

Anti-*Cryptosporidium* efficacy and metabolite profiling of *Schefflera arboricola* L. leaves extract

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ABSTRACT

Background: *Schefflera arboricola* L., (*S. arboricola* L) is a member of the Araliaceae family and exhibits various biological activities. *Cryptosporidium parvum*, a zoonotic protozoan infection, can infect a variety of vertebrate and invertebrate hosts, including humans.

Aim: This study aimed to explore the phytochemical constituents and efficacy of *S. arboricola* leaf extracts against *C. parvum* infection.

Methods: The assessment of chlorophyll, carotenoid, carbohydrate, total phenolic, and flavonoid contents in the leaves of *S. arboricola* was quantified using high-performance liquid chromatography and UPLC/ESI-MS. Parasitological, histological, deoxyribonucleic acid (DNA) fragmentation, liver functions, lipid profiles, and antioxidant parameter analysis will be used to assess the efficacy of the treatments after oral infection with *C. parvum* oocytes (2×10^3 /mouse). Fifty adult male mice were divided into five groups (10 each): non-treated *Cryptosporidium*-infected group (G1), infected and treated with low-dose *S. arboricola* extract (100 mg/kg) (G2), infected and treated with high-dose *S. arboricola* extract (200 mg/kg) (G3), Nitazoxanide treated (150 mg/kg) (G4), and non-infected group (G5).

Results: This study quantified the total phenolic and flavonoid contents 267.54 ± 0.61 mg GAE/g and 514.89 ± 0.20 mg rutin/g, respectively, and identified 51 compounds, including 14 phenolic acids and their derivatives, 30 flavonoids, 6 coumarins, and 1 anthocyanin. Both infected-treated mice groups (G2 & G3) showed a considerable improvement in *Cryptosporidium* oocyte shedding, with significant effectiveness ($p < 0.05$). In addition, the infected-treated groups showed less severe changes in the intestinal epithelium structures and a significant decrease in DNA fragmentation, hepatic biomarkers, and lipid indices compared with the infected non-treated control group.

Conclusion: *Schefflera arboricola* leaves contain a wide variety of pigments, carotenoid, carbohydrate, phenolic, and flavonoid compounds. Extracts of *S. arboricola* leaves are a new interesting prospective natural product that may be used in the treatment of *Cryptosporidium* infection.

Keywords: *Schefflera arboricola* L, Phytochemical, Natural product, Anti-*Cryptosporidium*.

Introduction

Schefflera arboricola L. (*S. arboricola* L) represents the most extensive genus within the Araliaceae family, encompassing more than 650 species found in tropical and subtropical regions. Various species have a documented history of traditional medicinal applications in China, India, and Indonesia (Wang *et al.*, 2020). *Schefflera arboricola*, which belongs to the Araliaceae family, is indigenous to Taiwan.

Commonly referred to as the "Dwarf umbrella tree" (Plunkett *et al.*, 2005), this evergreen shrub typically reaches heights of 3–4 m. Its leaves are palmately compound, featuring 7–9 leaflets that measure 10–20 cm in length and 4–6 cm in width (Zhao *et al.*, 2010). This ornamental plant is widely regarded as a favored choice for indoor gardening due to its adaptability to various unfavorable growing conditions (Wang *et al.*, 2021).

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The *S. arboricola* plant and its extracts have a long-standing history of possessing strong anti-inflammatory and analgesic effects, so they are utilized for alleviating stomach pain, headaches, trigeminal neuralgia, rheumatic arthritis, fractures, and sprains (Chang and Butt, 1986; Zhao *et al.*, 2010; Liu *et al.*, 2011). The leaves and bark of *Schefflera* are recognized for their antioxidant properties and are used as a treatment for coughs and as a diuretic (Deepa and Nalini, 2013). The phytochemical composition of *S. arboricola* leaves and stems has resulted in the isolation of two oleanolic acid glucuronides, specifically cynarasaponin H, olaxoside, lupine, triterpene, and saponins (Melek *et al.*, 2003; Guo *et al.*, 2006). Furthermore, *S. arboricola* L. leaves are thought to be a significant source of bioactive phenolic substances; Gas Chromatography-Mass Spectrometry and Liquid Chromatography–Electrospray Ionization–Mass Spectrometry (LC-ESI-MS) analysis revealed the presence of 29 phenolic compounds (El-Hagrassi *et al.*, 2022).

The apicomplexan parasite *Cryptosporidium* spp. mostly affects the gastrointestinal tracts of humans and animals. The infection is common in calves and children and can cause severe diarrhea, anorexia, and dehydration (Ghazy *et al.*, 2015a; Lombardelli *et al.*, 2019). Cryptosporidiosis is a zoonotic parasite that can spread from infected animals to humans. Transmission occurs through the ingestion of oocytes, through either direct contact or consumption of contaminated water or food (Toaleb and Shaapan, 2024). There is growing evidence that a variety of wildlife species, including fish, amphibians, reptiles, birds, and mammals, are infected with cryptosporidiosis (Saad-Alla *et al.*, 2022; Shaapan *et al.*, 2022). A panel of experts from the Food and Agriculture Organization and the World Health Organization have determined that *Cryptosporidium* is the fifth most important food-borne parasite in the world (Javed and Alkheraije, 2023).

The only treatment currently approved by the Food and Drug Administration (FDA) to treat *Cryptosporidium* infections in humans, particularly immunocompetent patients, is nitazoxanide (NTZ), which decreases oocyte shedding and shortens the severity of diarrhea (Shoukry *et al.*, 2025). In addition to NTZ, a few other commercially available medications, such as paromomycin and azithromycin, have some anti-*Cryptosporidium* efficacy, but the FDA has not licensed them to treat cryptosporidiosis (Caravedo and White, 2023). Consequently, the development of alternative, plant-based therapeutics with improved efficacy and safety profiles has become a research priority. Traditional plant extracts, such as onion (*Allium cepa*) and cinnamon (*Cinnamomum zeylanicum*) oils (Abu El Ezz *et al.*, 2011), ginger (*Zingiber officinale*), ginseng (*Panax ginseng*), and sage (*Salvia officinalis*) (Abouelsoued *et al.*, 2020), and *Citrus sinensis* peel extract (Abd El Wahab *et al.*, 2022).

Despite the extensive traditional use and recognized pharmacological potential of *S. arboricola*, comprehensive studies on its phytochemical composition, particularly detailed metabolite profiling of phenolic and flavonoid constituents, remain scarce. Moreover, no previous research has evaluated its efficacy against *Cryptosporidium parvum* infection. Therefore, the primary aim of the present study was to perform an in-depth phytochemical analysis of *S. arboricola* leaves and evaluate the anti-cryptosporidial efficacy of its extract in experimentally infected mice.

