



## Original Article

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Antibacterial and anti-parasitic activities of *Terfezia claveryi* methanolic extract against some common pathogenic agents of infectious diarrheaSultan F Alnomasy<sup>✉</sup>

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## ABSTRACT

**Objective:** To assess the antidiarrheal effects of *Terfezia claveryi* methanolic extract against *Escherichia coli*, *Salmonella typhimurium*, *Shigella flexneri*, and *Giardia lamblia*.

**Methods:** Antibacterial effects of the *Terfezia claveryi* methanolic extract were carried out by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration through micro broth dilution technique. Furthermore, reactive oxygen species production and protein leakage were evaluated. To evaluate the *in vitro* anti-giardial effects of *Terfezia claveryi* methanolic extract, *Giardia lamblia* WB (ATCC<sup>®</sup> 30957) trophozoites were treated with various concentrations of *Terfezia claveryi* methanolic extract for 10–360 min. In addition, the plasma membrane permeability of trophozoites treated with *Terfezia claveryi* methanolic extract was determined. The cytotoxicity effects of *Terfezia claveryi* methanolic extract against normal (HEK293T) and cancer (MCF-7) cells were also assessed using the MTT assay.

**Results:** The MIC and minimum bactericidal concentration of *Terfezia claveryi* methanolic extract against bacterial strains were in the range of 0.52–1.04 and 1.04–2.08 mg/mL, respectively. The results revealed that reactive oxygen species production and protein leakage were significantly increased after the bacteria were treated with the *Terfezia claveryi* methanolic extract, especially at 1/3 and 1/2 MICs ( $P < 0.001$ ). Furthermore, *Terfezia claveryi* methanolic extract decreased the viability of *Giardia lamblia* trophozoites in a dose-dependent manner. *Terfezia claveryi* methanolic extract at 1, 2, and 4 mg/mL resulted in 100% mortality in *Giardia lamblia* trophozoites after 360, 240, and 120 min, respectively. Moreover, *Terfezia claveryi* methanolic extract altered the permeability of plasma membrane of *Giardia lamblia* trophozoites by increasing the concentration. MTT assay revealed that the 50% cytotoxic concentrations values for HEK293T and MCF-7 cells were 4.32 mg/mL and 6.40 mg/mL, respectively, indicating that *Terfezia claveryi* methanolic extract had greater cytotoxicity against cancer cells than normal cells.

**Conclusions:** *Terfezia claveryi* methanolic extract had potent *in vitro* antibacterial and anti-parasitic effects on *Escherichia coli*, *Salmonella*

*typhimurium*, *Shigella flexneri*, and *Giardia lamblia* by affecting cell membrane permeability and reactive oxygen species generation with no significant cytotoxicity on normal cells.

**KEYWORDS:** *Terfezia claveryi*; Shigellosis; Giardiasis; Herbal medicines; Infectious diarrhea; Cellular mechanisms; Cytotoxicity; Microbiology

## 1. Introduction

Diarrheal diseases are mainly characterized by an intestinal transport disorder that is described by loose or watery stools, several times in several consecutive days which reduces the minerals needed by the body[1]. Diarrheal diseases with an annual incidence of 1.7 billion cases and more than 500 000 deaths are considered the

## Significance

At present, one of the main strategies for treating infectious diarrhea is therapy with antibiotics. However, they have some limitations due to their side effects and development of drug resistance. *Terfezia claveryi* methanolic extract had potent effects against *Escherichia coli*, *Salmonella typhimurium*, *Shigella flexneri*, and *Giardia lamblia* through affecting cell membrane permeability and reactive oxygen species generation with no significant cytotoxicity.

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second leading cause of death in infants under five years of age[2]. Generally, diarrheal diseases are caused by numerous pathogenic parasites [e.g., *Entamoeba histolytica*, *Cryptosporidium*, and *Giardia lamblia* (*G. lamblia*)], bacteria [e.g., *Salmonella* spp, *Campylobacter*, *Shigella* spp, and *Escherichia coli* (*E. coli*)] and viruses (e.g., rotavirus)[3]. Synthetic drugs such as antibiotics, anticancer drugs, and antiacids containing magnesium are also considered as another cause of diarrhea. However, chronic diarrhea can be frequently linked to diseases such as irritable bowel syndrome and Crohn's disease[3].

