



Antibacterial activity of ethanol extract and fractions obtained from *Taraxacum mongolicum* flower

H. Qiao, T.J. Sun*

School of Basic Medicine, Shanxi Medical University, Taiyuan 030001, PR China

Abstract

Background and objectives: Resistance towards revealing antibiotics has captured great interest in evaluating the antimicrobial properties of the natural plants. *Taraxacum mongolicum* is widely used as a folklore medicinal plant for its diuretic, antirheumatic and anti-inflammatory properties. Though there are some reports on antimicrobial properties of *Taraxacum mongolicum*, studies on antibacterial abilities of its flower are limited and it was decided to evaluate the antibacterial properties of the flowers in the present study. **Methods:** The antibacterial properties of ethanol extract of *Taraxacum mongolicum* flower, and its fractions (petroleum ether, ethyl acetate (ET), and aqueous fractions) were examined through agar disc diffusion method, and the minimum inhibitory concentration (MIC) was determined. Four Gram-negative and two Gram-positive bacteria were used in the study. **Results:** The antibacterial test results showed that the ET fraction strongly inhibited the growth of all of the microorganisms, especially *Pseudomonas aeruginosa* and *Bacillus subtilis* (with MIC values of 125 µg/mL and 62.5 µg/mL, respectively), whereas the ethanol extract and the other two fractions demonstrated moderate and weak activities, respectively. **Conclusion:** The ET fraction obtained from *Taraxacum mongolicum* flowers presented high antibacterial activity and might be suggested for use as a natural preservative ingredient in pharmaceutical industries.

Keywords: antibacterial activity, minimum inhibitory concentration, *Taraxacum mongolicum*

Introduction

Nowadays, multiple drug resistance has been developed due to the indiscriminate use of commercial antimicrobial drugs [1]. Therefore, there is a growing tendency for replacing synthetic additives with natural ones and this has brought about great interest in evaluating the antimicrobial properties of natural products [2-5]. *Taraxacum mongolicum* Hand.-Mazz. (Asteraceae) is a perennial weed and is widely distributed in the warmer temperate zones of the Northern Hemisphere, inhabiting fields,

roadsides and rural sites. It is a well-known traditional medicine with a long history, having diuretic, anti-inflammatory, anti-oxidative, anti-carcinogenic activity, etc. [6]. In China, *T. mongolicum* is used as medicine (treatment of mastitis, tonsillitis), food (nutritious plant) and livestock feed (for cows and goats). *T. mongolicum* produces antibacterial compounds that may act to reduce or control the bacterial growth and there are some reports describing its antibacterial activity [7,8]. For example, Gao has

reported that ethanol extracts of the dried aerial parts of *T. mongolicum* had demonstrated antibacterial activity which may be due to the presence of phenylpropanoids and sesquiterpene lactones [7]; meanwhile Qian has revealed that oligosaccharides derived from *T. mongolicum* showed high antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* [8]. However, studies on the antimicrobial properties of *Taraxacum mongolicum* flower were found to be limited. Thus, this work was aimed to assess the antibacterial potential of the ethanol extracts and its fractions of *T. mongolicum* flowers against selected Gram-negative and Gram-positive bacteria.

Experimental

Collection of samples

Fresh flowers of *Taraxacum mongolicum* with no apparent physical, insect and microbial damage were collected from the University Garden of Shanxi Medical University, Taiyuan. The flower petals were carefully removed and were freeze-dried (FD-1A-50, Bilon, Shanghai) for 48 h at -50 °C. Samples were powdered (mesh size 20), covered with aluminum foil and stored at 4 °C until analysis.

Extraction and fractionation

About 10 g of powdered *T. mongolicum* flower was soaked in 50 mL of 75% ethanol for 2 days and then filtered off using sterile Whatman No. 1 filter paper. The collected extract was concentrated to dryness under reduced pressure using a rotary flash evaporator (N-1000, Eyela, Tokyo) to yield 6.43% ethanol extract. The ethanol extract was sequentially partitioned with petroleum ether (PT), ethyl acetate (ET), and water (WT) to yield the following fractions: PT, ET and WT fractions which were evaporated to dryness. The extract and fractions were stored in tightly sealed collection bottles at -20 °C until the time of the experiments.