Materials and Methods

Chemicals

The chemicals and solvents used in this investigation were of analytical grade and were supplied by Merck and Co., Inc. (USA) and Sigma-Aldrich (USA). The high-performance liquid chromatography (HPLC) specifications and all tested compounds met the highest analytical purity criteria. Phenolic compounds and flavonoids analyzed in this study were acquired from Sigma-Aldrich Co. (USA).

Plant collection and extraction

Uniform young leaves of *S. arboricola* L. were collected from Orman Garden, Giza, Egypt in October 2024. Mrs. Trease Labib, a Plant Taxonomy Consultant at the Ministry of Agriculture, identified and authenticated the plant. A voucher specimen (Voucher No. M242) was deposited in the herbarium of the National Research Center, Cairo, Egypt, for future reference. The collected leaves were air-dried in the shade at room temperature to prevent the degradation of thermolabile constituents and then ground into a fine powder. A total of 400 g of the powdered leaves was subjected to cold maceration with absolute methanol (1.5 L × 6) to ensure exhaustive extraction of both polar and semipolar metabolites were extracted.

Detection of pigments and carbohydrates

The pigment concentration was quantified using spectrophotometry. Chlorophyll a and chlorophyll b concentrations were measured at wavelengths of 651 and 664 nm, respectively, while total carotenoids were evaluated at a wavelength of 470 nm. The calculations for total chlorophylls, total carotenoids, chlorophyll ratio (a/b), and pigment index (the ratio of total carotenoids to chlorophyll a) were based on the formulas provided by Bodnar *et al.* (2016). Furthermore, the total carbohydrate content was quantitatively determined as glucose using the phenol sulfuric acid method in accordance with Dubois *et al.* (1956).

Total phenolic and flavonoid detection

The Folin–Ciocalteu method was used to determine the total phenolic content in the methanol extract of *S. arboricola* L. leaves, as reported by El-Feky *et al.* (2017). The results were expressed in terms of the gallic acid equivalent. The total flavonoid content was evaluated using the aluminum chloride method, as

described by Baba and Malik (2015), with the flavonoid concentration represented as rutin equivalent. All results were presented as mean $\pm$ S.D.

Animals

Fifty adult male mice with an average body weight of 25–30 g were housed in the Laboratory Animal House at the National Research Center in Giza, Egypt. Mice are given a regular laboratory meal and unlimited access to water while being kept as 10 mice per cage with 12:12 hour day/night cycles at a temperature of (20°C–25°C) (Shaapan *et al.*, 2023).

Design of the experiments and infection

Mice were divided into five groups (10 each): *Cryptosporidium*-infected non-treated group (G1), infected and treated with a low dose of *S. arboricola* extract (100 mg/kg) (G2), infected and treated with a high dose of *S. arboricola* extract (200 mg/kg) (G3), infected and treated with NTZ (150 mg/kg) (G4), and non-infected non-treated group (G5). Oocytes from a stock of *Cryptosporidium* spp. that was kept in the Zoonotic Disease Department, National Research Centre, were genetically identified as *C. parvum* (Hassanain *et al.*, 2011). Mice infected by a single oral dosage of 2×10^3 *C. parvum* oocytes for each mouse using oral-gastric gavage treatment with *S. arboricola* extract and NTZ medications will start on the third day post-infection, and all tested mice will be sacrificed 3 weeks post-infection (Abouelsoued *et al.*, 2023).

Parasitological examination

Throughout the experiment, fecal samples from each group were separately collected every day, and the amount of *Cryptosporidium* oocytes was counted using MZN stain (Ghazy *et al.*, 2016). Centrifugation of the fecal suspension for 10 minutes at 3,000 rpm. 100 μ l of fecal sediment was used to measure the number of oocytes in 1 ml of fecal sample, which was then observed via an oil immersion lens ($\times 100$). The average of three computed counts, multiplied by 10, and the number of oocytes per gram of feces were stated (Elfadaly *et al.*, 2018).

Histopathological examination

The intestinal portion (ileum) of each mouse group was preserved in a 10% buffered formalin solution before being embedded in blocks of paraffin wax. Hematoxylin and eosin staining, sectioning, and light microscopy examination (Barakat *et al.*, 2024).

Quantification of deoxyribonucleic acid (DNA) fragmentation

The DNA fragmentation of the tested mice was detected in the intestinal epithelium using the PEG/

Hoechst fragmentation assay. This technique is based on the idea that centrifugal sedimentation can be used to separate broken-up double-stranded DNA from chromosomal DNA. High-molecular-weight DNA was preferentially precipitated using polyethylene glycol after cells were broken down with hypotonic acid. The fluorescent dye Hoechst 33258 was used in a fluorometer to measure the amount of fragmented DNA remaining in the supernatant (Loannou and Chen, 1996; Shaapan *et al.*, 2011).

Biochemical marker estimation

The sera collected from all examined groups of mice were analyzed for lipid profile: total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Sera liver function enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT). In addition, the oxidative stress marker malondialdehyde (MDA) was also estimated in the sera. The biochemical analysis was carried out in accordance with Lütjohann *et al.* (2023), and the test kits were supplied by Bio-diagnostic, Egypt, and BioMérieux-France.

Statistical analysis

A one-way analysis of variance (one-way ANOVA) was performed using the Statistical Package for Social Sciences (SPSS for Windows, version 11.0) to evaluate the positive and negative statistical relationships among different biological findings.

Ethical approval

The study was approved by the ethical review board of the Medical Research Ethics Committee at the National Research Centre in Egypt. The ethical approval certificate was documented under the registration number: (0441 in 29/04/2025).

Results

Content of pigments and carbohydrates

The chlorophyll and carotenoid levels in the leaves of *S. arboricola* L. were evaluated, and the results demonstrated that the chlorophyll a and b concentrations were 1.68 and 0.57 mg/g, respectively. The chlorophyll a/b ratio was calculated as 2.95 mg/g. The total chlorophyll content was 2.28 mg/g, while the total carotenoid content was 0.34 mg/g. The pigment index of the leaves was recorded at 0.20 mg/g. The total carbohydrate content in the leaves of *S. arboricola* L. was 23.5% (wt/wt), represented as glucose (Table 1).

Phenolic and flavonoid contents

The total phenolics and flavonoids were quantified in the methanol extract from the leaves of *S. arboricola*

Table1. Pigment contents in *S. arboricola* L. leaves.

Pigment concentration (mg/g)					
Chlorophyll a	Chlorophyll b	Chlorophylls a/b ratio	Total chlorophylls	Total carotenoids	Pigment index
1.68 $\pm$ 0.94	0.57 $\pm$ 1.20	2.95 $\pm$ 0.73	2.28 $\pm$ 0.91	0.34 $\pm$ 1.52	0.20 $\pm$ 1.34

Values calculated from three replicates and expressed as mean $\pm$ SE.

