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Cytotoxicity and anticoccidial activities of *Artemisia sieberi* leaf extract: an *in vitro* study

[*Citotoxicidade e atividades anticoccidianas do extrato da folha de Artemisia sieberi: um estudo* in vitro]

S.N. *Maodaa*[1], S. *Al-Quraishy*[1], A. *Alatawi*[1], S.A. *Alawwad*[2], R. *Abdel-Gaber*[1] E.M. *Al-Shaebi*[1]

[1]Department of Zoology, College o f Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia
[2]Department of Food Science and Nutrition, College of Food and Agricultural Science, King Saud University, Riyadh, Saudi Arabia

ABSTRACT

For centuries, medicinal plants with abundant supplies of phytochemicals that are physiologically active have been used in traditional medicine. Numerous of these contain anti-inflammatory and antioxidant qualities that help lower the risk of numerous diseases. The illness coccidiosis affects many animals and results in huge monetary losses. Drug-resistant strains of *Eimeria* spp. have emerged because of drug addiction and usage. Therefore, *Artemisia sieberi* (Asteraceae family) leaves methanolic extract (ASLE) was assessed for its Phytochemical components, in vitro cytotoxicity, and anticoccidial activity. Using infrared spectroscopy (FT-IR), the components of ASLE were detected. Additionally, different extract concentrations were tested for their anticancer activities when applied to breast cancer cell lines (MCF-7) and lung cancer cell lines (A549). ASLE was prepared and tested in vitro as anticoccidial using the oocyst of *Eimeria papillate*. Fifteen different functional groups were found to be present in ASLE using (FT-IR). Also, quantitative results showed phenolics and flavonoids of 235.5±2.7 and 47.89 ± 0.3 respectively in ASLE. Moreover, ASLE showed significant cytotoxicity against cancer cells. The LC_{50} of ASLE was obtained at 98.6± 1.8µg/mL for the A549 and 253.9±4.4µg/mL for the MCF-7 cell lines. At 96 h, significant inhibition of process sporulation for *E. papillata* oocysts was observed when exposed to ASLE (300mg/mL) and formalin 5%, while amprolium, Dettol™, and phenol showed different levels of inhibition. Our findings demonstrated the presence of anticoccidial in ASLE, which encourages the performance of multiple in vivo investigations to find an effective treatment.

Keywords: *Artemisia sieberi*, Cytotoxicity, Anticoccidial, *Eimeria papillata*

RESUMO

Durante séculos, as plantas medicinais com abundantes suprimentos de fitoquímicos fisiologicamente ativos têm sido usadas na medicina tradicional. Muitas delas contêm qualidades anti-inflamatórias e antioxidantes que ajudam a reduzir o risco de várias doenças. A coccidiose afeta um grande número de animais e resulta em enormes perdas monetárias. Cepas resistentes a medicamentos de Eimeria spp. surgiram como resultado da dependência e do uso de drogas. Portanto, o extrato metanólico das folhas de Artemisia sieberi (família Asteraceae) (ASLE) foi avaliado quanto a seus componentes fitoquímicos, citotoxicidade in vitro e atividade anticoccidiana. Usando a espectroscopia de infravermelho (FT-IR), os componentes do ASLE foram detectados. Além disso, diferentes concentrações de extrato foram testadas quanto às suas atividades anticancerígenas quando aplicadas a linhas celulares de câncer de mama (MCF-7) e linhas celulares de câncer de pulmão (A549). O ASLE foi preparado e testado in vitro como anticoccidiano usando o oocisto de Eimeria papillate. Verificou-se a presença de 15 grupos funcionais diferentes na LSA usando (FT-IR). Além disso, os resultados quantitativos mostraram fenólicos e flavonoides de 235,5 ± 2,7 e 47,89 ± 0,3, respectivamente, na LSA. Além disso, o ASLE apresentou citotoxicidade significativa contra células cancerígenas. A LC50 do ASLE foi obtida em 98,6±1,8µg/mL para a A549 e 253,9±4,4µg/mL para as linhas celulares MCF-7. Em 96 h, foi observada uma inibição significativa do processo de esporulação de oocistos de E. papillata quando expostos a ASLE (300 mg/mL) e formalina 5%, enquanto amprólio, DettolTM e fenol apresentaram diferentes níveis de inibição. Nossos achados demonstraram a presença de anticoccidianos no ASLE, o que incentiva a realização de várias investigações in vivo para encontrar um tratamento eficaz.

