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*Original Research*

# *Tordylium persicum* Boiss. & Hausskn extract: A possible alternative for treatment of pediatric infectious diseases

J. Sharifi-Rad[1, 2], F. Fallah[3, 4], W. N. Setzer[5], R. Entezari Heravi[1, 6], M. Sharifi-Rad[7*]

[1] Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran
[2] Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran
[3] Pediatric Infections Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5] Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA
[6] Department of Biotechnology, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran
[7] Zabol University of Medical Sciences, Zabol, Iran

**Abstract:** Antimicrobial herbal compounds are one of the important medical resources, and in order to help alleviate the spread of the pediatric infectious diseases, identification of additional bioactive phytochemicals and herbal extracts will be practical in treating illnesses. In the present work, antimicrobial activities various extracts of *Tordylium persicum* Boiss & Hausskn aerial parts were determined against five Gram-positive bacteria, five Gram-negative bacteria, two fungi, and *Echinococcus granulosus*. Antimicrobial activities were assayed using both disk diffusion and microbroth dilution methods. Scolicidal activity was assayed by the Smyth and Barrett method. Also total phenol and total flavonoid contents for plant extracts were assayed. Results showed that the methanolic extract was more effective on all microbes. The results showed that *Streptococcus pyogenes* was the most susceptible to the methanolic extract (MIC = 25.9 ± 0.0 µg/mL), while *Proteus vulgaris* was the most resistant strain (MIC = 295.3 ± 0.0 µg/mL) among all bacteria evaluated. The extracts showed significant activity versus *E. granulosus* ($P < 0.5$) with dose-dependent inhibitions of the protoscolices. The high concentration of total polyphenolics (294.5 ± 0.1 GAE/g DW) and flavonoids (105.7 ± 0.3 mg CE/g DW) may be responsible for these activities. Our study is first evaluation on antimicrobial and scolicidal activities of *T. persicum*. Due to the appearance of antibiotic-resistance, our study suggested that methanol extracts of this plant are appropriate candidate for traditional curative uses and it can be utilized in the pediatric infectious disease therapy, especially pediatric infectious disease.

***Key words:*** *Tordylium persicum*, antimicrobial, disk diffusion method, microbroth dilution method, *Echinococcus granulosus*.

## Introduction

In 2011, World Health Organization reported that infectious diseases were responsible for almost 18 million deaths in worldwide (1). Bacterial infections are most frequent (70%), followed by viral (20%) and fungal infections (8%) (2,3). Liu et al. (4) reported that in 2013, 51.8% (3.257 million) from 6.3 million children who died in their first 5 years of life were related to infectious diseases.

In recently years, the extreme and continual use of drugs in medicine has resulted to the development of antibiotic-resistant microbial strains, therefore diminishing the antimicrobial chemotherapeutics available to cure clinical infections (5-10). Antibiotic resistance is one of the most significant and challenging problems in global health (11). Also, pathogenic bacteria resistance too many antibiotics (multidrug-resistant strains), also known as 'superbugs', are appearing at a rapid pace which has led to an increase in morbidity and mortality from microbial infections. The strategies recently developed to fight increasing drug resistance include genomic approaches, vaccine development, and modification of existing agents. Hence, during the recent decades, considerable attention has been paid to finding and further developing natural antimicrobial agents that can target multiple organisms, are highly effective, and have adverse side effects (12-15).

For many years, people from all of different regions of the earth have used plants and herbs to treat disease (16- 21). Higher plants have shown great promise in synthesis of antimicrobial agents as their protective mechanisms to alleviate biotic stresses. The phytochemical antimicrobials can be classified into various classes, inducing flavonoids, polypeptides, polyphenolics, polyacetylenes, alkaloids, lectins, and terpenoids (22-24). Also, in traditional medicine, a large number of medicinal plants have shown antimicrobial effects and many of these have been utilized for treatment of different infectious diseases (25-27).

Apiaceae (Umbelliferae) is a well-known family of aromatic and economically important plants, and is comprised of more than 2500-3000 species worldwide. *Tordylium* is a genus of Apiaceae, described by an annual habit, 1-3-pinnate leaves, thickened mericarp margins, and dorsally compressed mericarps (28). There are only a few phytochemical and biological activity studies on some *Tordylium* species, and apparently no previous studies on *T. persicum* have appeared in the literature. The purpose of our study, therefore, was to perform *in-vitro* examination of the antibacterial, antifungal, and scolicidal activities of different extracts of *T. persicum*

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*** Corresponding author:** Mehdi Sharifi-Rad, Zabol University of Medical Sciences, Zabol, Iran. Email: mehdi_sharifirad@yahoo.com

grown in Iran. The findings from this study may add to the overall value of this important herb.