L., resulting in measurements of 267.54 ± 0.61 mg GAE/g and 514.89 ± 0.20 mg rutin/g, respectively. These findings affirm the considerable presence of total phenolics and flavonoids in the leaves of *S. arboricola* L., which serves as a basis for a diverse array of biological functions.

The methanol extract from the leaves of *S. arboricola* L. was subjected to HPLC to ascertain the presence of various phenolic acids and flavonoids, as shown in Table 2 and Figure 1. The results of the analysis identified 19 distinct compounds, with rosmarinic acid being the predominant one, recorded at a retention time of 11.830 and a concentration of 17,926.41 μ g/g. Other notable compounds included quercetin, caffeic acid, naringenin, gallic acid, kaempferol, and hesperetin, with respective concentrations of 2,657.63, 2,071.95, 1,946.32, 1,531.69, 1,061.24, and 1,052.41 μ g/g.

Furthermore, the Ultra-Performance Liquid Chromatography–Electrospray Ionization–Mass Spectrometry (UPLC/ESI–MS) analysis performed in both negative and positive ionization modes to thoroughly characterize the diverse phytochemical classes found in the leaves of *S. arboricola* L. This investigation revealed the existence of 51 compounds, including 14 phenolic acids and their derivatives, 30 flavonoids, 6 coumarins, and 1 anthocyanin (Fig. 2).

Morphological identification of *Cryptosporidium* oocytes

The obtained mZN-stained *C. parvum* oocytes shed in the feces of experimentally infected mice showed a smooth wall, ovoid to spherical-shaped oocytes, and occasionally showed an incomplete suture line at the oocytes wall and presented as red-pink (acid fast) with green-back (Fig. 3).

Cryptosporidium oocyte shedding

The count of *Cryptosporidium* oocytes was significantly higher in the fecal samples of the infected non-treated (positive control) group (G1) than in the other treated infected groups during all experimental periods. Oocyte shedding gradually decreased until few oocytes were detected on day 7 PI. In the groups treated with *S. arboricola* L. extract (G2 & G3) and NTZ (G4), there was a statistically significant decrease in oocyte shedding. Additionally, the higher-dose *S. arboricola* L (200 mg/kg)-treated group (G3) revealed decreased oocyte shedding throughout time, followed by the NTZ-treated group (G4), whereas the low-dose *S. arboricola* L (100 mg/kg)-treated group (G2) showed the highest oocyte shedding than the other two treatment groups (Table 3).

Histopathological investigation

The intestinal epithelial mucosa of each mouse group had a markedly different look at the end of the experiment (PI, 3 weeks). The infected non-treated

Table 2. HPLC identification of polyphenolic compounds in the methanol extract from *S. arboricola* L.

No.	Compound	R _t	Conc. (μ g/g)
1	Gallic acid	3.570	1,531.69
2	Chlorogenic acid	4.326	620.14
3	Catechin	4.516	39.76
4	Methyl gallate	5.632	438.62
5	Caffeic acid	5.941	2,071.95
6	Syringic acid	6.454	268.21
7	Pyro catechol	6.803	27.34
8	Rutin	7.002	220.39
9	Ellagic acid	7.408	395.67
10	Coumaric acid	8.711	114.63
11	Vanillin	9.338	215.64
12	Ferulic acid	9.989	156.78
13	Naringenin	10.448	1,946.32
14	Rosmarinic acid	11.830	17,926.41
15	Daidzein	16.140	240.37
16	Quercetin	17.362	2,657.63
17	Cinnamic acid	19.390	62.89
18	Kaempferol	20.672	1,061.24
19	Hesperetin	21.237	1,052.41

R_t: retention time, ND: Not detected.

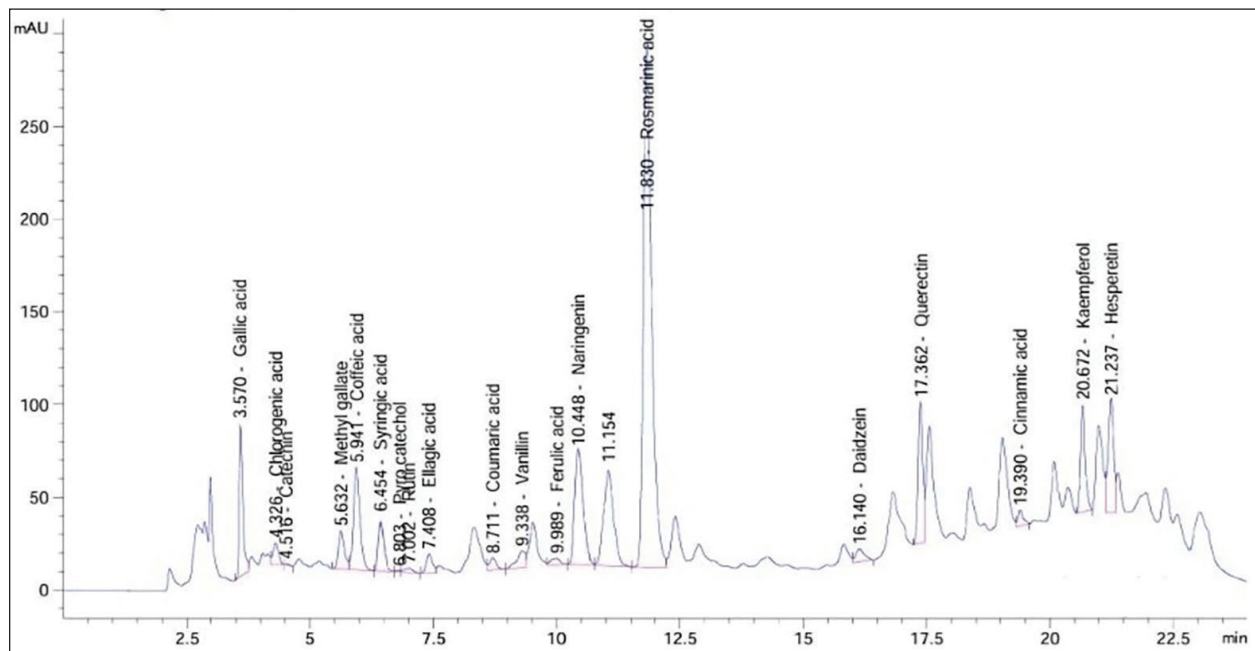


Fig. 1. High-performance liquid chromatography (HPLC) chromatogram for polyphenolic compounds in the methanol extract from *S. arboricola* L.