Palavras-chave: Artemisia sieberi, Citotoxicidade, Anticoccidiano, Eimeria papillate

Corresponding author: maodaa_28@yahoo.com
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INTRODUCTION

Coccidiosis is a protozoan infection caused by intestinal parasites of the genus Eimeria (subclass: Coccidia). Eimeria infecting animals causes gastrointestinal problems such as diarrhea, reduces development performance, and, in severe cases, results in death (Allen and Fetterer, 2002; Kulkarni *et al*., 2019). Furthermore, Eimeria spp. infections can result in secondary infections with other pathogens such as bacteria (Collier *et al*., 2008). In addition, this illness generates significant losses worldwide (Chapman, 2014) where, drug-resistant strains cause enormous global economic losses due to low weight increase and excessive food consumption. Eimeria has an asexual and sexual reproduction cycle, and it produces resistant parasite stages known as oocysts that are released into the environment, facilitating the spread of infection (Graat *et al*., 1994). As a result, deactivating the sporulation operation is an essential step in controlling these parasites (Mai *et al*., 2009).

In recent decades, coccidiosis control has relied primarily on the use of chemical medicine; however, the use of prebiotics, probiotics, and natural products has been preferred to improve overcome drug resistance, immune system and reduce unwanted side effects of synthetic medicine in the food chain (Abbas *et al*., 2012; Brisibe *et al*., 2008; Drăgan *et al*., 2014; Gholamrezaie *et al*., 2013; Kostadinovic *et al*., 2012). Additionally, drug-resistant strains of Eimeria spp. have appeared because of the overuse and abuse of these medication. Plant extracts are now being evaluated as viable sustainable alternatives to new medications (Hema *et al*., 2015) as a result of this. It has been demonstrated that herbal extracts from plants like *Curcuma longa, Artemisia absinthium, Saussurea lappa, Ageratum conyzoides, Olea europaea, Ruta pinnata,* and *Trachyspermum ammi* have antiparasitic properties as well as the ability to boost the immune system and growth capacity, assisting the host in recovering from coccidiosis infection (Debbou-Iouknane *et al*., 2019; Zaman *et al*., 2015) Besides, Vaccine effectiveness is limited partly due to high production costs and ineffectiveness under poor management conditions. As a result, there is a significant desire to replace existing treatments with some natural alternative agents (Abudabos *et al*., 2017).

Scientists all over the world are currently investigating the use of natural therapies, such as plants and plant-derived compounds, to mitigate the effects of coccidiosis (Abbas *et al*., 2012). During coccidiosis, the effect of various medicinal plants, either alone or in combination, has been studied (Arab *et al*., 2006). *Artemisia* species are high in natural compounds, and their anticoccidial activity has been proven.

Terpenoiod is abundant in all *Artemisia* species. Environmental factors such as latitude and longitude, altitude, humidity, temperature, climate, and soil, as well as metabolic pathways and biosynthesis, affect the number of compounds in the genus *Artemisia*. As a result, secondary metabolites are also biosynthesized under various environmental conditions (Zehra *et al*., 2020).

Artemisia (Asteraceae), sometimes known as "Sage Brush" or “Wormwood," is a genus of roughly 500 species of tiny herbs and shrubs native to Asia, Europe, and North America (Bora and Sharma, 2011). It is commonly used in traditional medicine for a variety of diseases, including lowering pain (Morshedi *et al*., 2011), coughing (Tan *et al*., 1998), hypertension (Ben-Nasr *et al*., 2013) and reducing phlegm (Martínez *et al*., 2012). The *Artemisia* species have played a vital role in both traditional and modern medicine (Ekiert *et al*., 2022).

There are some species of the genus *Artemisia* L. grown in the northern part of Saudi Arabia, including *Artemisia judaica, Artemisia monosperma,* and *Artemisia sieberi*. (El-Sayed *et al*., 2013; Guetat *et al*., 2017). They are commonly used in traditional medicine (El-Sayed *et al*., 2013) because these species have antipyretic, anthelmintic, anti-inflammatory, antibacterial effects (El-Sayed *et al*., 2013; Guetat *et al*., 2017; Moharram *et al*., 2021). The *Artemisia judaica* and *A. sieberi* species that thrive in Saudi Arabia also possess anticancer effects, according to documented results. (Nasr *et al*., 2020) In addition, these species demonstrated strong antibacterial activity against (Salmonella enteritidis and Escherichia coli) two human diseases (Guetat *et al*., 2017).

