

## Original Research Article

# **Taraxacum mongolicum extract exhibits antimicrobial activity against respiratory tract bacterial strains *in vitro* and in neonatal rats by enhancing systemic Th1 immunity**

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### Abstract

**Purpose:** To study the antimicrobial activity of the *Taraxacum mongolicum* extract against respiratory infection-causing bacterial strains *in vitro* and in neonatal rats.

**Methods:** The *in vitro* antibacterial activity was assessed by micro-dilution method. Antioxidant activity was determined by ferric reducing antioxidant power (FRAP), nitro blue tetrazolium (NBT) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays. *In vivo* antimicrobial activity was evaluated in neonatal rat model. Interleukin (IL)-2 (IL-2) and gamma interferon (IFN- $\gamma$ ) were estimated using enzyme-linked immunosorbent assay (ELISA).

**Results:** The hydro-methanol extract of *T. mongolicum* contained high levels of phenolics and flavonoids, and exhibited strong antimicrobial activity against respiratory infection-causing bacterial species with MICs of 25 - 100  $\mu$ g/ml, and MBCs of 55 - 215  $\mu$ g/ml. The highest and lowest antimicrobial activities were observed against *Streptococcus pneumonia* and *Haemophilus influenza*, respectively. The extract at doses of 25 and 50 mg/kg exerted protective effects against *Streptococcus pneumonia*-infected neonatal rats by boosting their Th1 immunity. It enhanced the production of interleukin (IL)-2, concomitant with decreased production of interferon (IFN)- $\gamma$  in neonatal rats. The extract contained isoetin, hesperidin, naringenin, kaempferol, sinapinic and gallic acid.

**Conclusion:** These results suggest that the hydro-methanolic extract of *Taraxacum mongolicum* and its constituents can be potentially developed for use in the management of respiratory bacterial infections.

**Keywords:** Respiratory tract infection, Interleukin, *Taraxacum mongolicum*, Immunity, Neonatal rats

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## INTRODUCTION

Plants manufacture a wide array of metabolites that have been utilised as medicine since ages [1]. The plants of genus *Taraxacum* have been reported to be of immense pharmacological potential, having been utilised for the management of different diseases and disorders

[2]. A wide array of pharmacological properties such as anticancer and antimicrobial effects has been attributed to different extracts of *Taraxacum* species [3]. In the present study, the antimicrobial activity of *Taraxacum mongolicum* was evaluated against the common bacterial strains that cause respiratory infections. Respiratory infections are common and cause

significant mortality and morbidity in humans. More than four million deaths across the globe were attributed to respiratory infections in 1990 alone [4].

Respiratory infections are caused mainly by several pathogenic bacteria which include but are not limited to *Streptococcus pneumoniae* [5]. It has been reported that in infants, the immature immune system is subjected to antigens that are quite different from those it is challenged with later in life [6]. The development of immunity against antigens prevents the onset of diseases. However, failure to develop immunity against such antigenic challenges leads to development of respiratory infections. It has been reported that during the exposure of the infants to antigenic challenges, there is an imbalance between T-helper 1 (Th1) and Th2 immune responses [7].

Furthermore, due to control mechanisms involving gamma interferon (IFN- $\gamma$ ), the Th1 immune response is suppressed and the immune system of the infants becomes more biased towards Th2. This imbalance is often addressed after biological weaning. However, the production of IFN- $\gamma$  and IL-2 boosts the Th1 immunity which is beneficial to the health of the infants [8]. The present study was carried out to investigate the antimicrobial activity of hydro-methanol extract of *Taraxacum mongolicum* *in vitro*, as well as in neonatal rats. The study also determined the phytochemical composition of the extract.

## EXPERIMENTAL

### Plant material and preparation of extract

The fresh leaves of *Taraxacum mongolicum* were collected from natural habitats. The leaves were cleaned by washing in running water and then shade-dried and ground to fine powder. Hydro-methanol extract of the powder was prepared and the extracts was then stored at 4 °C under vacuum for further experimentation.

### Phytochemical analysis and antioxidant activity assays

Total phenolic content was determined with Folin–Ciocalteu reagent as described earlier [9], while total flavonoid content was determined by the AlCl<sub>3</sub> method of Chang *et al* [10]. Antioxidant activities were determined using DPPH, NBT and FRAP assays as described previously [11].

### Bacterial strains and antimicrobial activity

The respiratory infection-causing bacterial strains *Streptococcus pneumoniae*, *Legionella*

*pneumophila*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Haemophilus influenzae* were obtained from the Department of Infectious Diseases, Beijing Jishuitan Hospital. For assessment of the antibacterial activity of the extract, the microdilution method was used. In essence, media containing varied concentrations of the extract (256 to 1.25  $\mu$ g/ml) were placed in a 96-well microplate. This was followed by the addition of the microbial cultures, with pure cultures and DMSO as control. Streptomycin was taken as positive control. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations were determined as described previously [12].