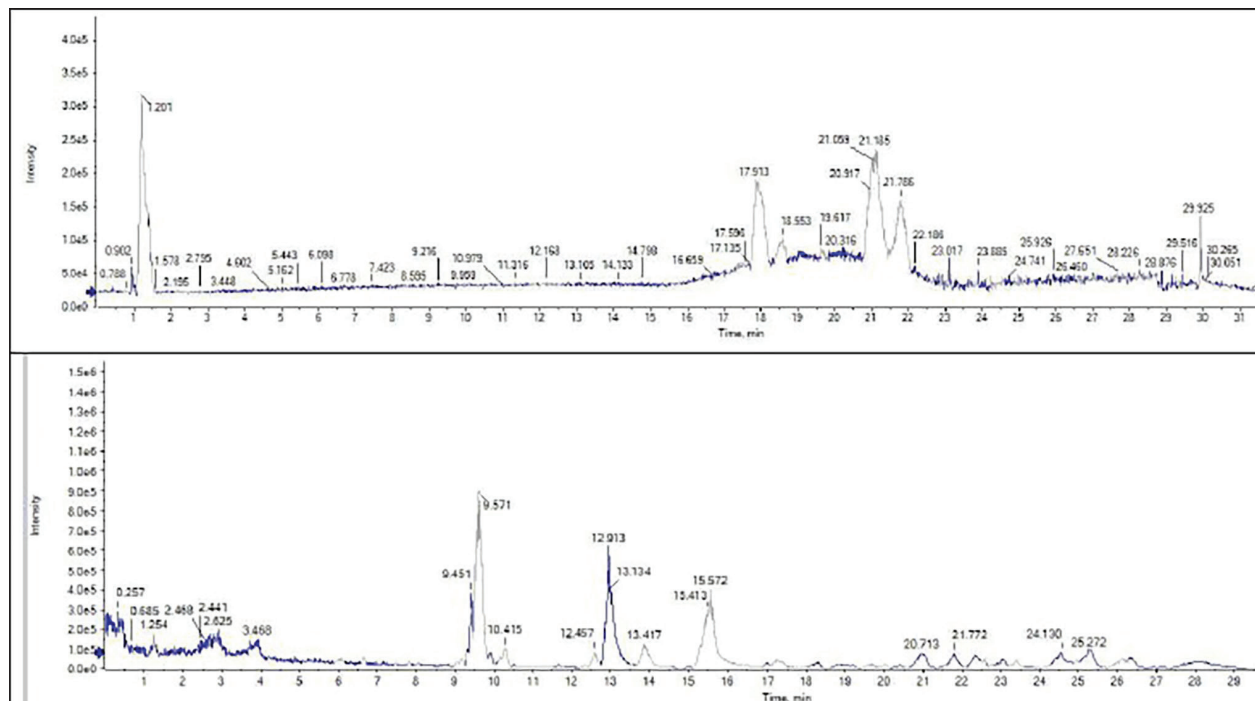


Fig. 2. UPLC/ESI-MS chromatogram for the polyphenolic profile in the methanol extract from *S. arboricola* L.

mice (positive control) (Fig. 4A) exhibited prominent histopathological changes in the intestinal mucosa, including enlarged, shortened, and desquamated intestinal villi, indicating a significant degree of villous atrophy with chronic inflammatory cell infiltration in the

lamina propria. Conversely, the histological alterations were more pronounced in the other treatment groups (Fig. 4 B–D), and there was evidence of a higher number of goblet cells and a modest degree of villous atrophy with little inflammatory cell and modest edema

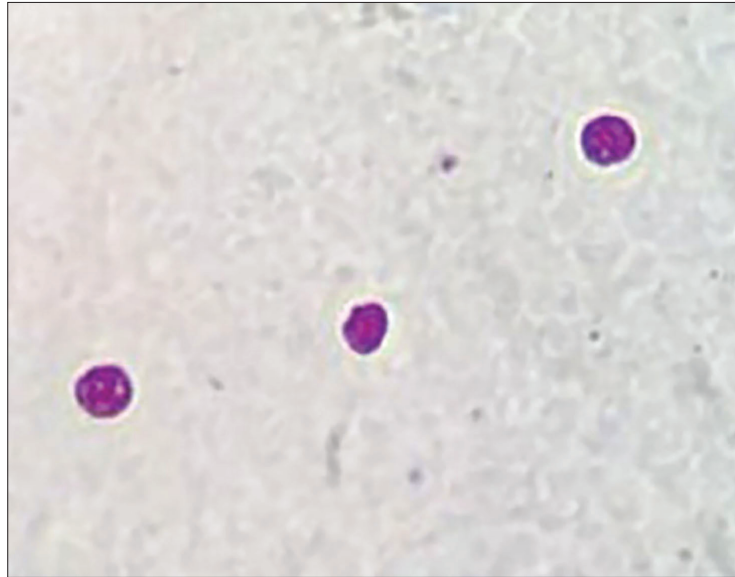


Fig. 3. *Cryptosporidium* oocysts in mice fecal smears stained with Modified Ziehl-Neelsen (MZN) (x400).

Table 3. *Cryptosporidium* oocysts in mice fecal smears stained with Modified Ziehl-Neelsen (MZN) (x400).

Group Days PI	G1 (+ve Control)	G2 Low Dose <i>S. arboricola</i> extract	G3 High Dose <i>S. arboricola</i> extract	G4 NTZ treated	p-value
Day 3	97.3 ± 3.5 ^{a,c}	94.9 ± 5.5 ^{qd}	90.1 ± 7.2 ^{cd}	92.6 ± 5.3 ^{cd}	0.017*
Day 4	102.6 ± 4.5 ^{a,c}	100.1 ± 5.5 ^{qd}	95.7 ± 3.4 ^{cd}	97.3 ± 6.2 ^{cd}	0.016*
Day 5	107.3 ± 3.7 ^{a,c}	104.6 ± 7.5 ^{qd}	98.2 ± 6.7 ^{cd}	102.1 ± 7.6 ^{cd}	0.014*
Day 6	117.6 ± 3.91 ^{a,b}	113.5 ± 3.5	103.8 ± 7.6 ^d	107.9 ± 6.3 ^d	0.033*
Day 7	98.9 ± 4.6	95.7 ± 9.2	89.1 ± 6.5 ^a	91.8 ± 7.6	0.031*
Day 8	90.6 ± 7.6	86.7 ± 6.7 ^a	78.5 ± 6.3 ^a	81.9 ± 4.7 ^{b,c}	0.022 *
Day 9	82.0 ± 6.4 ^a	77.9 ± 7.7 ^a	67.6 ± 7.4 ^a	72.3 ± 1.7 ^{b,c}	0.007*
Day 10	74.3 ± 9.1 ^a	70.8 ± 3.9 ^a	57.4 ± 9.2 ^a	64.2 ± 3.9 ^{b,c,d}	0.003 *
Day 11	62.5 ± 5.4 ^{a,b}	54.5 ± 7.6 ^{a,d}	46.9 ± 9.2 ^d	49.8 ± 6.3 ^{b,c,d}	<0.004*
Day 12	51.2 ± 6.5 ^{a,b,c}	43.1 ± 7.9 ^{a,b,d}	35.9 ± 3.9 ^{a,c,d}	38.1 ± 5.3 ^{b,c,d}	<0.002*
Day 13	42.5 ± 6.6 ^{a,b,c}	35.3 ± 5.7 ^{a,b,d}	27.2 ± 6.3 ^{c,d}	30.5 ± 0.5 ^{8,c,d}	<0.001*
Day 14	33.7 ± 4.3 ^{a,b,c}	26.7 ± 6.8 ^{a,b,d}	20.1 ± 6.3 ^{c,d}	23.2 ± 6.4 ^{b,c,d}	<0.002*
Day 15	28.4 ± 4.6 ^{a,b,c}	22.1 ± 7.4 ^{a,b,d}	15.5 ± 5.4 ^{c,d}	18.2 ± 5.7 ^{c,d}	<0.002*
Day 16	20.3 ± 3.6 ^{a,b,c}	16.3 ± 3.6 ^{a,b,d}	9.9 ± 5.2 ^{c,d}	12.2 ± 6.1 ^{c,d}	<0.001*
Day 17	14.8 ± 2.7 ^{a,b,c}	10.8 ± 5.8 ^{a,b,d}	5.1 ± 2.7 ^{c,d}	7.2 ± 5.4 ^{c,d}	<0.001*
Day 18	11.2 ± 6.3 ^{a,b,c}	7.6 ± 4.8 ^{a,b,d}	4.9 ± 3.7 ^{c,d}	5.9 ± 4.2 ^{c,d}	<0.002*
Day 19	8.1 ± 3.6 ^{a,b,c}	6.3 ± 8.5 ^{a,b,d}	3.7 ± 5.6 ^{c,d}	4.4 ± 4.5 ^{c,d}	<0.002*
Day 20	5.7 ± 6.5 ^{a,b,c}	4.0 ± 8.3 ^{a,b,d}	2.4 ± 7.6 ^{c,d}	3.3 ± 5.6 ^{c,d}	<0.001*
Day 21	2.9 ± 4.4 ^{a,b,c}	1.7 ± 6.6 ^{a,b,d}	0.5 ± 7.6 ^{c,d}	1.0 ± 5.6 ^{c,d}	<0.001*