A. sieberi is a well-known medicinal herb in traditional Middle Eastern medicine as an anthelmintic. Locally known as "Shih" in Arabic countries, *A. sieberi* (Artemisia herba alba), also known as "the desert worm wood". The genus *Artemisia* contains more than 160 secondary metabolites that have already been isolated from the genus *Artemisia*, including flavonoids. One-third of flavonoids are flavones, apigenin and luteolin derivatives. Santonin, lactones, sesquiterpene and bicyclic monoterpene glycosides (Marco *et al*., 1993). Abdolmaleki *et al*. (2015) mentioned that the ethanolic extract of A. sieberi has a strong cytotoxic effect on the HCT116- cells line and is a potent inhibitor of angiogenesis in cultured cells.

Artemisinin is a sesquiterpene lactone that was isolated for the first time from *Artemisia annua* and is also found in *A. sieberi*. The leaves and blooming branches were boiled in normal saline for external use, and the extracted solution was used to treat gangrenous ulcers, inflammations and infectious ulcers (Zargari, 1989). It has been used as a carminative, to reduce inflammation and abscesses, and to prevent leprosy (Sînâ, 1985). Arab *et al*. (2006) have discovered the artemisinin content of this plant for the first time and found that *A. sieberi's* amount of artemisinin (0.14–0.2 % of dry weight during various seasons), which is comparable to that of other species, including *Artemisia annua* (Arab *et al*., 2006).The beneficial effects of *A. sieberi* essential oil as insecticidal (Negahban *et al*., 2006), antimicrobial (Irshaid *et al*., 2010), anti-malaria, nematocidal (Ardakani and Parhizkar, 2012) and anticoccidiosis effects (Arab *et al*., 2006). Also, it is used as an anthelminthic in traditional Middle Eastern medicine (Mahboubi, 2017) and to treat a variety of ailments, including diabetes mellitus in Jordan (Irshaid *et al*., 2012). Moreover, *A. sieberi* has also been used as a herbal remedy to treat gastrointestinal ailments and high blood pressure (Bidgoli *et al*., 2013).

Thus, the present study aimed to determine the following: (i) the phytochemical constituents; (ii) the *in vitro* cytotoxic activities of ASLE; (iii) the *in vitro* anticoccidial activity of ASLE against *E. papillata*.

MATERIALS AND METHODS

The leaves of *A. sieberi* were collected in Al Badiya - Tabuk, Saudi Arabia. A taxonomist from the Botany Department (King Saud University, Riyadh, Saudi Arabia) recognized and confirmed the plant material in the herbarium. The methanol extract of *A. sieberi* leaves (70% methanol- 30 % distilled water) was prepared using the method reported by Manikandan (2008), with some changes as follows: the air-dried leaves (for 5 days) of *A. sieberi* were ground into a powder with an electric blender (Senses, MG-503T, Korea). The dried powder (100 g) of Shih leaves was macerated in 70% methanol (Sigma-Aldrich/32213- CAS-No:67-56-1, Poland) on volume 700 ml methanol/300mL distilled water for 24 hours at 4ºC, followed by percolation 5-7 times until complete extraction. Following filtering, ethanol was isolated from the extract using a vacuum evaporator set at 50 °C and low pressure. The crude extract (2.7 g) was lyophilized and kept at -20°C until further usage.

A very little part of the material was mixed with an excess of potassium bromide powder (1: 99 wt.%), homogenized, finely powdered, and then put in a die for pellet formation. The Fourier-transform infrared spectrometer (FT-IR) NICOLET 6700 optical spectrometer from Thermo Scientific is the tool used to analyze infrared (IR). Maximum absorption was recorded as waves (cm^{-1}) in number. From 400 to 4000 cm^{-1}, spectra were recorded (Abu Hawsah *et al*., 2023).

The Ainsworth and Gillespie, 2007 method was used to estimate the leaf extract's total phenolic content (TPC).300 µL of sodium carbonate solution (20%) and 100µL of the Folin-Ciocalteu reagent were added to 100µL of the leaf extract. Then, the sample was incubated at room temperature for 30 minutes in the dark. A UV-Visible spectrophotometer (SHIMADZU, UV-1800) was used to measure the wavelength, which was 765nm. Based on a standard curve created using various gallic acid concentrations (25-400g/mL), the total phenolic in the samples was calculated from the following linear equation ($y = 0.0021x + 0.0021$ with $R2 = 0.9995$). The total phenolic content was represented as mg/g DW.

Using the Ordonez *et al*., 2006 method, the total flavonoid content (TFC) of plant materials was determined. The same volume of a 2% $AlCl_3$ water solution was mixed with 0.5mL of

methanol extract. At 25℃, the wavelength was measured at 420 nm after two hours. The TFC was calculated using a calibration curve constructed from various quercetin standard concentrations (50-0400µg/mL) using the equation (y = 0.0172x + 0.0507 with R2 = 0.995). Quercetin (mg/g DW) has been used to represent the estimated TFC.