At present, one of the main strategies for treating infectious diarrhea is therapy with antibiotics such as trimethoprim/sulfamethoxazole, metronidazole, ampicillin, nalidixic acid, ciprofloxacin, and fluoroquinolone[4]. However, the use of these antimicrobial agents has limitations due to their side effects and the development of drug resistance. This, accordingly, leads researchers to search for new sources of promising antibacterial agents from natural products for treatment of infectious diarrhea[5].

Today, mushrooms are well-known as a widespread food in different regions of the world and they have become very pleasant as a functional food[6]. Truffles are considered a wide family of hypogeous fungi, generally containing the genera of *Picoa*, *Tirmania*, *Tuber*, and *Terfezia*[7]. Genus of the *Terfezia* (belonging to the family Tubraceae) have four species including *Terfezia boudieri* Chatin, *Terfezia claveryi* (*T. claveryi*) Chatin, *Terfezia leonis* Tul, and *Terfezia metaxasi* Chatin[8]. *T. claveryi* as a black-color truffle is the most frequent and widely used truffle in Saudi Arabia[8]. A study considering the nutritional contents of *T. claveryi* revealed that *T. claveryi* provided from different regions of Saudi Arabia contained 28%, 16%, 4%, and 2% of carbohydrates, proteins, fibers, and fats, respectively[9]. In addition to the nutritional benefits of *T. claveryi*, many studies showed different pharmacological uses of this truffle such as antioxidant, anti-angiogenic, antidiabetic, antimicrobial, and hepatoprotective activities[8,10]. Considering the biological and pharmacological activities of this mushroom, the current study intended to assess the anti-diarrheal effects of *T. claveryi* methanolic extract (TCME) against *E. coli*, *Salmonella typhimurium* (*S. typhimurium*), *Shigella flexneri* (*S. flexneri*), and *G. lamblia* as the most common agents of infectious diarrhea.

## 2. Materials and methods

### 2.1. Reagents and drugs

Folin-Ciocalteu's reagent, Mayer and Dragendorff's, catechin, 2',7'-dichlorofluorescein diacetate (DCFH-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT), and Triton X-100 were provided from Sigma-Aldrich (St. Louis, MO). Tryptic Soy Broth and Bradford reagent were purchased from Merck, Darmstadt, Germany. All the chemicals used were of analytical grade.

### 2.2. Plant materials

*T. claveryi* aerial parts were purchased from a market in Riyadh, Saudi Arabia. The obtained materials were then determined by a

mycologist and a voucher example was archived at Department of Medical Laboratories Sciences, College of Applied Medical Sciences in Al-Quwayyah, Shaqra University, Saudi Arabia, for further experiments.

### 2.3. Preparation of the extract

Dried materials (250 g) were extracted by using the percolation technique with methanol for 72 h at 21 °C. Then, the extract was filtered by a filter paper and evaporated at 50 °C in a vacuum and reserved at -20 °C for further examinations[11].

### 2.4. Phytochemical analysis

The primary phytochemical examination of TCME was carried out to assess the presence of tannins, saponins, alkaloids, flavonoids, glycosides, etc based on the previous investigations[12].

### 2.5. Secondary metabolite analysis

Total phenol content was measured based on Folin-Ciocalteu's reagent colorimetric technique using gallic acid as standard[13] and the content amount was presented as mg gallic acid equivalents/g dry weight (mg GAE/g DW). Total flavonoid content was determined by aluminum chloride ( $AlCl_3$ , 2%) colorimetric assay using quercetin as standard based on the methods described by Phuyal *et al*[14]. The total flavonoid was exhibited as mg quercetin equivalent/g DW (mg QE/g DW). The tannin condensed contents were measured according to the technique defined by Broadhurst and Jones[15] and the content was represented as mg catechin equivalent/g DW (mg CE/g DW).

### 2.6. Antibacterial effects

#### 2.6.1. Bacteria cells

The strains of *E. coli* (ATCC 8739), *S. typhimurium* (ATCC 13311), and *S. flexneri* (ATCC 12022) were cultured into Tryptic Soy Broth improved with 0.6% yeast extract (Merck) and incubated at 37 °C for 24 h.