Preparation of stock and sample solutions

Stock solutions of the ethanol extract and fractions (10 mg/mL) were prepared using dimethyl sulfoxide (DMSO). For assessing the minimum inhibitory concentration (MIC), the stock solutions were serially diluted with Mueller Hinton broth containing 1% Tween 20 to obtain concentrations between 62.5 and 8000 µg/mL. The extract and fractions were filtered with 0.45 µm syringe filters (Minisart, Sartorius, Gottingen) prior to use.

Antibacterial activity assay

Preparation of bacterial culture

The following bacterial strains were obtained from the Department of Microbiology & Immunology, Shanxi Medical University (Taiyuan, China): Gram-negative bacteria: (*Escherichia coli*, *Proteus Vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), Gram-positive bacteria: (*Staphylococcus aureus*, *Bacillus subtilis*). Bacterial strains were grown in Muller Hinton broth for 24 h at 37 °C. Before using the culture for antibacterial assay, culture broth was serially diluted using above sterile fresh broth medium to get a cell number of 1.0×10^6 CFU/mL.

Disc diffusion assay

The standard disc diffusion method described by Bauer *et al.* [9] was followed. Whatman filter paper (No. 1) discs of 6 mm diameter were impregnated with 10 µL of the solution containing ethanol extract and fractions (at a concentration of 10 mg/mL). And then these discs were evaporated at 37 °C for 24 h. The solvents used for dissolving the extracts served as the negative controls, while reference antibiotics gentamicin (10 µg per disc) and tetracycline (10 µg per disc) were used as the positive controls for Gram-negative bacteria and Gram-positive bacteria, respectively. Discs of *T. mongolicum* flower extract/fractions, gentamicin and tetracycline were placed on Muller Hinton agar plates where the bacterial culture was swabbed on the surface of the agar and incubated for 24 h

at 37 °C. The antibacterial activity was evaluated by measuring the zones of inhibition, and the diameters of these zones were measured in millimeters against the test organisms. All experiments were carried out in triplicate and the mean values were accounted for results.

Determination of minimum inhibitory concentration (MIC)

The MIC values of the ethanol extract, fractions and reference antibiotics were determined for bacterial cultures. About 95 µL of Muller Hinton broth and 5 µL of inoculum containing 1.0×10^6 CFU/mL was pipetted into designated wells of the 96-well microtiter plate, except for the negative control wells which consisted of 100 µL of Muller Hinton broth. Then 100 µL different concentrations of the extract/fractions (ranging from 62.5 to 8000 µg/mL) were added to the designated wells. The final volume in each well was 200 µL. Growth inhibitions or microbial growth was determined by measuring the optical density of the culture in the micro wells with different concentrations of *T. mongolicum* flower extracts at 590 nm using a microplate reader.

Two controls were considered: Muller Hinton broth + bacterial suspensions to verify microbial growth (normal); aqueous gentamicin or tetracycline solutions (at concentrations 0.625, 1.25, 2.5, 5.0, 10.0, 20.0 and 40.0 µg/mL) as positive control. Values obtained for *T. mongolicum* flower extracts were compared with the values from normal and the difference was considered as growth inhibition activity. MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. All experiments were conducted in triplicate and the mean values were accounted for results.

Results and discussion

Antibacterial activity

The *in vitro* antibacterial activity of the ethanol extract and the three fractions were assessed by the zone diameters and results were listed in table

1. The ethanol extract and the ET fraction exhibited inhibitory activity against both Gram-negative and Gram-positive bacteria, while the PT and WT fractions were inactive against all six tested bacteria.

The results showed that *P. aeruginosa* and *B. subtilis* were highly susceptible to the ET fraction, though this fraction was also active against *E. coli*, *P. vulgaris*, *K. Pneumoniae* and *S. aureus*. On the other hand, the ethanol extract was found to be active against *P. aeruginosa* and *B. subtilis*, and partially against *E. coli*, *P. vulgaris*, *K. pneumoniae*, and *S. aureus*. This agreement may be explained by the presence of similar compounds in the ethanol extract and the ET fraction, whereas the higher antibacterial activity might be attributed to the presence of high flavonoids and phenolic acids in the ET fraction [10]. The activity against both types of bacteria may be indicative of the presence of broad spectrum of antibiotic compounds. Both positive controls, gentamicin and tetracycline, demonstrated the greatest inhibitory activities against all tested bacteria, while the negative control DMSO did not show any inhibition zone (table 1).