Breast (MCF-7) and lung (A549) cancer cell lines were routinely cultivated in DMEM medium (Gibco, USA) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco, USA). In an incubator with a humidified environment of 5% CO_2, the cells were incubated at 37°C.

Using an MTT test, the cytotoxic potential of plant extract was assessed. In a nutshell, cells were plated in a 96-well culture plate at a density of 5 x 10^4 per ml and given 24 hours to grow. Next, doxorubicin was utilized as a positive control while cells were treated to various concentrations of plant extract (500, 250, 125, 62.5, 31,125, and 15.625g/mL).

After the 48 hours of incubation, each well received 10 µL of MTT solution (5 mg/mL in PBS), which was then incubated for an additional 4 hours. The formazan product was then solubilized by adding 100µL of acidified isopropanol to each well, and the plate was shaken for 10 minutes. The absorbance was measured using a microplate reader (BioTek, USA) to measure it at 570nm.

%Cell Viability = Mean absorbance [(treated cells / untreated cells] × 100

Using OriginPro software, the IC_{50} values (concentration of extract that caused 50% inhibition) were calculated from the dose-response curve of cell viability percentage.

The parasite was obtained from fresh fecal cells of infected mice. Feces were collected, and oocysts were then separated using the flotation method and employed in an in vitro experiment.

The effect of different ASLE concentrations on the sporulation of *E. papillate* oocysts was studied in vitro. In this assay, we tested four concentrations (300, 200, 100, and 50mg/mL)/5mL potassium dichromate containing 1×10^5 oocysts. Untreated control oocysts were left untreated, positive control oocyst treated with 5mL 2.5% potassium dichromate ($K_2Cr_2O_7$). Also, 8.3mg amprolium (Veterinary Agriculture Products Company [VAPCO], Jordan), 109µL Dettol ™, 25µL phenol, and formalin (5%) were tested, and each test was done in triplicate. All petri utilized for these treatments were incubated for 72 and 96 hours at 25 to 29°C and 80% relative humidity. The oocysts were rinsed in distilled water at the end of the incubation period, as described by Fatemi *et al.* (2015). The samples were then kept at 4°C. The sporulation % and sporulation inhibition percentage were recorded and counted with a haemocytometer as done by Thagfan *et al.* (2020).

$$\text{Sporulation (Sp)}\% = \frac{\text{Number of sporulated oocyts}}{\text{Total number of oocysts}}\ X\ 100$$

$$Sporulation\ (Sp) inhibition\ percentage = \frac{Sp\%\ of\ control - Sp\%\ of\ extract}{Sp\%\ of\ control}\ X\ 100$$

One-way analysis of variance (ANOVA) was used to examine the data in SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL, USA). Differences between groups were considered significant at a p-value ≤ 0.01.

RESULT

Major bands were revealed by the FT-IR analysis of ASLE at 3390.30 cm^{-1}, 2934.71cm^{-1}, 1763.99cm^{-1}, 1606.14cm^{-1}, 1514.66cm^{-1}, 1451.34cm^{-1}, 1383.43cm^{-1}, 1266.30cm^{-1}, 1175.69cm^{-1}, 1118.10cm^{-1}, 1069.40cm^{-1}, 832.42cm^{-1}, 795.02cm^{-1}, 769.00cm^{-1}, 612.83cm^{-1}. (Figure 1 and Table 1). N-H stretching was indicated by the band at 3390.30 cm^{-1} confirming the presence of aliphatic primary amine. The band at 2934.71cm^{-1} implicit C-H stretching for the presence of alkane. C=O stretching at 1763.99cm^{-1} emphasizes the presence of carboxylic acid. The band at 1606.14cm^{-1} coincides with C=C stretching for the presence

of conjugated α, β-unsaturated ketone. N-O stretching at the band 1514.66cm^{-1} confirmed the presence of nitro compound. The band at 1451.34cm^{-1} implied (C-H bending) for the presence of alkane and the band at 1383.43cm^{-1} corresponds to C-H bending for the presence of alkane. 1266.30cm^{-1} (C-N stretching), 1175.69cm^{-1} (C-O stretching), 1118.10cm^{-1} (C-O stretching), 1069.40cm^{-1} (S=O stretching), 832.42cm^{-1} (C=C bending), 795.02cm^{-1} (C-H bending), 769.00cm^{-1} (C-H bending) and 612.83cm^{-1} (C-I bending) assigned to aromatic ester, ester, secondary alcohol, sulfoxide, alkene, 1,2,3-trisubstituted, 1,2-disubstituted and halo compound respectively (Table 1).