## Materials and Methods

### Plant collection and preparation

The aerial parts (leaves, stems and flowers) of *Tordylium persicum* Boiss & Hausskn were collected between April-May 2015 from area of Hamun Lake of Zabol, Sistan and Baluchestan Province, Iran. The plant was identified taxonomically at the Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran, where a voucher specimen was preserved. Aerial parts of *T. persicum* (3 kg) were dried in shade for three days and then stored at 4°C in desiccators until further testing.

### Preparation of Polar and Non-polar Extracts

For preparation of polar and non-polar extracts, 300 g dry powdered aerial parts of *T. persicum* was sequentially extracted (Soxhlet extractor with water bath) with polar (methanol; MeOH) and non-polar (dichloromethane) for 12 h. Each extract was then filtered (Whatman No. 2 filter paper), and the solvent removed from the filtrate under reduced pressure (rotary evaporator) at 35 °C. The concentrated extracts so obtained were then stored at -20 °C in labeled sterile bottles and kept as aliquots until further evaluation. For preparation of the aqueous extract, 300 g of dry powdered plant sample was extracted by soaking in 1 L distilled water (DW) in a round-bottom flask, stirred for 5 min, tightly stoppered and left overnight at room temperature (25 ± 1 °C). Afterwards, the extract solution was filtered (Whatman No. 2 filter paper) and the extract was freeze dried and stored in labeled sterile bottles at -20 °C.

### Total Phenol Content

In this study, total phenols were assayed based on Dewanto *et al*. (29). From each extract, an aliquot was added to 0.125 mL of Folin–Ciocalteu reagent and 0.5 mL of DW. The mixture was mixed, allowed to stand for 15 min, and 1.25 mL 5% $Na_2CO_3$ solution was added. The solutions were then adjusted with DW to a final volume of 4 mL and mixed thoroughly. Absorbance at 760 nm was read versus a prepared blank after incubation in the dark. The total phenol concentration of each plant extract was expressed in terms of milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) from a calibration curve with gallic acid.

### Total Flavonoid Content

The colorimetric assay was used for assay total flavonoids based on method of Dewanto *et al*. (29). One aliquot of standard solution of (+)-catechin or diluted sample was added to 50 mL of 5% $NaNO_2$ solution and mixed for 5 min, followed by addition of 0.15 mL 10% $AlCl_3$. After 5 min, 0.5 mL of NaOH was added, and the final volume was adjusted to 2.5 mL with DW and mixed thoroughly. Absorbance was determined at 510 nm against a blank. Total flavonoid concentration is expressed as milligrams of catechin per gram of dry weight (mg CE/g DW) against the calibration curve of (+)-catechin, from 0 to 400 mg/mL.

### Antimicrobial Activities

#### *Microorganisms*

The all of microorganisms that were used in this study were purchased from the Persian Type Culture Collection (PTCC), Tehran, Iran. The extracts were examined against five Gram-positive bacteria: *Staphylococcus aureus* PTCC 1112 (American Type Culture Collection ATCC 6538), *Staphylococcus epidermis* PTCC 1114 (ATCC 12228), *Staphylococcus saprophyticus* subsp. *saprophyticus* PTCC 1440 (ATCC 15305), *Enterococcus faecalis* PTCC 1774 (ATCC 19433) and *Streptococcus pyogenes* PTCC 1447 (ATCC 8668), five Gram-negative bacteria: *Pseudomonas aeruginosa* PTCC 1074 (ATCC 9027), *Klebsiella pneumoniae* PTCC 1053 (ATCC 10031), *Escherichia coli* PTCC 1330 (ATCC 8739), *Proteus vulgaris* PTCC 1312 (ATCC 7829) and *Proteus mirabilis* PTCC1776 (ATCC 43071); and two fungi: *Aspergillus niger* PTCC 5010 (ATCC 9142) and *Candida albicans* PTCC 5027 (ATCC 10231).