Each value represents mean of 5 values ± SD, *p-value ≤ 0.05 was statistically significant. ^a Significantly different from oral NTZ at $p \leq 0.05$. ^b Significantly different from G2 and G3 dose treated at $p \leq 0.05$. ^c Significantly different from G1 dose at $p \leq 0.05$. ^d Significantly different from Positive Control at $p \leq 0.05$.

in the lamina propria. Mice that were neither infected nor receiving treatment (negative control) (Fig. 4E)

had normal intestinal mucosa morphology, with several finger-like villi and without any pathological changes

in the mucosa or lamina propria. Additionally, the histopathological changes improved more in high-dose (200 mg/kg) (Fig. 4C) than low-dose (100 mg/kg) *S. arboricola* L-treated mice (Fig 4B).

DNA fragmentation

The high-dose *S. arboricola* L-treated mice showed a decline in the DNA fragmentation quantitative results (54.47), followed by NTZ-treated (74.92) and low-dose *S. arboricola* L-treated (103.95) mice compared with the infected non-treated mice (209.03) (Fig. 5).

Biochemical parameters

The results showed that *C. parvum*-infected non-treated mice (G1) had a higher lipid profile (TC, TG, HDL, and LDL), liver enzymes (ALT and AST), and oxidative stress marker MDA compared to non-infected mice (G5). Meanwhile, the groups infected with *S. arboricola* and NTZ (G2, G3, and G4) demonstrated a significant decline in all biochemical parameters (Fig. 6).

Discussion

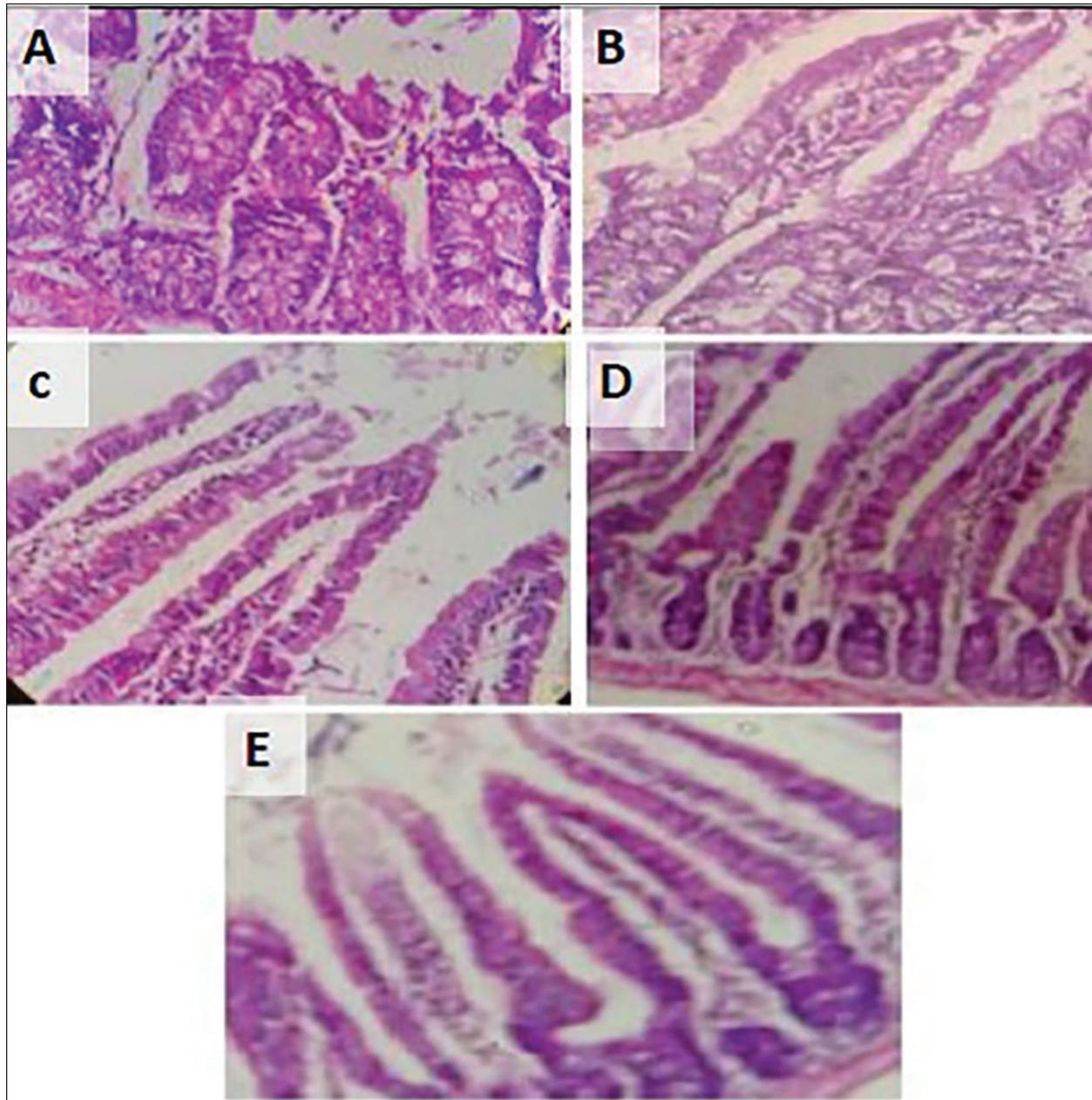


Fig. 4. Mice intestine; *C. parvum* infected nonrated (A), infected low dose treated with plant extract (B), infected treated with high dose of plant extract (C), infected treated with NTZ (D), and non-infected (E).