The amounts of secondary metabolites in the ASLE were measured, like flavonoids and phenolics. Figure (2) shows that the amount of phenolic concentration (235.5 ± 2.7 mg/g DW) was high compared to the flavonoid's concentration (47.89 ± 0.3 mg/g DW).

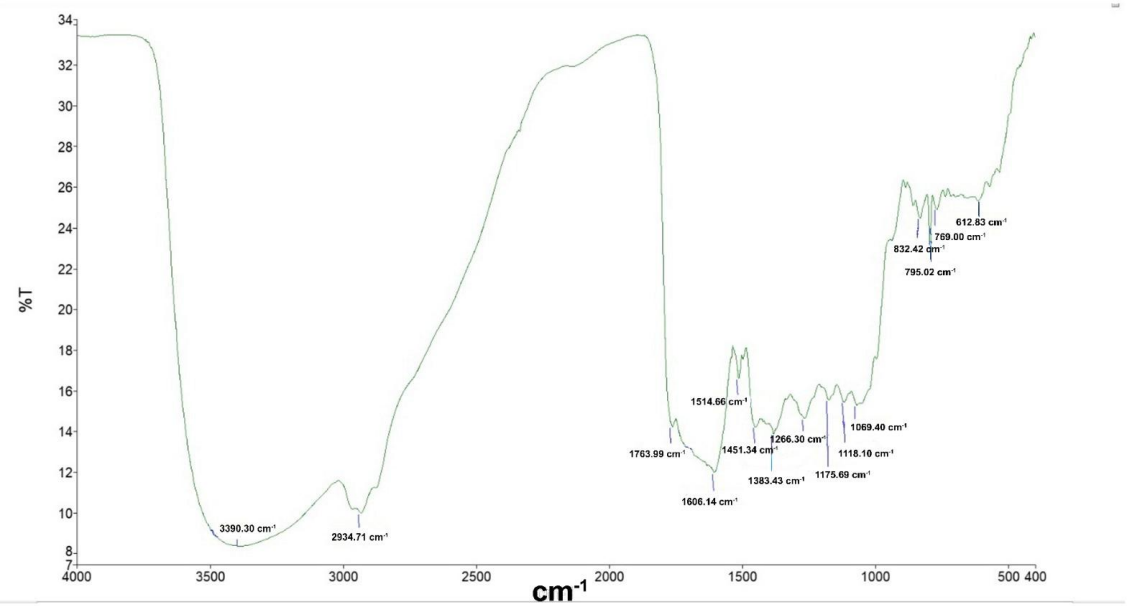

Figure 1. FTIR of ASLE shows the material's functional characteristics.

Table 1. FT-IR for the extract of *A. sieberi* leaves

Absorption (cm^{-1})	Transmittance (%)	Appearance	Group	Compound class
3390.30	11.13726	medium	N-H stretching	aliphatic primary amine
2934.71	4.068068	medium	C-H stretching	alkane
1763.99	3.403546	strong	C=O stretching	carboxylic acid
1606.14	4.278121	strong	C=C stretching	α,β-unsaturated ketone
1514.66	9.217903	strong	N-O stretching	nitro compound
1451.34	4.927714	medium	C-H bending	alkane
1383.43	4.108677	medium	C-H bending	alkane
1266.30	4.831751	strong	C-N stretching	aromatic ester
1175.69	6.214668	strong	C-O stretching	ester
1118.10	4.295302	strong	C-O stretching	secondary alcohol
1069.40	3.638029	strong	S=O stretching	sulfoxide
832.42	10.90045	medium	C=C bending	alkene
795.02	10.64206	strong	C-H bending	1,2,3-trisubstituted
769.00	9.613958	strong	C-H bending	1,2-disubstituted
612.83	8.722485	strong	C-I bending	halo compound