#### 2.6.2. Preparation of 0.5 McFarland standard solution

The 0.5 McFarland standard solution was provided through adding 0.5 mL of  $BaCl_2$  (0.048 mol/L) ( $2H_2O$  w/v  $BaCl_{20}$  1/175%) to 99.5 mL of sulfuric acid (0.18 mol/L) (v/v 1%). The suspension was stirred constantly, and the standard optical density was evaluated by absorbance measurement using a spectrophotometer at an optical length of 1 cm. The absorbance of 625 nm should be between 0.8 and 0.13. After dissolving several colonies of bacteria physiological serum, the turbidity of the mixture was compared with the 0.5 McFarland standard solution[16].

#### 2.6.3. Micro broth dilution

The minimum inhibitory concentration (MIC) of TCME against *E. coli*, *S. typhimurium*, and *S. flexneri* was evaluated by micro broth dilution method based on the Clinical and Laboratory Standards Institute orders[17]. Briefly, a stock of TCME was prepared with normal saline solvent and sterile Müller-Hinton broth culture

medium. Then, 50 µL of sterile Müller Hinton broth culture medium was added to the rows of 3 to 12. From the stock made, 100 µL was added to rows 1 and 2 and the dilution operation was performed from the second to the tenth rows. In this way, from the second row, 50 µL to the third row and from the third row 50 µL to the fourth row and up to the tenth row will be diluted in the same way. Finally, after 24 h of culture, the desired microorganism was added in the amount of 50 µL equivalent to half McFarland turbidity ( $1.5 \times 10^8$  CFU/mL) of two to ten rows. The plates were placed in a shaker incubator for 24 h at 37 °C and then salts of 2, 3, and 5-triphenyltetrazolium chloride were used as a visual indicator for bacterial growth. The lowest concentrations at which no visible bacterial growth was noted were reported as MICs. The lowest concentrations of the TCME in which no bacteria survived were considered as minimum bactericidal concentrations (MBC) of TCME. Normal saline was used as negative control and gentamicin, chloramphenicol, and ampicillin antibiotics were used as positive controls.

### 2.7. Effects of the TCME on protein leakage

The effects of the TCME on protein leakage in tested bacteria were determined based on the technique defined by Du *et al.*[18]. Briefly, the suspension of each bacteria was treated with the TCME at the concentrations of 1/4 MIC, 1/3 MIC, and 1/2 MIC and was incubated at 37 °C with shaking for 2 h. In the next step, after centrifuging the bacteria suspensions at 4000 rpm for 240 s, 0.05 mL of the suspension supernatants were mixed with 0.95 mL of Bradford reagent. Finally, the protein content was assessed according to Bradford's method[19]. The negative and positive control were medium and sodium dodecyl sulfate (0.1%). The absorbance of suspensions was determined at 590 nm by a microplate reader spectrophotometer (BioTek Winooski, VT, USA).

### 2.8. Effects of the TCME on reactive oxygen species (ROS) generation

In the present investigation, DCFH-DA was used to determine the level of bacterial ROS prompted by TCME. Briefly, bacterial strains were separately incubated with 10 µM of DCFH-DA at 37 °C for 30 min. Next, bacteria were separately treated with and without the TCME at the concentrations of 1/4 MIC, 1/3 MIC, and 1/2 MIC for 3 h. Finally, the fluorescence intensity of the mixture was determined at excitation and emission wavelengths of 488 and 525 nm, respectively[20].

### 2.9. Giardia cell

The standard strains of *G. lamblia* WB (ATCC® 30957) were axenically cultured to 37 °C and 5% CO<sub>2</sub> in modified Diamond's TYI-S-33 media complemented with 10% fetal bovine serum. Cells in the logarithmic phase were harvested using refrigerated flasks after 72-96 h routine subcultures. Next, trophozoites were washed with phosphate buffer saline and the cell number was adjusted as  $2 \times 10^6$ /mL using a haemocytometer.