Minimum inhibitory concentration

The ethanol extract and the ET fraction that showed antibacterial activities against Gram-positive and Gram-negative bacteria were evaluated for their MIC. The results have been listed in table 2. The data indicated that the ethanol extract and the ET fraction exhibited variable levels of antimicrobial activity against the investigated pathogens. The MIC values of the ET fraction against the tested Gram-negative bacteria ranged from 125 to 250 µg/mL and for Gram-positive bacteria from 62.5 to 250 µg/mL. Though these MIC values were higher than that of the reference antibiotics gentamicin (against Gram-negative bacteria) and tetracycline (against Gram-positive bacteria), it paves way for the potential use of *T. mongolicum* flower as a new source of effective antibacterial compounds.

Table 1. Antibacterial activity (mm) of *Taraxacum mongolicum* flower extract/fractions against human pathogens

Microorganism	Ethanol extract	PT	ET	WT	Gentamicin	Tetracycline	DMSO
Gram-negative bacteria							
<i>E. coli</i>	12.05±1.29	7.12±0.79	14.21±1.14	8.42±0.88	18.92±1.45	nd	6.00±0.00
<i>P. vulgaris</i>	13.38±1.26	8.34±0.82	18.33±1.29	9.65±1.22	19.47±1.02	nd	6.00±0.00
<i>P. aeruginosa</i>	16.52±1.07	8.78±0.80	19.19±1.23	8.83±1.09	19.98±1.18	nd	6.00±0.00
<i>K. pneumoniae</i>	13.24±1.18	7.59±1.03	17.72±0.89	8.36±0.96	18.86±1.23	nd	6.00±0.00
Gram-positive bacteria							
<i>S. aureus</i>	11.22±0.96	7.88±1.07	15.07±0.98	8.05±0.97	nd	38.79±0.87	6.00±0.00
<i>B. subtilis</i>	14.06±0.86	8.76±0.92	19.56±1.07	9.71±1.20	nd	23.62±0.94	6.00±0.00

Zone of inhibition was determined by agar disc diffusion assay. Results were presented as mean±SD. nd: not determined; PT: petroleum ether fraction; ET: ethyl acetate fraction; WT: aqueous fraction. Zone of inhibition <10 mm: inactive; 10-13 mm: partially active; 14-19 mm: active; >19 mm very active.

Table 2. Minimum inhibitory concentration of *T. mongolicum* flower extracts against microorganisms (µg/mL)

Extract/fractions/controls	MIC (mg/mL)					
	<i>E. coli</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>B. subtilis</i>
ethanol extract	500±2	500±3	250±2	250±2	250±2	250±2
ET	250±1	250±2	125±2	125±2	250±3	62.5±3
Gentamicin	1.25±0.05	2.5±0.2	2.5±0.2	5.0±0.3	nd	nd
Tetracycline	nd	nd	nd	nd	2.5±0.2	5.0±0.2

Results were presented as mean±SD. nd: not determined; ET: the ethyl acetate fraction.

Antibacterial potency of the ET fraction against these bacteria expressed in MIC indicated that this fraction was more effective against Gram-positive (lowest 62.5 µg/mL) at lower concentration compared to Gram-negative bacteria (lowest 125 µg/mL). Saha *et al.* have reported similar results for essential oils and methanol extracts of *Ocimum* species [11]. A possible explanation may reside in the possession of an outer membrane of Gram-negative bacteria, which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide and protects the bacteria cell wall from leakage [12,13].

Phytochemical analysis have revealed the presence of flavonoids, phenolic acids, and terpenoids in the *T. mongolicum* [14,15]. The antibacterial nature of the ethanol extract and the ET fraction might be related to the high flavonoid and phenolic acid contents [16], particularly luteolin and chlorogenic acid. The findings of the present study are in agreement with previous reports [17-19].

This is the first study to provide data about *T.*

mongolicum flower extract/ fractions antibacterial activity against the six mentioned microorganisms. The results indicated that the ET fraction of the ethanol extract could be suggested as a natural alternative to synthetic antimicrobial drugs.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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