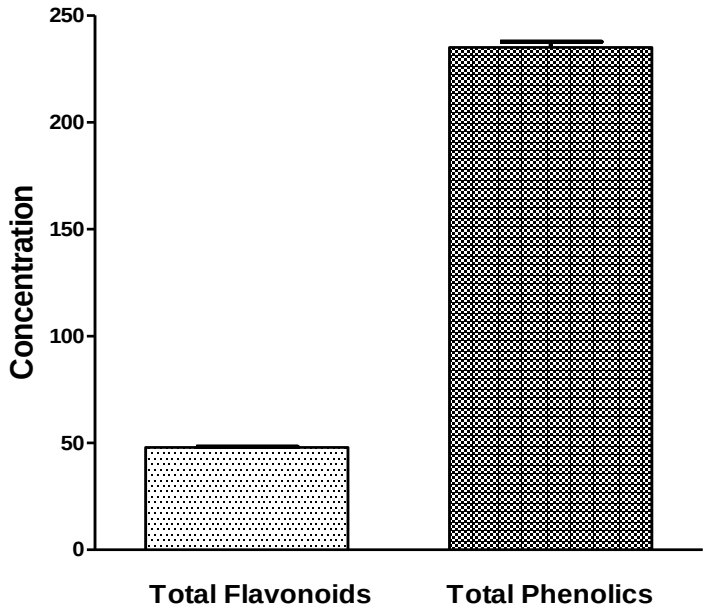

Figure 2. Methanolic extract of the *A. sieberi* plant leaves contains flavonoids and total polyphenols.

Cell viability was affected by the highest concentrations of ASLE, whereby the concentrations of 125, 250 and 100µg/mL showed toxicity against 60, 70, 75, % of A549 (Figure 3). Additionally, this extract was shown to be safe for normal cells up to a concentration of 62.5µg/mL with LC_{50} attributed at 98.6±1.8µg/mL. ASLE demonstrated clear cytotoxic effects on the MCF-7 cell line, at a high concentration only of 500, causing cell death at a rate of 70%, and LC_{50} at 253.9±4.4µg/mL.

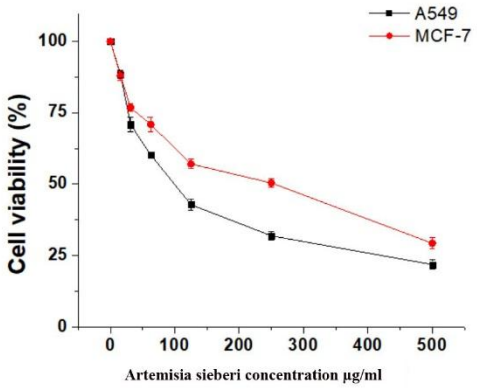

Figure 3. (MTT) assay for tested Cytotoxicity of ASLE at various concentrations (µg/mL) against breast (MCF-7) cancer cell lines and Lung (A549) after 48 h of incubation. A549 (98.6 ± 1.8 g/mL) and MCF-7 (253.9±4.4g/mL) cancer cell growth inhibition at 50% of the studied plant extract dose is indicated by the (LC_{50}).

In vitro studies on ASLE and a few other materials revealed sporulation of the oocyst (%) and sporulation inhibition (%) for *E. papillata* at 72 and 96h. A significant degree of oocyst sporulation (%) in distributed H_2O was found to be (66.6%) when compared to the ASLE, which had sporulation levels of 0%, 12.1%, 61.4%, and 80.5% at 72h (Figure 4), while at 96h, were 2.4%, 80.3%, 89.5%, and 93.7% at concentrations of 300, 200, 100, and 50mg/mL,

respectively (Figure 5) also, the rate of sporulation (%) varied in each of the Dettol™, phenol, and formalin 5% were 23.08%, 7.7%, and 0 %, respectively, at 72 h (Figure 4), while at 96h, were 18.67%, 10.67%, and 0%, respectively (Figure 5).

On the other hand, the highest sporulation inhibition (100 %) was obtained for ASLE at a concentration of 300 mg in 72 h and 96h (Figures 6 and 7, respectively). While the levels of sporulation inhibition for amprolium, Dettol™, phenol, and formalin 5% were 37. 33 %, 81.33 %, 89.33 %, and 100 %, respectively, at 96 h (Figure 7), while, at 72 h it was 34.61%, 76.92%, 92.30%, and 100% (Figure 6).