#### 2.9.1. Anti-giardial effects of TCME

To evaluate the anti-giardial effects, 0.5 mL of trophozoites suspension was added to each test tube containing TCME at the concentrations of 1, 2, and 4 mg/mL, and then tubes were incubated 37 °C for 15, 30, 60, 120, 240, and 360 min. In the next step, the supernatant of the solution was discarded and then 50 µL of 0.1% eosin stain was added to the remaining settled trophozoites. Finally, the remaining pellet was smeared on a glass slide separately, covered with a cover glass, and studied by a light microscope at 400× magnification[21,22]. The percentages of viability of treated trophozoites were calculated by counting 300 parasites; whereas the dead and live parasites were observed in pink and colorless, respectively[23]. Non-treated trophozoites and trophozoites treated with metronidazole (50 µg/mL) were considered as negative and positive controls.

#### 2.9.2. Effects of TCME on the plasma membrane permeability of Giardia trophozoites

The effects of various concentrations of TCME (1, 2, and 4 mg/mL) on the plasma membrane permeability of *Giardia* trophozoites ( $1 \times 10^6$  cells/mL) were studied by Sytox green stain test according to the manufacturer's protocol. Non-trophozoites and the trophozoites treated with 2.5% of Triton X-100 were considered as the negative and positive control, respectively. The plasma membrane permeability was measured using a microplate reader (BMG Labtech, Ortenberg, Germany) for 4 h[24].

### 2.10. Cytotoxicity effects of TCME

The cells of human embryonic kidney 293 (normal HEK293T cells) and breast cancer cell line (MCF-7) prepared from American Type Tissue Culture Collection (Manassas, USA) were used for evaluation of the cytotoxicity effects by the colorimetric MTT assay[25]. HEK293T and MCF-7 cell lines were cultured in Dulbecco's Modified Eagle Medium, supplemented with 10% fetal bovine serum and streptomycin (100 µg/mL), and penicillin (200 IU/mL). Then, cells ( $5 \times 10^4$ /mL) were treated with the TCME at concentrations of 10-200 µg/mL for 48 h at 37 °C with 5% CO<sub>2</sub>. Lastly, the absorbance of the tested plate was calculated by a microplate reader (BioTek Winooski, VT, USA), at 570 nm wavelength. The experiments were repeated in triplicate. The 50% cytotoxic concentrations (CC<sub>50</sub> values) were calculated by means of the Probit test in SPSS software[25].

### 2.11. Statistical analysis

The data were presented as mean±SD and analyzed using the SPSS statistical package, version 25.0 (SPSS, Inc.). The unpaired samples *t*-test, and one-way analysis of variance (ANOVA) were used to compare the outcomes between tested groups. *P*<0.05 was considered statistically significant.

### 3. Results

#### 3.1. Phytochemical analysis of TCME

Based on the results of the primary phytochemical analysis of the TCME, the presence of flavonoids, tannins, glycosides, terpenoids was confirmed in this plant with the lack of alkaloids and saponins. The results of the secondary metabolite contents of TCME demonstrated that total flavonoid, phenolic, and tannin content was 43.78 (mg QE/g DW), 57.26 (mg GAE/g DW), and 12.60 (mg CE/g DW), respectively.

#### 3.2. Antibacterial effects

The antibacterial effects of TCME against *E. coli*, *S. typhimurium*, and *S. flexneri* as the most main agents of diarrheal diseases were evaluated by measuring the MIC and MBC values. As shown in Table 1, TCME displayed varying antibacterial activity against these tested strains. The MIC and MBC values of bacterial strains were in the range of 0.52–1.04 and 1.04–2.08 mg/mL, respectively. The results demonstrated that *S. flexneri* and *S. typhimurium* were the most sensitive and resistant strains against TCME, respectively.

#### 3.3. Effects of TCME on protein leakage

Figure 1 exhibits the protein content after treatment with the TCME at the concentrations of 1/4 MIC, 1/3 MIC, and 1/2 MIC. The findings demonstrated that TCME increased the protein leakage of tested bacteria, especially at the concentrations of 1/3 MIC and 1/2 MIC ( $P < 0.001$ ).