#### *Antibacterial and Antifungal Activities*

Different concentrations of each extract plant were screened against microorganisms using the disc diffusion method (30). Briefly, bacterial and fungi were cultured at 37 °C for 14–24 h and the densities were adjusted to $10^8$ CFU/mL (a McFarland turbidity of 0.5 at absorbance of 530 nm). Afterwards, 100µL aliquots of each microbial suspension were applied to nutrient agar (Merck, Germany) plates (100 mm × 15 mm). The discs (6 mm diameter) were singly infused with 10 µL of each extract at different concentrations (150, 300, 600, and 1000 µg/mL) and positioned onto the inoculated agar plates. Each of the inoculated plates was incubated at 37 °C for 24 h. Gentamicin (10 mg/disc), ampicillin (10 mg/disc), and ketoconazole (10 mg/disc) were used as positive controls for Gram-negative, Gram-positive bacteria, and fungi, respectively. The negative control for this assay was dimethyl sulfoxide (DMSO). Antibacterial and antifungal activities were determined by estimating the inhibition zone (mm). Minimal inhibitory concentrations (MICs) for active extracts in disc diffusion method were determined using the standard procedure of the Clinical and Laboratory Standards Institute using the microbroth dilution assay in 96-well microtiter plates (31). The microbial strains tested were each suspended in Luria-Bertani medium and the densities were adjusted to a McFarland turbidity of 0.5 at 570 nm ($10^8$ CFU/mL). Each extract was dissolved in 50% aqueous DMSO to a final concentration of 10 mL. Each microbe was screened with extracts that were serially diluted in medium to secure final concentrations ranging from 512.0 to 0.06 µg/mL. The broth cultures of each microorganism were prepared and the concentrations in each well were adjusted to $10^6$ CFU/mL. The microtiter plates were incubated at 37 °C for 24 h. The medium with bacteria and fungi, but without extract, served as the growth control; medium without bacteria or fungi was the sterility control. The bacterial and fungal growth was compared to those of the controls. The MICs of the extracts in disc diffusion method were determined visually as the lowest extract concentration that showed > 95% growth inhibition of the appraised microbes.

**Scolicidal Activity**

The protoscolices of *Echinococcus granulosus*, prepared from the infected livers of calves killed in a slaughterhouse, were used to assay scolicidal activity. The Helsinki Declaration guidelines were followed to ensure the ethical treatment of animals. The Smyth and Barrett method was used to collect the hydatid fluid along with protoscolices (32). In brief, hydatid fluid added to a glass cylinder allowing the protoscolices to settle to the bottom (ca. 40 min). The protoscolices were washed three times with normal saline and their motility; visualized using a light microscope (Nikon Eclipse E200, Tokyo, Japan) was used to determine viability. Protoscolices were transferred to a dark receptacle containing normal saline and stored at 4 °C. The various methanolic extract concentrations (10, 20, 25, 50, and 100 mg/mL, dissolved in 9.7 mL of normal saline supplemented with 0.5 mL of Tween-80 under continuous stirring) were tested for 10, 20, 30, and 60 min. In each assay, one drop of protoscolices-rich solution was added to 3 mL of extract solution, mixed slowly, and incubated at 37 °C. Following each incubation period, the upper phase was slowly removed so as to not disturb the protoscolices; afterwards, 1 mL of 0.1% eosin stain was added to the protoscolices that remained and was mixed slowly. After incubating for 20 min at 25 °C, the supernatant was discarded, and the remaining pellet of protoscolices was smeared on a manually scaled glass slide, covered with a cover slip, and appraised under a light microscope. After counting a minimum of 600 protoscolices, the percentage of dead protoscolices was determined. The control consisted of protoscolices treated only with normal saline + Tween-80.

**Statistical Analysis**

Each extract was assayed in triplicate for total polyphenol, flavonoid contents analysis, as well as and biological activities assays. Analysis of variance (ANOVA) of the data, following a completely randomized design, was used to determine the least significant difference (LSD) at $P < 0.05$, utilizing statistical software package (SPSS v. 11.5). The all results are expressed as mean ± SD.

**Results**

The total polyphenol and flavonoid contents in plant extracts are shown in Table 1. The total polyphenolics content for dichloromethane, methanolic, and aqueous extracts were found to be 195.9 ± 0.2, 294.5 ± 0.1, and 212.7 ± 0.3 GAE/g DW, respectively. Flavonoids concentrations were 66.6 ± 0.1, 105.7 ± 0.3, 74.9 ± 0.3 mg CE/g DW, respectively. There were significant differences among the different extracts for total polyphenolics as well as flavonoids contents ($P < 0.5$). The methanolic extract showed maximum concentrations for both total polyphenolics and flavonoids.