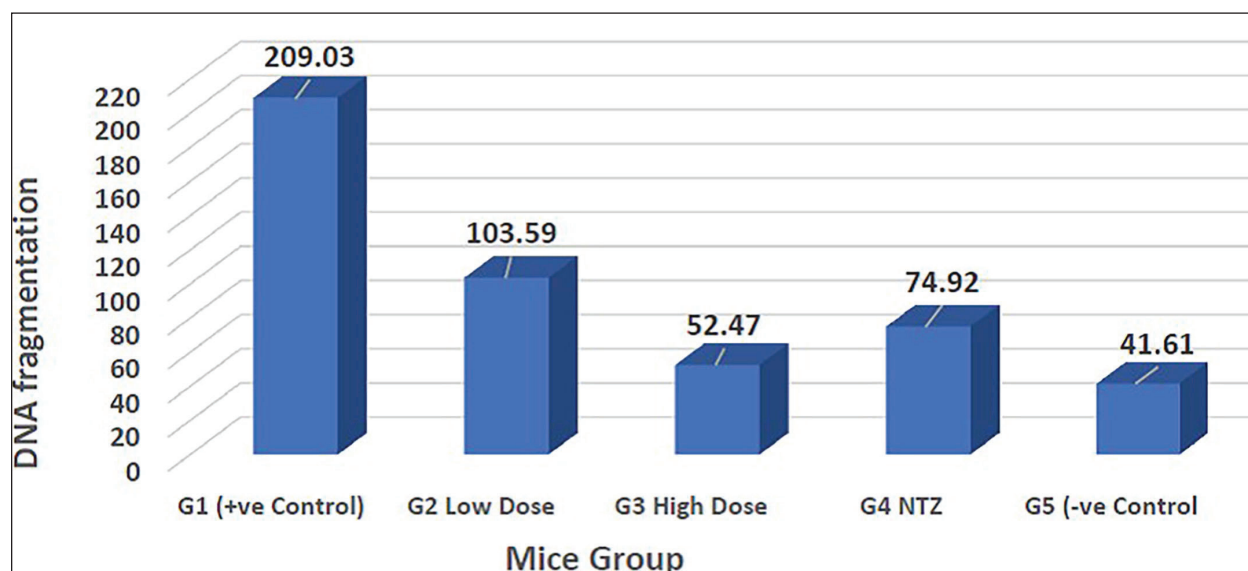


Fig. 5. DNA fragmentation in *Cryptosporidium* experimentally infected non-treated (G1), infected treated with low-dose *S. arboricola* (G2), infected treated with high-dose *S. arboricola* (G3), infected treated with NTZ (G4), and non-infected non-treated (G5).

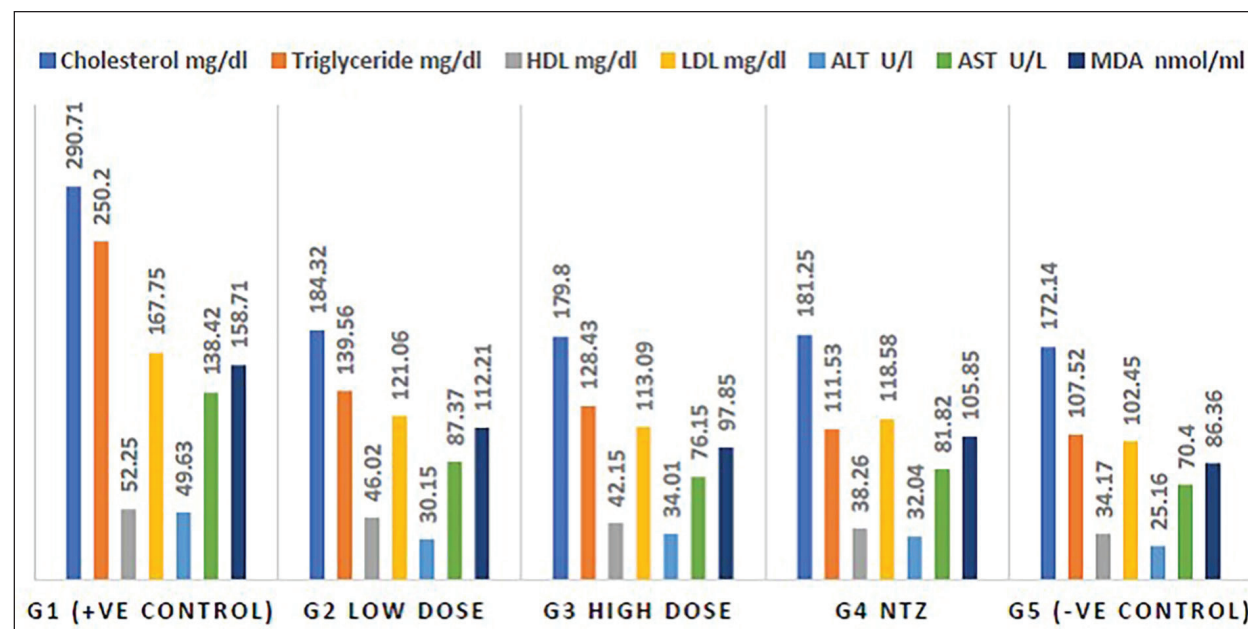


Fig. 6. Biochemical parameters in mice *Cryptosporidium* experimentally infected non-treated (G1), infected treated with low-dose *S. arboricola* (G2), infected treated with high-dose *S. arboricola* (G3), infected treated with NTZ (G4), and non-infected non-treated (G5).