DISCUSSION

In this work, we reported that A. sieberi is a promising source of phenolic and flavonoid chemicals. Our findings are consistent with previous research that found same elements in the same species cultivated in different parts of the world (Azimian and Roshandel, 2015; Ranjbar *et al*., 2020). Also, Previous research has shown total phenolics and flavonoids in various *Artemisia* species (Erel *et al*., 2012; Iqbal *et al*., 2012; Singh *et al*., 2009). Numerous in vitro studies have established the phenolic and flavonoid compounds' ability to act as antioxidants, and they have also demonstrated their potent capacity of scavenging a number of non-physiological radicals, including DPPH and ABTS (Cai *et al*., 2006; Kosar *et al*., 2003; Kumar and Pandey, 2013; Payet *et al*., 2005; Pietta, 2000). It is generally known that phenolic compounds, including phenol, flavonoid, and flavanol, have antioxidant action and benefit human health. The total phenolics and flavonoids in various *Artemisia* species have already been described in previously reported (Erel *et al*., 2012; Iqbal *et al*., 2012; Singh *et al*., 2009). According to a number of research, phenol concentration and antioxidant activity are related (Khezrilu and Heidari, 2014; Kiselova *et al*., 2006). The presence of reductions has been demonstrated to have reducing capabilities, which have been linked to their ability to act as antioxidants by donating an atom of hydrogen to break the chain of free radicals (Geckil *et al*., 2005).

This study assessed the number of total phenols and flavonoids in the aerial section of *A. sieberi*. Additionally, different species of *Artemsia* varied in their ability to act as antioxidants (Lopes-Lutz *et al*., 2008), which can be ascribed to the presence of phenolic compounds, including flavonoids, and climate and edaphic properties of the geographical regions.

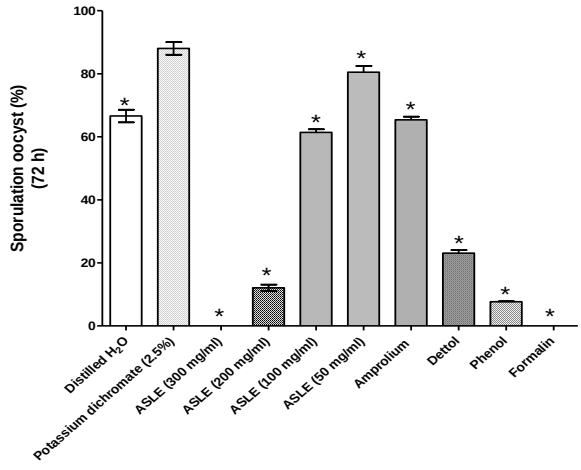

Figure 4. Effects of *A. sieberi* leaves extract (ASLE) on the anticoccidial of *E. papillata* oocyst sporulation at 72 hours. *Significance compared to the Potassium dichromate (2.5%) group ($p \leq 0.01$).

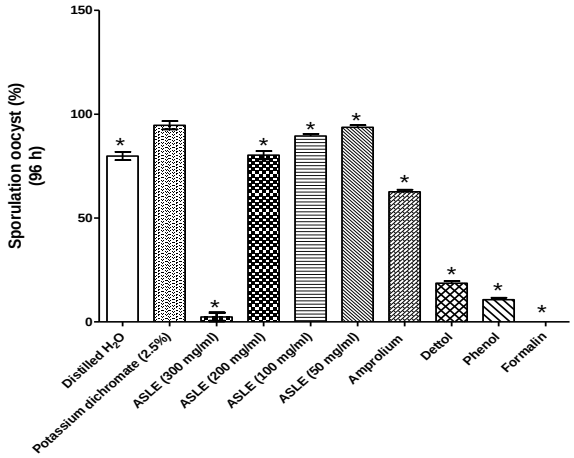

Figure 5. Effects of *A. sieberi* leaves extract (ASLE) on the anticoccidial of *E. papillata* oocyst sporulation at 96 hours. *Significance compared to the Potassium dichromate (2.5%) group ($p \leq 0.01$).

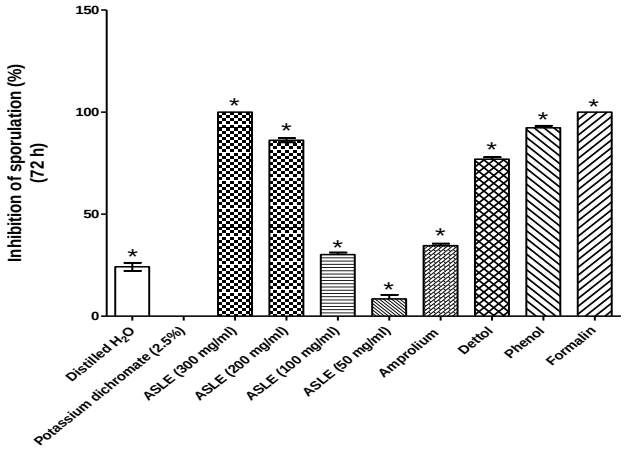

Figure 6. Anti-coccidial effects of *A. sieberi* leaves extract (ASLE) on the sporulation Inhibition (%) of *E. papillata* oocysts at 72 h. *Significance compared to the Potassium dichromate (2.5%) group ($p \leq 0.01$).