The antibacterial activities are summarized in Table 2. The results showed that the dichloromethane, methanol, and aqueous extracts of *T. persicum* exhibited a dose-dependent antibacterial effect on the growth of all tested bacteria. The *T. persicum* extracts showed the maximum zones of inhibition at a concentration of 1000 µg/mL of methanol extracts on the growth of all bacteria. Inhibition zones at concentration of 1000 µg/mL of the methanol extracts were 28.4 ± 0.3, 21.9 ± 0.0, 23.8 ± 0.0, 22.9 ± 0.3, 24.8 ± 0.0, 17.3 ± 0.2, 14.5 ± 0.0, 19.5 ± 0.2, 17.7 ± 0.5, and 19.3 ± 0.0 mm for *S. aureus*, *S. epidermis, S. saprophyticus, E. faecalis, S. pyogenes, K. pneumonia, P. aeruginosa, E. coli, P. vulgaris, P. mirabilis*, respectively. Among bacteria, *S. pyogenes* (MIC of methanolic extract = 25.9 ± 0.0 µg/mL) was the most susceptible to the extracts of *T. persicum*, because of its very low MIC. The results of antifungal tests of plant extracts are shown in Table 3. The different *T. persicum* extracts inhibited the growth of *C. albicans* and *A. niger* in all assayed concentrations. The maximum inhibition zone observed in concentration 600 and 1000 µg/mL of methanol extracts for the fungi. The methanol extracts showed strong activity against *C. albicans* with an inhibition zone of 13.5 ± 0.0 mm at 1000 µg/mL concentration. MICs for *A. niger* and *C. albicans* were 196.5 ± 0.2 and 93.9 ± 0.5 µg/mL of methanolic extract, respectively.

Mortality rates of *E. granulosus* protoscolices after treatment with various concentrations of *T. persicum* extracts are shown in Table 4. As exposure time and extracts concentration increased, % mortality was also increased. Hence, exposure to the extracts for 60 min, at 10, 20, 25, 50, and 100 mg/mL resulted in 24.95%, 32.52%, 37.19%, 42.43% and 64.86% inhibition, respectively. The mortality in the control was 56.41%, after 60 min.

**Discussion**

Plants have been the foundation of traditional medicinal systems worldwide for thousands of years to cure or prevent disease. Antimicrobial herbal compounds are one of the important medical resources, and in line with the spread of pediatric infectious diseases, identification of more of these extracts and compounds will be practical in treating patients (33, 34). The significant advantage claimed for therapeutic use of medicinal plants is their safety in addition to being economical, efficient and readily accessible. The mechanism of plant antimicrobials action has not been entirely elucidated. Efflux mechanisms like multidrug efflux pumps (MEPs) have become recognized as an important mechanism of resistance to numerous classes of antibiotics (35, 36). A new

**Table 1.** Total polyphenol (GAE/g DW) and flavonoids (mg CE/g DW) contents in the dichloromethane, methanolic and aqueous extracts of *Tordylium persicum.*

| Extracts | Total phenolic content | Total flavonoid content |
|---|---|---|
| Dichloromethane | 195.9 ± 0.2 c[§] | 66.6 ± 0.1 c |
| Methanolic | 294.5 ± 0.1 a | 105.7 ± 0.3 a |
| Aqueous | 212.7 ± 0.3 b | 74.9 ± 0.3 b |

[§]Values are expressed as mean ± SD. Different letters show significant differences for each plant in each column at *P* value less than 0.05.

**Table 2.** Antibacterial activity of dichloromethane, methanol and aqueous extracts of *Tordylium persicum* against gram-positive and gram negative bacterial strains.