### *In vivo* antibacterial activity

A neonatal rat model was employed for the assessment of the antibacterial activity of the extract. Pregnant Wistar rats obtained from the animal holding capacity of Beijing Jishuitan Hospital, Beijing, China were subjected to standard animal holding conditions and sterilized diet. After the gestation period, each dam littered 10 pups.

The pups were randomly put into four different groups. Group I received normal saline, group II was given 1 ml of *S. pneumoniae* (10<sup>9</sup> CFU/mL), group III received 1 ml of *S. pneumoniae* plus 25 mg of the extract, while group IV received 1ml of *S. pneumoniae* plus 50 mg of the extract once daily for four days. The degree of survival and weight of the pups after 9 days of treatment were determined.

### Isolation of spleen and primary cell preparations

The neonatal rats were sacrificed and the spleen were isolated under aseptic conditions. The cells were extracted and the IL-2 and IFN- $\gamma$  levels were determined by ELISA as described previously [13].

### LC/MS analysis of the extract

In order to identify its active constituents, chemoprofiling of the *Taraxacum* extract was performed by LC/MS analysis as described previously [14].

### Statistical analysis

All experiments were carried out in triplicate, and the data are presented as mean  $\pm$  standard deviation (SD). Student's *t*-test was used for statistical analysis with the aid of GraphPad Prism 7 software. Values of *p* < 0.05 were taken as indicative of significant difference.

## RESULTS

### Hydro-methanol extract of *T. mongolicum* exhibited strong antioxidant activity

Phytochemical screening of the hydro-methanol extract of *T. mongolicum* revealed total phenolic content of 23.65 GAE/g dry weight (DW), while the total flavonoid content was 19.65 QE/g DW. Given the high amounts of phenolics and flavonoid contents, the antioxidant potential of the *T. mongolicum* extract was assessed by 3 different assays. DPPH assay showed that the extract inhibited DPPH radical in a concentration-dependent manner, with more than 70 % inhibition at 50 µg/ml (Table 1).

In the DPPH assay, the antioxidant activity was comparable to that of ascorbic acid used as positive control. Similar observations were made in NBT assay (Table 2). However, in FRAP assay, the antioxidant activity was significantly lower than that of chlorogenic acid (positive control; Table 3).

### Hydro-methanol extract of *T. mongolicum* inhibited the growth of respiratory infection-causing bacterial strains

The results of antimicrobial assays revealed that the MIC of the extract was between 25 and 100 µg/ml. The lowest MIC of 25 µg/ml obtained was against *S. pneumonia*, while the highest MIC of 100 µg/ml was observed against *H. influenzae* (Table 4). The MBC values also showed the same trend and ranged between 55µg/ml (against *Streptococcus pneumonia*) and 215 µg/ml (against *Haemophilus influenzae*; Table 4).

### Hydro-methanol extract of *T. mongolicum* exhibited antimicrobial activity in neonatal rats

The *in vivo* antibacterial activity of the extract of *T. mongolicum* was carried out in the neonatal rat model. The neonatal rats were orally administered the *S. pneumonia* alone or together with the extract at 25 mg or 50 mg, while the control group received normal saline. It was observed that the percentage survival of the rats administered only normal saline was 90 (group 1), while a sudden decline in the % survival was observed for neonatal rats orally administered *S. pneumonia* (Group II).

The % survival in the group II rats was 40 only. However, the % survival of the *S. pneumonia*-treated neonatal rats improved upon subsequent administration of 25 and 50 mg/kg of the extract.

The survival percentage of the rats administered *S. pneumonia* plus 25 mg/kg extract (group III) and *S. pneumonia* plus 50 mg/kg (group V) were 50 and 60 %, respectively (Figure 1).

Next, the effect of the extract on the Th1 immunity of the neonatal rats was evaluated. It has been reported that interleukin (IL)-2 production leads to the development of Th1 cells, and that interferon (IFN)-γ suppresses the Th1 cell development. Therefore, the productions of IL-2 and IFN-γ were assessed in all the neonatal rat groups.

The results showed that the production of IL-2 was enhanced while that of IFN-γ was reduced in *S. pneumonia*-infected neonatal rats concomitantly administered 25 or 50 mg/kg of the hydro-methanol extract of *T. mongolicum* (Figure 2).