In the current study, LC-ESI-MS analysis of *S. arboricola* leaf extract revealed a complex profile of phenolic acids and their derivatives. These compounds are characterized by high polarity, which accounts for their early elution during CS. Most phenolic acids were identified in the negative ionization mode, with limited detection in the positive mode,

corresponding to compounds 1–6, 8–14, and 37, respectively. According to Ristivojević *et al.* (2015), the fragmentation behavior of phenolic acids typically involves the release of CO₂ and H₂O groups, a pattern also observed in this study. For instance, ferulic acid (compound 1, R_f 1.05) displayed a molecular ion [M–H][–] at *m/z* 193.989 and a fragment ion at *m/z* 178,

resulting from the loss of a methyl group (Gardana *et al.*, 2007). Similarly, compound 9 exhibited precursor ions at m/z 353.007 (negative mode) and 355.103 (positive mode), consistent with that of caffeoylquinic acid (a chlorogenic acid derivative). Its MS/MS fragmentation pattern was characterized by ester bond cleavage between caffeoyl and quinic acid moieties, dehydration of quinic acid to m/z 173 [quinic acid-H-H₂O], and decarboxylation of caffeoyl yielding m/z 135 [caffeoyl-H-CO₂] (Ruan *et al.*, 2019). Compound 12, identified as dicaffeoylquinic acid, appeared in the negative mode at R_t 4.84 with a molecular ion $[M-H]^-$ at m/z 515.176, producing fragment ions at m/z 353, 191, and 179, reflecting successive caffeoyl losses and quinic dehydration (Ruan *et al.*, 2019).

In addition to phenolic acids, the LC-ESI-MS profiling of *S. arboricola* leaves revealed a rich diversity of flavonoid subclasses in both negative and positive ionization modes. The identified compounds included six flavonoid aglycones, 14 flavonoid monoglycosides, 5 flavonoid diglycosides, 4 methoxy flavonoids, and a single bioflavonoid, diosmin (compound 43). These findings are consistent with those of previous reports (El-Hagrassi *et al.*, 2022), confirming the wide spectrum of phenolic and flavonoid constituents in this species. Methoxy flavones exhibit characteristic methyl loss during fragmentation, with m/z values of 298, 330, 358, and 328, respectively (Pellati *et al.*, 2011). A detailed examination of the negative ionization mode spectra revealed that quercetin (compound 42), kaempferol (compound 46), and isorhamnetin (compound 47) followed the classical Recommended Dietary Allowance fragmentation pathway, generating dominant $[1,3A]^-$ and $[1,2A]^-$ fragments at m/z 179 and 151, respectively (Fabre *et al.*, 2001). Flavonoid glycosides identified through the cleavage of glycosidic *O*-linkages in both ionization modes, leading to the loss of monosaccharide residues and H-rearrangement. In compounds 21, 29, 34, 39, 43, 48, and 50, the removal of rutinose (m/z 308 amu) yielded aglycone-specific fragments (Kečkeš *et al.*, 2013). Similarly, compounds 16, 17, 18, 20, 22, 28, and 41 underwent fragmentation through the loss of hexosides (glucose or galactose, m/z 162 amu), whereas compounds 35 and 36 eliminated rhamnose (m/z 146 amu). Compounds 38 and 45 (arabinose or xylose, m/z 132 amu) were lost (Hossain *et al.*, 2010). Characteristic fragmentation of *C*-glycosides involves dehydration and (0.2)/(0.3)-ring cleavages with elimination of ions at m/z 120 and 150, a hallmark of *C*-linkages (Farag *et al.*, 2016). In addition to flavonoids, six coumarins—esculetin, 6,7-dimethoxycoumarin, scopoletin, and esculin—were identified in both ionization modes. For example, scopoletin (compound 23, R_t 11.95) displayed a molecular weight of 193.158 in positive mode and produced fragment ions at m/z 178 (loss of CH₃), 149 (loss of CO), and 133 (loss of CH₃COOH) (Zeng *et al.*, 2015).

Using a modified Ziehl-Neelsen stain, we discovered that *Cryptosporidium* oocytes in the mucosa of intestinally scraped mice had a smooth, circular, bright red wall. Identification based on standards, such as oocyte size and morphology (Xiao *et al.*, 2004). *Cryptosporidium parvum* mostly infects the gastrointestinal epithelium of humans and animals. It lives at the apical portion of the epithelial cells and does not cause systemic infection or deep tissue penetration (Ghazy *et al.*, 2015b). The ileum was chosen for this investigation because it is the region most affected by cryptosporidiosis in mice. Cryptosporidiosis severity depends on the level of immunosuppression (Oliveira *et al.*, 2019). According to the parasitological results of the investigation, the fecal samples of the infected non-treated group (positive control) had significantly higher levels of *Cryptosporidium* oocytes shedding than those of the other *Moringa oleifera*-treated infected groups across all experimental periods (Ghazy *et al.*, 2016). This finding, as well as the fact that the infected non-treated mice exhibited fewer oocytes shedding and pathological lesions compared to the infected treated groups, was noted in oral NTZ-treated groups on days 6–15 following infection (Abouelsoued *et al.*, 2020). These modifications to the histopathological appearance of *Cryptosporidium*-infected mice are in line with previous research. The intestinal mucosa of the untreated infected mice showed obvious histopathological changes, such as shortened and widened intestinal villi. This indicated a significant level of villous atrophy and desquamation of the epithelial lining layer in the majority of the villi (Abu El Ezz *et al.*, 2011; Abd El Wahab *et al.*, 2022). Cryptosporidiosis management is difficult due to the low infectious dosage, the enormous number of sporulated oocytes that shed from different hosts, and the stability of the oocytes in the environment. Certain drugs, such as lasalocid, nitazoxanide, aminoglycoside, and dinitroanilineoryzalin, have been shown to reduce the number of *Cryptosporidium* oocytes *in vitro* (Innes *et al.*, 2020). There are numerous explanations for why the intestinal disorders and total quantity of *Cryptosporidium* oocytes in mice given *S. arboricola* may have strong potential antioxidant, immunomodulatory, and anti-inflammatory effects, which could account for the study's notably beneficial and corrective outcomes (Abouelsoued *et al.*, 2023). This could account for *S. arboricola*'s apparent anti-*Cryptosporidium* action, particularly at higher doses (200 mg/kg).

The observed anti-*Cryptosporidium* activity of *S. arboricola* leaf extract is most likely due to its rich phenolic and flavonoid composition rather than a single bioactive constituent. Phenolic acids such as rosmarinic acid, one of the predominant compounds identified in the extract, have demonstrated antiparasitic and antimicrobial properties and represent promising scaffolds for development against protozoan targets,

including *Giardia* species, through enzyme inhibition and oxidative stress mechanisms. (Kyriakopoulou *et al.*, 2025). Flavonoids such as quercetin and kaempferol have been reported to exert antiparasitic effects against a range of protozoa. *In vitro* studies have shown that certain flavonoids—including quercetin and naringenin—display activity against *C. parvum*, supporting their relevance to the anti-*Cryptosporidium* activity observed in this study (Mead and McNair, 2006). In addition, quercetin and kaempferol are known for their broad antiprotozoal activity against other gastrointestinal parasites, such as *Giardia lamblia*, *Leishmania* spp., and *Entamoeba* spp., frequently acting through mechanisms involving disruption of parasite metabolism, oxidative damage, or interference with enzyme systems essential for survival. Caffeic acid and chlorogenic acid derivatives—also identified in the extract—have shown antiparasitic effects *in vitro*, including growth inhibition of *Giardia* trophozoites, likely via reactive oxygen species induction and parasite energy metabolism disruption. Although flavanones such as naringenin and hesperetin often exhibit weaker direct anti-parasitic activity, they may contribute through antioxidant and host immunomodulatory effects that enhance defense mechanisms against infection (Alday-Provencio *et al.*, 2015). Collectively, these findings suggest that the anti-*Cryptosporidium* activity of *S. arboricola* leaf extract arises from the synergistic action of multiple phenolic acids and flavonoids, consistent with previous reports on plant-derived antiparasitic extracts.