| Plant Extracts (µg/mL) | Dichloromethane | | | | Methanol | | | | Aqueous | | | | DMSO * | Ampicillin | Gentamicin | MIC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 150 | 300 | 600 | 1000 | 150 | 300 | 600 | 1000 | 150 | 300 | 600 | 1000 | | | | |
| *Staphylococcus aureus* | 12.1± 0.0[§] | 15.3 ± 0.1 | 18.5 ± 0.9 | 19.4 ± 0.5 | 16.2 ± 1.1 | 23.1 ± 0.2 | 26.5 ± 0.2 | 28.4 ± 0.3 | 14.23 ± 0.1 | 18.3 ± 0.2 | 19.1 ± 0.0 | 19.3 ± 0.2 | 0.0 ± 0.0 | 22.5 ±0.0 | - | 34.5 ± 0.2 |
| *Staphylococcus epidermis* | 10.3 ± 0.1 | 14.2 ± 0.0 | 18.5 ± 0.2 | 19.8 ± 0.1 | 12.7 ± 0.0 | 14.9 ± 0.1 | 15.4 ± 0.2 | 21.9 ± 0.0 | 12.5 ± 0.2 | 16.7 ± 0.1 | 18.3 ± 0.1 | 20.6 ± 0.2 | 0.0 ± 0.0 | 24.9 ± 0.0 | - | 115.8 ± 0.3 |
| *Staphylococcus saprophyticus* | 11.5 ± 0.0 | 13.5 ± 0.2 | 13.9 ± 0.0 | 14.5 ± 0.1 | 16.5 ± 0.2 | 18.6 ± 0.0 | 21.5 ± 0.4 | 23.8 ± 0.0 | 14.4 ± 0.0 | 15.8 ± 0.4 | 16.2 ± 0.0 | 18.7 ± 0.0 | 0.0 ± 0.0 | 26.5 ± 0.0 | - | 154.5 ± 0.0 |
| *Enterococus faecalis* | 14.3 ± 0.5 | 17.8 ± 0.2 | 18.5 ± 0.4 | 19.9 ± 0.0 | 15.6 ± 0.0 | 17.8 ± 0.2 | 19.9 ± 0.3 | 22.9 ± 0.3 | 13.5 ± 0.0 | 16.4 ± 0.1 | 18.4 ± 0.2 | 20.3 ± 0.0 | 0.0 ± 0.0 | 25.3 ± 0.0 | - | 165.2 ± 0.1 |
| *Streptococcus pyogenes* | 15.8 ± 0.2 | 16.4 ± 0.0 | 18.7 ± 0.5 | 19.6 ± 0.2 | 16.8 ± 0.0 | 19.4 ± 0.3 | 23.9 ± 0.3 | 24.8 ± 0.0 | 14.5 ± 0.1 | 14.5 ± 0.0 | 15.5 ± 0.2 | 19.5± 0.0 | 0.0 ± 0.0 | 24.5 ± 0.0 | - | 25.9 ± 0.0 |
| *Klebsiella pneumoniae* | 8.5 ± 0.0 | 11.7 ± 0.2 | 11.4 ± 0.1 | 12.5 ± 0.1 | 12.4 ± 0.1 | 12.5 ± 0.0 | 14.7 ± 0.2 | 17.3 ± 0.2 | 10.2 ± 0.0 | 10.8 ± 0.1 | 12.4 ± 0.2 | 16.6 ± 0.2 | 0.0 ± 0.0 | - | 22.8 ± 0.0 | 225.3 ± 0.0 |
| *Pseudomonas aeruginosa* | 7.7 ± 0.2 | 9.3 ± 0.2 | 10.5 ± 0.0 | 11.3 ± 0.1 | 9.5 ± 0.0 | 12.3 ± 0.2 | 12.5 ± 0.0 | 14.5 ± 0.0 | 10.2 ± 0.0 | 11.5 ± 0.2 | 13.2 ± 0.2 | 13.8 ± 0.0 | 0.0 ± 0.0 | - | 19.5 ± 0.0 | 240.5 ± 0.1 |
| *Escherichia coli* | 10.6 ± 0.2 | 14.5 ± 0.0 | 15.2 ± 0.1 | 16.3 ± 0.0 | 13.2 ± 0.0 | 15.8 ± 0.5 | 17.9 ± 0.0 | 19.5 ± 0.2 | 11.5 ± 0.0 | 12.0 ± 0.0 | 14.2 ± 0.1 | 14.5 ± 0.0 | 0.0 ± 0.0 | - | 23.9 ± 0.0 | 210.9 ± 0.0 |
| *Proteus vulgaris* | 12.5 ± 0.3 | 12.9 ± 0.0 | 14.2 ± 0.0 | 15.3 ± 0.2 | 13.2 ± 0.0 | 14.7 ± 0.5 | 15.9 ± 0.3 | 17.7 ± 0.5 | 10.4 ± 0.1 | 12.3 ± 0.0 | 14.5 ± 0.2 | 17.2 ± 0.0 | 0.0 ± 0.0 | - | 26.2 ± 0.0 | 295.3 ± 0.0 |
| *Proteus mirabilis* | 13.5 ± 0.0 | 13.8 ± 0.5 | 14.5 ± 0.0 | 14.5 ± 0.0 | 13.9 ± 0.0 | 15.8 ± 0.1 | 16.4 ± 0.2 | 19.3 ± 0.0 | 12.9 ± 0.2 | 14.5 ± 0.2 | 14.8 ± 0.0 | 17.9 ± 0.2 | 0.0 ± 0.0 | - | 25.3 ± 0.0 | 290.8 ± 0.2 |