### LC/MS analysis revealed the presence of active constituents in the hydro-methanol extract of *T. mongolicum*

The active constituents of the hydro-methanol extract of *T. mongolicum* that could be potentially responsible for its antibacterial activity were identified by LC-MS analysis. The main compounds identified from the extract were isoetin, hesperidin, naringenin, Kaempferol, sinapinic and gallic acid (Figure 3).

**Table 1:** DPPH antioxidant potential of the hydro-methanol extract of *T. mongolicum*

Concentration (µg/ml)	Inhibition (%)	
	Extract	BHT
10	27 ± 2	67 ± 2
20	45 ± 2	73 ± 1
30	56 ± 3	79 ± 2
40	62 ± 2	82 ± 4
50	72 ± 4	84 ± 3

The results are presented as mean ± SD (n = 3)

**Table 2:** NBT assay showing the antioxidant potential of the hydro-methanol extract of *T. mongolicum*

Concentration (µg/ml)	Inhibition (%)	
	Extract	Ascorbic acid
10	28 ± 2	67 ± 2
20	47 ± 1	73 ± 1
30	54 ± 2	79 ± 2
40	63 ± 4	82 ± 4
50	73 ± 3	84 ± 3

The results are presented as mean ± SD (n = 3)

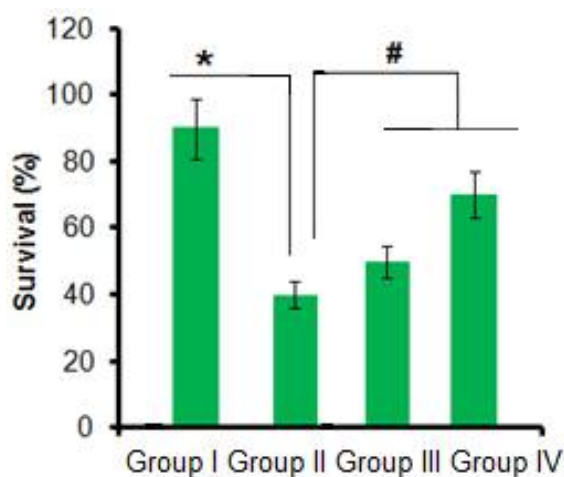
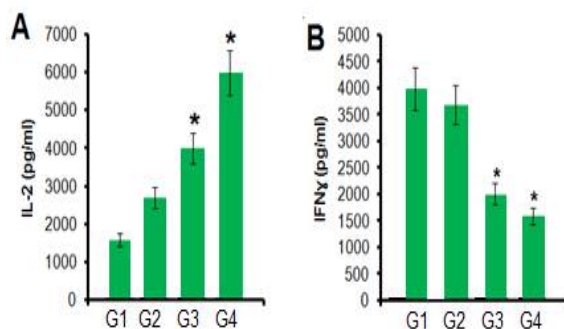
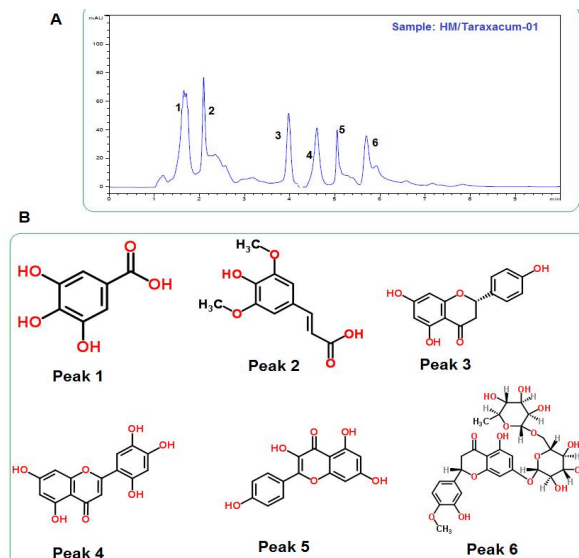
**Table 3:** DPPH antioxidant potential of the hydro-methanol extract of *T. mongolicum*

Concentration (µg/ml)	Absorbance (700 nm)	
	Extract	Chlorogenic acid
10	0.32 ± 0.02	1.05 ± 0.07
20	0.51 ± 0.02	1.66 ± 0.01
30	0.75 ± 0.01	2.56 ± 0.05
40	0.77 ± 0.04	2.85 ± 0.09
50	0.83 ± 0.02	3.56 ± 0.08

The results are presented as mean ± SD of 3 replicates

**Table 4:** Antibacterial activity of hydro-methanol extract of *Taraxacum mongolicum* (MICs and MBCs are expressed in µg/ml)