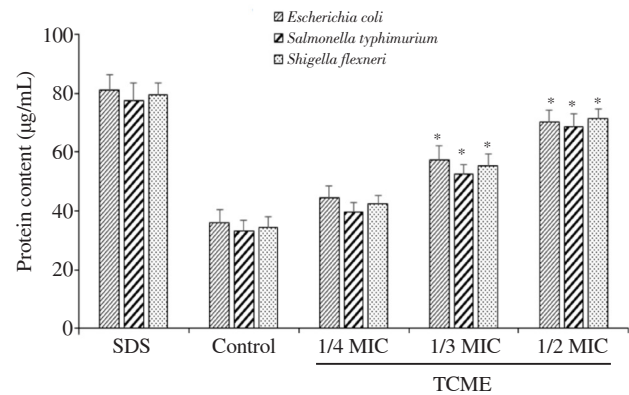
#### 3.4. Effect of TCME on ROS generation

The effects of TCME on ROS generation in *E. coli*, *S. typhimurium*, and *S. flexneri* were evaluated. The results revealed that the fluorescence intensity was increased in a dose-dependent manner; nevertheless, a significant increase ( $P < 0.05$ ) was found at the concentrations of 1/3 MIC and 1/2 MIC (Figure 2). These results demonstrated that the TCME mediated ROS production.

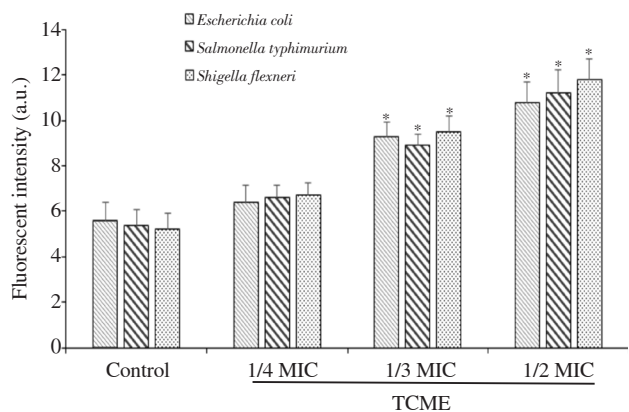
#### 3.5. Anti-giardial effects

In this study, we evaluated the *in vitro* anti-giardial effects of various concentrations of TCME on *G. lamblia* trophozoites at different times. The findings revealed that TCME decreased the viability of *G. lamblia* trophozoites in a dose-dependent manner. In

addition, TCME at the concentrations of 1, 2, and 4 mg/mL resulted in 100% mortality of *G. lamblia* trophozoites after 360, 240, and 120 min, respectively (Figure 3). The mortality of *G. lamblia* trophozoites in the negative and positive control groups was 2.6% and 100% after 360 and 120 min incubation, respectively.



**Figure 1.** Protein content after exposure of *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri* to *Terfezia claveryi* methanolic extract (TCME) at the concentrations of 1/4 MIC, 1/3 MIC, and 1/2 MIC. The data are presented as mean±SD ( $n=3$ ) and analyzed using SPSS. The unpaired samples *t*-test, and one-way analysis of variance were used to compare the outcomes between tested groups. \* $P < 0.001$  compared with control. MIC: minimum inhibitory concentration; SDS: sodium dodecyl sulfate.

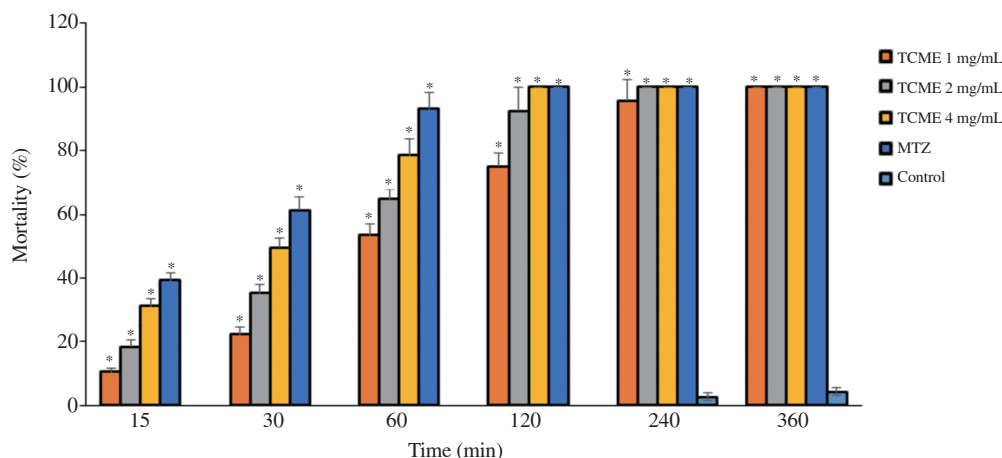


**Figure 2.** Effects of TCME on the production of reactive oxygen species in *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri*. The data are presented as mean±SD ( $n=3$ ) and analyzed using SPSS. The unpaired samples *t*-test, and one-way analysis of variance were used to compare the outcomes between tested groups. \* $P < 0.001$  compared with control. a.u.: arbitrary units.