The biochemical findings in the present study demonstrated that *C. parvum* infection induced significant alterations in hepatic function markers, lipid profile, and oxidative stress parameters in infected mice. The infected non-treated group exhibited elevated serum levels of ALT, AST, total cholesterol, triglycerides, LDL, and MDA compared with the non-infected control group, reflecting hepatic injury, metabolic disturbance, and enhanced lipid peroxidation. For instance, Taha *et al.* (2023) reported that *C. parvum* infection in immunosuppressed mice caused a marked elevation in liver transaminases, lipid peroxidation, and antioxidant defense impairment. Similarly, Rahman *et al.* (2022) observed that cryptosporidiosis disrupted lipid metabolism and increased oxidative stress biomarkers in infected hosts, indicating that oxidative injury plays a central role in *Cryptosporidium*-induced pathogenesis. Conversely, treatment with *S. arboricola* extract, particularly at a higher dose (200 mg/kg), significantly reduced these altered biochemical parameters. The observed hepatoprotective and antioxidant effects can be attributed to the rich phytochemical composition of *S. arboricola*, notably its phenolic acids and flavonoids such as rosmarinic acid, quercetin, and kaempferol, which are well known for their free radical scavenging and lipid-stabilizing properties (Sharma *et al.*, 2021;

Pellati *et al.*, 2004). These bioactive metabolites likely modulate antioxidant enzyme activity, suppress lipid peroxidation, and improve cellular redox balance (El-Feky and El Batanony, 2022).

Furthermore, the quantitative DNA fragmentation assay revealed that *C. parvum* infection induced marked DNA damage in the intestinal tissues of infected mice. This observation aligns with earlier reports indicating that *Cryptosporidium* infection can trigger apoptosis and DNA strand breaks through oxidative stress-mediated pathways and inflammatory responses (Atwa *et al.*, 2020). In contrast, *S. arboricola* extract-treated mice exhibited a substantial reduction in DNA fragmentation. This improvement may be attributed to the strong antioxidant defense provided by *S. arboricola* phenolic constituents, which mitigate oxidative DNA damage by scavenging free radicals and stabilizing cellular membranes. Compounds such as rosmarinic acid, caffeic acid, and quercetin have been reported to attenuate DNA fragmentation by inhibiting ROS generation and modulating apoptotic signaling cascades (Ayna, 2021). Thus, the decline in DNA fragmentation following *S. arboricola* treatment indicates the genoprotective potential of the extract against *Cryptosporidium*-induced oxidative injury. Based on the parasitological, biochemical, antioxidant, and histopathological findings, the anti-*Cryptosporidium* activity of *S. arboricola* leaf extract is likely mediated through multiple complementary mechanisms. *Cryptosporidium parvum* infection is associated with pronounced intestinal oxidative stress and inflammation, which contribute to epithelial damage and parasite persistence. The improvement in antioxidant parameters observed in treated groups suggests that phenolic acids and flavonoids in the extract may mitigate oxidative injury by scavenging reactive oxygen species and restoring redox balance (Bhagat *et al.*, 2017). Flavonoids such as quercetin, kaempferol, naringenin, and hesperetin are known to suppress pro-inflammatory signaling pathways, including Nuclear Factor kappa-light-chain-enhancer of activated B cells and Cyclooxygenase-2, which may account for the marked histopathological recovery of intestinal tissues (Hämäläinen *et al.*, 2007). Additionally, several of the identified phenolic compounds have been reported to exert direct antiprotozoal effects by interfering with parasite metabolism and viability, suggesting a possible direct inhibitory action against *Cryptosporidium* developmental stages (Soto-Sánchez, 2022). Collectively, the anti-*Cryptosporidium* efficacy of *S. arboricola* appears to arise from a synergistic interplay between antioxidant, anti-inflammatory, and direct antiparasitic effects.

In addition to its therapeutic efficacy, *S. arboricola* leaf extract demonstrated a favorable tolerability profile under the experimental conditions. The absence of elevations in serum ALT and AST levels in the treated groups, together with the normalization of lipid profile

parameters and oxidative stress markers, indicates that the extract did not exert hepatotoxic or metabolic adverse effects. In contrast, treatment—particularly at the higher dose (200 mg/kg)—ameliorated the biochemical disturbances induced by *C. parvum* infection, suggesting a hepatoprotective rather than toxic effect. No abnormal clinical signs, behavioral changes, or mortality were observed in extract-treated mice throughout the experimental period. These findings are consistent with previous reports highlighting the relative safety of therapeutic doses of phenolic- and flavonoid-rich plant extracts and support the potential advantage of *S. arboricola* as a well-tolerated natural alternative or complementary agent in the management of cryptosporidiosis.

Although the present study demonstrated the promising anti-*Cryptosporidium* efficacy and favorable tolerability of *S. arboricola* leaf extract in an experimental mouse model, further investigations are required to fully confirm its therapeutic potential. Sub-chronic and chronic toxicity assessments should be included in future studies to establish long-term safety, as well as bioactivity-guided fractionation to identify the most active constituents.

Conclusion

The leaves of *S. arboricola* L. extract were found to contain high concentrations of numerous carbohydrates and pigments, such as chlorophyll, carotene, phenolics, and flavonoids. The extract demonstrated significant anti-*C. parvum* activity in experimentally infected mice, as evidenced by reduced oocyst shedding, improved histopathological architecture, and amelioration of oxidative stress and biochemical disturbances. *Schefflera arboricola* is a promising natural therapeutic candidate for the management of cryptosporidiosis.

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Conflict of interest

The author declares no conflict of interest.

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Authors' contributions

Amal M. El-Feky performed the phytochemical analyses, interpreted the data, and prepared the original manuscript draft. Amany M. Mohamed, Nehal M. Khairy, and Raafat M. Shaapan conducted the biological experiments, evaluated the therapeutic efficacy, and analyzed and interpreted the data. Magdy A. A. Ayad as a corresponding author. All authors participated in the revision of the manuscript, approved

the final version, and agreed to be accountable for all aspects of the work.

Data availability

The data supporting the findings of this study are available on request from the corresponding author, and no additional data sources are required.

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