Bacterial strain	MIC	MBC
<i>Legionella pneumophila</i>	100 ± 5	210 ± 3
<i>Streptococcus pneumoniae</i>	25 ± 1	55 ± 3
<i>Haemophilus influenzae</i>	30 ± 2	70 ± 4
<i>Klebsiella pneumoniae</i>	50 ± 3	90 ± 3
<i>Staphylococcus aureus</i>	50 ± 3	95 ± 3
<i>Streptomycin</i>	14 ± 3	22.5 ± 2

**Figure 1:** Effect of the *T. mongolicum* extract on normal and *Streptococcus pneumoniae*-infected neonatal rats. The experiments were repeated three times and the results are expressed as mean ± SD; \**p* < 0.05: Group I vs Group II; and #*p* < 0.05 for Group II vs Groups III and IV)**Figure 2:** Effect of *T. mongolicum* extract on the production of (A) IL-2 and (B) INF-γ in different groups of neonatal rats (\**p* < 0.05)**Figure 3:** Tentative identification of the active constituents of the hydro-methanol extract of *T. mongolicum*. (A) LC-MS chromatogram showing different peaks; (B) structures of the tentatively identified compounds. Peak 1: gallic acid, Peak 2: sinapinic acid, Peak 3: naringenin, Peak 4: kaempferol, Peak 5: isoetin, Peak 6: hesperidin

## DISCUSSION

Respiratory infections are commonly caused by a number of bacterial species, and they lead to considerable mortality worldwide [15]. Although a number of antibiotics are used to overcome these bacterial infections, the emergence of drug resistance is prevalent among microorganisms. Drug-resistant bacterial strains are often difficult to treat [16]. Over the years, plants and plant-derived products have been considered beneficial in the treatment of bacterial infections. Since a number of active compounds are present in plant extracts, it is believed that each antibacterial molecule may have a different target. Thus, these active components of plant extracts may act synergistically to suppress the growth of pathogenic bacteria and also prevent the emergence of drug resistance [17].

In the present study, the antibacterial activity of the hydro-methanol extract of *T. mongolicum* was evaluated against several bacterial strains *in vitro* and in neonatal rats *in vivo*. The result revealed that the *T. mongolicum* extract inhibited the growth of all the respiratory infection-causing bacterial species used in the study. These results are in agreement with previous findings. Indeed, *T. mongolicum* has been reported to exhibit broad spectrum antimicrobial activity [18]. *Taraxacum* has been shown to suppress the growth of pathogenic bacteria under *in vitro* conditions [19]. It is believed that plants produce a diversity to metabolites to prevent the growth of

pathogens. Metabolites that inhibit the growth of plant pathogens also exhibit antimicrobial activity against human pathogens.

Flavonoids and phenolic compounds inhibit important bacterial enzymes, and also inhibit the cell wall biosynthesis and DNA replication in bacteria, thereby considerably suppressing bacterial growth [20]. Since *T. mongolicum* extract contains considerable amounts of phenolic compounds and flavonoids, these metabolites may be responsible for the observed antimicrobial activity against the tested bacterial strains. In order to further confirm the antibacterial effect of the extract, its antibacterial activity was assessed in neonatal rats. The results showed that administration of the *T. mongolicum* extract to *S. pneumonia*-infected neonatal rats improved their survival rate. Moreover, the extract boosted the Th1 immunity of the neonatal rats by enhancing the production of IL-2 and reducing the production of IFN- $\gamma$ . It has been reported that IL-2 production leads to the development of Th1 cells, while interferon (IFN)- $\gamma$  suppresses development of Th1 cells [6].

These results indicate that *T. mongolicum* exerts protective effects in neonatal rats by enhancing their Th1 immunity. Finally, to identify the active components of the extract, chemo-profiling led to the identification of isoetin, hesperidin, naringenin, kaempferol, sinapic and gallic acid. Previous studies have also reported the presence of these compounds in different species of *Taraxacum*. Moreover, these metabolites have known antimicrobial activities. Thus, they are likely to be responsible for the antimicrobial activity of the hydro-methanol extract of *T. mongolicum*.

## CONCLUSION

Hydro-methanol extract of *T. mongolicum* exhibits significant antimicrobial activity against respiratory infection-causing bacteria. The extract also boosts the Th1 immunity of neonatal rats infected with respiratory infection-causing bacteria. The active constituents of the extract are mainly flavonoids and phenolic compounds. Thus, the hydro-methanol extract and its constituents may be useful in the management of bacterial infections.

## DECLARATIONS

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We thank Beijing Jishuitan Hospital for providing access to the facilities used during this study.

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. This study was designed and mainly performed by Chengdong Sun. Yan Wang and Xuemei Zhang assisted some parts of the experiments, including collected materials and manuscript revision.

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