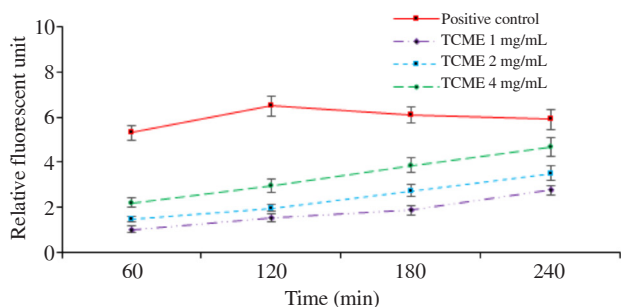
**Table 1.** The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of *Terfezia claveryi* methanolic extract (TCME) against *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri* (mg/mL).

Bacteria	TCME		Gentamicin		Chloramphenicol		Ampicillin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Escherichia coli</i>	0.80±0.36	1.66±0.72	0.065±0.020	0.13±0.04	0.083±0.36	0.16±0.07	0.25±0.00	0.41±0.14
<i>Salmonella typhimurium</i>	1.04±0.36	2.08±0.72	0.130±0.040	0.26±0.04	0.125±0.00	0.25±0.00	0.33±0.14	0.66±0.28
<i>Shigella flexneri</i>	0.52±0.18	1.04±0.36	0.100±0.040	0.20±0.09	0.083±0.36	0.16±0.07	0.33±0.14	0.66±0.28

The data are expressed as mean±SD ( $n=3$ ).



**Figure 3.** The *in vitro* anti-giardial effects of various concentrations of TCME (1, 2, and 4 mg/mL) on *Giardia lamblia* trophozoites at different times (0-360 min). The data are presented as mean±SD ( $n=3$ ) and analyzed using SPSS. The unpaired samples *t*-test, and one-way analysis of variance were used to compare the outcomes between tested groups. \* $P<0.001$  shows a significant difference compared with the control group. MTZ: metronidazole.



**Figure 4.** Effects of TCME on the plasma membrane permeability of *Giardia lamblia* trophozoites. The data are presented as mean±SD ( $n=3$ ) and analyzed using SPSS. The unpaired samples *t*-test, and one-way analysis of variance were used to compare the outcomes between tested groups.

### 3.6. Effects of TCME on the plasma membrane permeability of *Giardia* trophozoites

Figure 4 shows that TCME can affect the plasma membrane permeability of the *Giardia* trophozoites. The results revealed that the plasma membrane permeability of the trophozoites was changed after exposure to various concentrations of TCME.

### 3.7. Cytotoxicity effects of TCME

The findings revealed that the  $CC_{50}$  values for HEK293T and MCF-7 cells were 4.32 mg/mL and 6.40 mg/mL, respectively, indicating that TCME has more cytotoxicity against cancer cells than normal cells.

## 4. Discussion

Based on the results of the primary phytochemical analysis of the TCME, the presence of flavonoids, tannins, glycosides, terpenoids, and lack of alkaloids and saponins was confirmed. The results of

the secondary metabolites contents of TCME demonstrated that total flavonoid, phenolic, and tannin content was 43.78 mg QE/g DW, 57.26 mg GAE/g DW, and 12.6 mg CE/g DW, respectively. The study conducted by Saddiq *et al.* demonstrated that the total phenolic content of the *T. claveryi* water extract was 48 mg gallic acid/g by using Folin-Ciocalteu reagent[26]. Kivrak reported the total flavonoid contents of the ethyl acetate extracts of *T. claveryi* was  $(4.71 \pm 0.11)$   $\mu$ g QEs/mg[27]. This difference in the amount of phenolic compounds may be related to some factors such as analysis method, type of extract, place of sample collection, *etc*[8].