[§] Data are expressed as mean ± SD of inhibition zone diameter (mm) for different concentrations of extract, controls and minimum inhibitory concentration (MIC) (µg/mL); * DMSO: dimethyl sulfoxide.

**Table 3.** Antifungal activity of *Tordylium persicum* extracts against fungal strains.

| Plant Extracts (µg/mL) | Dichloromethane | | | | Methanol | | | | Aqueous | | | | DMSO * | Ketoconazole | MIC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 150 | 300 | 600 | 1000 | 150 | 300 | 600 | 1000 | 150 | 300 | 600 | 1000 | | | |
| *Candida albicans* | 8.5 ± 0.1[§] | 9.9 ± 0.0 | 11.4 ± 0.2 | 11.5 ± 0.2 | 11.4 ± 0.1 | 11.5 ± 0.1 | 12.5 ± 0.2 | 13.5 ± 0.0 | 9.4 ± 0.2 | 10.5 ± 0.0 | 11.0 ± 0.0 | 11.2 ± 0.2 | 0.0 ± 0.0 | 14.5 ± 0.0 | 93.9 ± 0.5 |
| *Aspergillus niger* | 4.4 ± 0.1 | 4.8 ± 0.5 | 5.5 ± 0.4 | 6.2 ± 0.3 | 5.3 ± 0.0 | 6.2 ± 0.1 | 6.8 ± 0.2 | 7.9 ± 0.5 | 4.1 ± 0.0 | 4.5 ± 0.0 | 6.4 ± 0.2 | 6.5 ± 0.3 | 0.0 ± 0.0 | 13.5 ± 0.0 | 196.5 ± 0.2 |

[§] Data are expressed as mean ± SD of inhibition zone diameter (mm) for different concentrations of extracts, controls and minimum inhibitory concentration (MIC) (µg/mL); * DMSO: dimethyl sulfoxide.

**Table 4.** Scolicidal activity of *Tordylium persicum* extracts against *Echinococcus granulosus*.

| Concentration(mg/mL) | Exposure Time(min) | Protoscolices | Dead Protoscolices | Mortality (%) |
|---|---|---|---|---|
| | 10 | 889.5 ± 12.00 § | 102.31 ± 67.66 | 11.5 |
| | 20 | 1265.34 ± 18.95 | 245.38 ± 15.62 | 19.39 |
| 10 | 30 | 1211.14 ± 23.45 | 269.48 ± 82.14 | 22.25 |
| | 60 | 985.44 ± 57.84 | 245.09 ± 46.00 | 24.95 |
| | Control * | 1477.00 | 833.22 | 56.41 |
| | 10 | 860.22 ± 25.25 | 152.44 ± 25.00 | 17.72 |
| | 20 | 1014.88 ± 20.5 | 215.41 ± 25.98 | 21.22 |
| 20 | 30 | 1385.18 ± 49.14 | 399.85 ± 23.18 | 28.86 |
| | 60 | 889.00 ± 89.00 | 289.12 ± 18.19 | 32.52 |
| | Control | 1477.00 | 833.22 | 56.41 |
| | 10 | 1542.42± 11.89 | 325.12 ± 14.00 | 21.07 |
| | 20 | 1215.11 ± 14.88 | 320.19 ± 45.37 | 26.35 |
| 25 | 30 | 999.47 ± 21.00 | 357.8 ± 11.2 | 35.79 |
| | 60 | 1483.16 ± 58.14 | 551.71 ± 15.12 | 37.19 |
| | Control | 1477.00 | 833.22 | 56.41 |
| | 10 | 1122.46 ± 12.11 | 352.12 ± 25.46 | 31.37 |
| | 20 | 991.44 ± 11.98 | 367.49 ± 44.00 | 37.06 |
| 50 | 30 | 924.15 ± 12.79 | 367.14 ± 14.35 | 39.72 |
| | 60 | 1245.13 ± 78.49 | 528.32 ± 73.4 | 42.43 |
| | Control | 1477.00 | 833.22 | 56.41 |
| | 10 | 944.22 ± 83.25 | 384.25 ± 42.49 | 40.69 |
| | 20 | 1271.44 ± 77.17 | 528.99 ± 65.66 | 41.60 |
| 100 | 30 | 844.65 ± 22.12 | 518.47 ± 15.31 | 61.38 |
| | 60 | 1377.45 ± 12.00 | 893.45 ± 24.46 | 64.86 |
| | Control | 1477.00 | 833.22 | 56.41 |

