol that according to research Ultee et al [26] showed carvacrol is active against *S. typhi*. This compounds permeation in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable [27].

CONCLUSION

Aqueous extracts of *Scrophularia khorassanica* is a medicinal plant native in Iran (Sarakhs), so may be used as an anti typhoid agent and natural antibacterial preservatives in food industry.

ACKNOWLEDGEMENTS

The authors thank Islamic Azad University, Quchan Branch, Iran, for support of this research.

REFERENCES

- [1] World Health organization. The diagnosis, treatment and prevention of typhoid fever. 2003, Pp1-30.
- [2] Cabrera R, Ruiz J, Marco F, Oliveira I, Usera M, De Anta M. T., Gascon J., and Vila J. Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing Travellers' diarrhoea. *Antimicrob Agents Chemother.* 48 (10), 2004, 3934 – 9.
- [3] Breuil J, Brisabois A, Casin I, Armand-Lefevre L, Fremy S & Collatz E. Antibiotic Resistance in *Salmonellae* Isolated from Humans and Animals in France: Comparative Data from 1994 and 1997. *Journal of Antimicrobial Chemotherapy.* 46 (6), 2000, pp.965-971.
- [4] Dorman HJD & Deans SG. Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils. *Journal of Applied Bacteriology*, 88(2), 2000, pp. 308-316.
- [5] Elgayyar M, Draughon F.A, Golden D.A & Mount J.R. Antimicrobial Activity of Essential Oils from Plants Against Selected Pathogenic and Saprophytic Microorganisms. *Journal of Food Protection*, 64 (7), 2001, pp. 1019-1024.
- [6] Plotkin M.J. Conservation, Ethnobotany and the Search for New Jungle Medicines: Pharmacognosy Comes of

- Ageagain. *Pharmacotherapy*, 8(5), 1998, pp. 257-262.
- [7] Ahmed B, Al-Rehaily A.J, El-Sayed KA, Ahmad MS. Scropolioside-D2 and Harpagoside-B: two new iridoid glycosides from *Scrophulariadeserti* and their antidiabetic and antiinflammatory activity. *Biological and Pharmaceutical Bulletin*, 26(4), 2003, 462-467.
- [8] Tasdemir D, Burn R, Franzbla SG, Sezgin Y, Calis L. Evaluation of antiprotozoal and antimycobacterial activities of the resin glycosides and the other methabolities of *Scrophulariacryptophila*. *Phytomedicine*, 15(3), 2008, 209-15.
- [9] Diaz AM, Abad MJ, Fernandez L, Silvan AM, De Santos J, Bermejo P. Phenylpropanoid glycosides from *Scrophulariascorodonia*: In vitro anti-inflammatory activity. *Life Science* 74(20), 2004, 2515-26.
- [10] Attar F, Joharchi MR, Nowrouzi M, Hatami A. New species of *Scrophularia*L.(*Scrophulariaceae*) from Iran. -Iran. *Journal Botanical*, 12(2), 2006, 193-202.
- [11] Lajimi AA, Barzegar M, TaviraniMR,Ahmadi S, Entezari M, Mahdavi M. Study of antimicrobial the *Scrophulariastriata*extractt. *Medical Science Journal of Islamic Azad University*, 23(3), 2013, 190-195.
- [12] Abbasi N, AziziJalilian F, Abdi M, Saifmanesh M. A comparative study of the antimicrobial effect of *Scrophulariastriata*Boiss: extract and selective antibiotics against *Staphylococcus aureus* and *Pesudomonasaeruginosa*. *Journal Med Plants*, 1(6), 2007, 10-18.
- [13] Havasian MR, Panahi J, Pakzad I, Davoudian A, Jalilian A, ZamanianAzodi M. Study of Inhibitory effect of alcoholic and aqueous extract of *Scrophulariastriata* (tashnedari) on *candida albicans* in vitro.Pejouhesh, 36(5), 2013, 19-23.
- [14] GhasemiPirbalouti A, Bahmani M, Avijgan M. Anti-*Candida* Activity of Some of the Iranian Medicinal Plants.Electronic Journal of Biology, 5(4), 2009, 85-88.

- [15] Iran Herbal Pharmacopoeia Commission. Iranian herbal pharmacopoeia. Tehran : Ministry of Health and Medical Education; 2002. P 84-92.
- [16] Andrews JM. Journal of Antimicrobial Chemotherapy, 48, 2001, 5-16.
- [17] Mahboubi M, Kazempour N, Boland Nazar AR. Total Phenolic, Total Flavonoids, Antioxidant and Antimicrobial Activities of *Scrophularia Striata* Boiss Extracts. Jundishapur journal of natural Pharmaceutical products. 8(1), 2013, 15–19.
- [18] Alo MN, Anyim C, Igwe JC, Elom M and Uchenna DS. Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta*. Advances in Applied Science Research, 3(2), 2012, 844-848
- [19] Mahida Y & Mohan J.S.S. Screening of Plants for Their Potential Antibacterial Activity Against *Staphylococcus* and *Salmonella* spp. Natural Product Radiance, 6(4), 2007, 301-305.
- [20] Evans CE, Banso A, Samuel O.A. Efficacy of Some Nupe Medicinal Plants Against *Salmonella typhi*: An in Vitro Study. Journal of Ethnopharmacology, 2000; p. 21–24.
- [21] Oluwafemi F and Debiri F. Antimicrobial effect of *Phyllanthus amarus* and *parquetina nigrescens* on *Salmonella typhi*. African Journal of Biomedical Research, 11 (2), 2008, 215-219.
- [22] Mbatchou VC., Ayeabila AJ and Apea OB. Antibacterial activity of phytochemicals from *Acacia nilotica*, *Entada africana* and *Mimosa pigra* L on *Salmonella typhi*. Journal of Animal & Plant Sciences, 10(1), 2011, 1248-1258.
- [23] Doughari JH and Okafor NB. Antibacterial activity of *Sennasiamae* leaf extracts on *Salmonella typhi*. African Journal of Microbiology Research. V(2), 2008, 042-046.
- [24] Fernandez MA, Garcia MD, Saenz MT. Antibacterial activity of the phenolic acids fractions of *Scrophularia frutescens* and *Scrophularia sambucifolia*. Journal

- of Ethnopharmacology, 53(1), 1996, 11-14.
- [25] Burt, Sara . Essential oils: their antibacterial properties and potential application in food- a review. International Journal of Food Microbiology, 94(3), 2004, pp223-253.
- [26] Ultee A, Kets EP, Alberda M, Hoekstra FA, Smid EJ. Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. Arch Microbiol, 74(4), 2000, 233-8.
- [27] Burt, SA.; Fledderman, MJ.; Haagsman, HP, van Knapen, F., & Veldhuizen, E.J.A. Inhibition of *Salmonella enterica* Serotype Enteritidis on Agar and Raw Chicken by Carvacrol Vapour. International Journal of Food Microbiology, 119(3), 2007, 346–350.