The antibacterial assay showed that *S. flexneri* and *S. typhimurium* were the most sensitive and resistant strains against TCME. In line with our results, previous studies have demonstrated the *in vitro* and *in vivo* antibacterial activity of *T. claveryi* against a number of Gram negative and Gram positive pathogenic bacteria[26–30]. Aldebasi *et al.* showed that *T. claveryi* had relevant antibacterial activity against all clinical isolates of corneal ulcer (*e.g.*, *Proteus vulgaris*, *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*) with the MIC and MBC values ranging from 0.4–1.25 mg/mL and 0.024–0.240 mg/mL, respectively[28]. In this study, we also assessed the *in vitro* anti-giardial effects of various concentrations of TCME on *G. lamblia* trophozoites at different times. The findings revealed that TCME decreased the viability of *G. lamblia* trophozoites dose-dependently. Moreover, the plasma membrane permeability of *Giardia* trophozoites was highly altered by TCME.

Proteins are considered as the key functional compound in the living cells as it is a key factor for the maintenance of life. In this investigation, we assessed the effects of TCME on cell membrane of tested bacteria by determining the leakage of intracellular protein of the bacteria solution. The present findings exhibited that the protein leakage in the tested bacteria after treatment with TCME was increased significantly at the concentrations of 1/3 MIC and 1/2 MIC. ROS are considered as active molecules comprising oxygen which affects almost all biomolecules, including lipids, proteins, and nucleic acids, causing bacterial death[31]. Besides,

studies have revealed that various types of antibiotics are able to kill bacteria through various mechanisms such as the triggering of cell metabolisms or the synergistic communication between antibiotics and constituents to induce ROS<sup>[32,33]</sup>. In the present study, we evaluated the effects of the TCME on ROS generation in *E. coli*, *S. typhimurium*, and *S. flexneri*. The results revealed that the fluorescence intensity was increased in a dose-dependent manner. These results demonstrated that the TCME caused bacterial death via the mediation of ROS production.

Tannins and phenolic compounds (such as polyphenols and flavonoids) are considered the most important secondary metabolites found in most plants, fruits, and vegetables<sup>[34–36]</sup>. These compounds exhibit several pharmacological activities such as anti-inflammatory, antioxidant, anticancer, anti-allergic, antihypertensive, and antimicrobial properties<sup>[34–36]</sup>. Several reviews have confirmed the antimicrobial effects of phenolic compounds against numerous pathogenic bacteria (Gram-negative and Gram-positive bacteria), parasites (e.g., *Leishmania* spp., *Trypanosoma* spp., and *Plasmodium* spp.), fungi (e.g., *Candida* spp., *Trichophyton* spp., and *Aspergillus* spp.), and viral (e.g., dengue virus, HIV, HCV virus, and arboviruses) strains<sup>[37–39]</sup>. Some previous studies also reported that these compounds exhibit their antimicrobial mechanisms by affecting the permeability of cell membranes, disrupting membrane integrity, promoting the leakage of vital intracellular elements, inhibiting virulence aspects of bacteria such as enzymes and toxins, and suppressing biofilm formation, thus increasing a synergistic effect with antibiotics<sup>[40–43]</sup>. Therefore, the antibacterial and anti-parasitic effects of TCME may be attributed to the presence of polyphenols, flavonoids, and tannin compounds.

We also evaluated the cytotoxic effects of TCME against normal and cancer cells. The MTT assay revealed that TCME has more cytotoxicity against cancer cells than normal cells. However, there are some limitations of this study. The effective compounds of the plant should be further identified in the future. *In vivo* studies should be conducted to further verify the effect of TCME and other mechanisms of cellular action of antibacterial and anti-parasitic activity of this plant need to be investigated.

In conclusion, the findings of the present study showed the promising effects of TCME against *E. coli*, *S. typhimurium*, *S. flexneri*, and *G. lamblia* as the most common agents of diarrheal diseases. Based on the results of the present study, TCME displayed its antibacterial and anti-parasitic effects through affecting cell membrane permeability and ROS generation with no significant cytotoxic effect on normal cells.

### Conflict of interest statement

The author declares that there are no competing interests.

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