§ Values are mean ± SD of three replicates; * in the control, protoscolices were treated only with saline + Tween-80 solution.

and encouraging approach to deal with multidrug resistance is to enhance the clinical performance of different antibiotics by utilizing MEP inhibitors. Plants have greatly been investigated as possible sources of these inhibitors (37).

Results of this study showed among the different extracts of *T. persicum,* the methanolic extract was the most effective on all bacteria. Matejic *et al*. (38) reported methanol extracts of *T. maximum* showed inhibitory antimicrobial activity against *Bacillus cereus*, *P. aeruginosa*, *E. coli*, *S. aureus, Salmonella enteritidis*, *Listeria monocytogenes*, and *C. albicans*. The antibacterial assays in our study showed that *S. pyogenes* was the most susceptible to the *T. persicum* methanolic extract. Beta-hemolytic *Streptococcus pyogenes* can cause a range of types and sensitivity of infections in childhood comprising toxin-mediated, invasive, and immune-mediated diseases. In our study, *S. aureus* and *K. pneumoniae* were other bacteria that the plant extract had demonstrated notable effects. *S. aureus* is the most common cause of musculoskeletal infections in pediatric patients. Martínez-Aguilar *et al*. (39) reported that the *pvl* gene presence may be having a relationship and raised the probability of complications in *S. aureus* musculoskeletal infections in children. In each year, 1-9 million children with age of under 5 year die from pneumonia (40). *K. pneumoniae* is usually a nosocomial pathogen, being the fifth and fourth most common causes of bacteremia and pneumonia, respectively, in intensive care patients.

In our study, the plant extracts showed antifungal effects on the two fungi. *Aspergillus* and *Candida* are the main genera of fungi associated with human infectious diseases. Cystic hydatid disease caused by the *Echinococcus granulosus* is medically and economically one of the most significant of the zoonoses. Surgery and the administration of chemotherapeutic agents are the principal hydatid disease treatments (41). However, most of them are accompanied by adverse side effects. Hence, new scolicidal agents are needed with no local or systemic side effects.

Generally, antimicrobial activities of the methanolic plant extract in our study can be related to presence of high concentrations of polyphenolics and flavonoids compared with the other type of extracts. Higher plants produce polyphenols as secondary metabolites, which play various essential roles in plant physiology and have potential healthy characteristic on human organism, principally as antimicrobial agents, anti-allergic, antioxidants, anti-inflammatory, antihypertensive, and anticancer (42). Regarding chemical structure, polyphenolics comprise a wide diversity of compounds, which are usually divided into nonflavonoids and flavonoids (38). These compounds can interpose by means of their lipophilic moiety with the lipophilic membrane bilayer of the pathogens after forming a complex with cholesterol, while the hydrophilic sugar part remains outside of the cell and interacts with glycolipids or glycoproteins (43). The membrane integrity destruction enables other highly polar flavonoid glycosides to penetrate the pathogen and exert their effects. On this principle, the antimicrobial activity is probably to be affected by the synergistic and supplemental effects of all ingredients (43).

This study shows that methanol extracts of *T. persicum* have extensive antimicrobial effects against various strains of Gram-positive and Gram-negative bacteria, some species of fungi, and Echinococcosis. The results suggest that the antimicrobial potential of *T. persicum* can be attributed to the presence of polyphenolics and flavonoids compounds. Finally, the antimicrobial poten-

tial showed by *T. persicum* warrants further exploration for the development of novel effective chemotherapeutic agents for traditional therapeutic uses, and can be used in the therapy of pediatric infectious disease, especially pediatric infectious disease as well as an antimicrobial additive in foods.

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