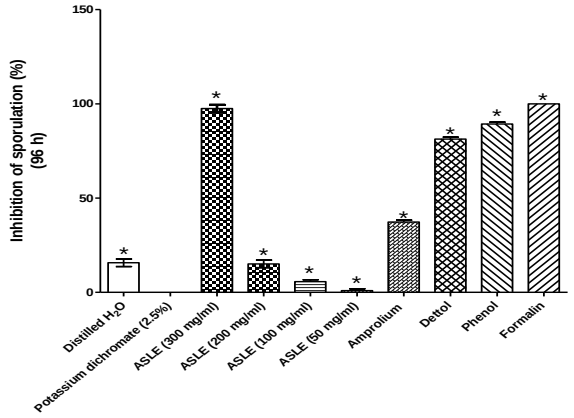

Figure 7. Anti-coccidial effects of *A. sieberi* leaves extract (ASLE) on the sporulation Inhibition (%) *of E. papillata* oocysts at 96 h. *Significance compared to the Potassium dichromate (2.5%) group ($p \leq 0.01$).

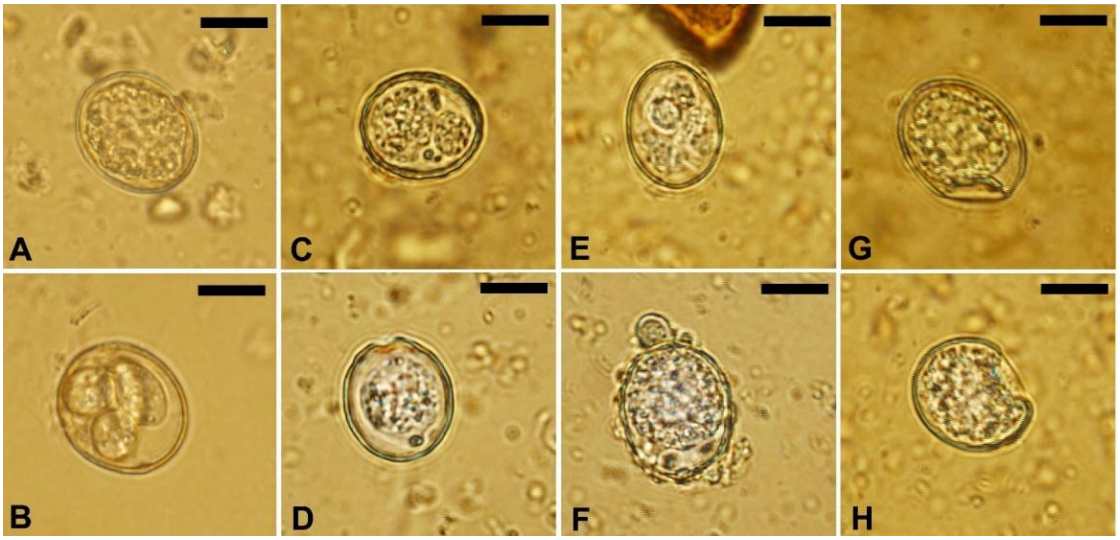

Figure 8. Changes observed after exposure of E. papillata oocytes to different treatment. (a) normal nonpopulated oocysts in H2O; (b) normal sporulated oocysts in $K2Cr_2O_7$; (c–h) abnormal oocytes in the ASLE (300 mg/mL). Scale bar = 12.5µm.

Sesquiterpene lactones, tocopherols, flavonoids, polyphenols, and sulfoxide are examples of biological chemicals that have showed substantial anticoccidial activities, both in vitro and in vivo, and can therefore be utilized as alternatives to commercial disinfectants (Alhotan and Abudabos, 2019; Allen *et al*., 1997; Mo *et al*., 2014; Nahed *et al*., 2022).

The methanol extracts of *A. sieberi* in this study shown cytotoxic efficacy against all examined cancer cell lines. The Lung (A549) cell line's IC_{50} for ASLE was found to be 98.6 1.8g/mL, whereas the MCF-7 cell line's IC_{50} was 253.9 4.4g/mL (Figure 3). in the vitro cytotoxicity of *A. sieberi* was examined against the breast cancer (MCF-7) and lung (A549) cell lines at various concentrations. Our results supported the notion that cell viability is directly dose dependent. The results demonstrated that, when compared to untreated cells, the viability of (A549) and (MCF-7) cells were significantly reduced after 48 hours of incubation with different concentrations of *A. sieberi*. Also, the methanolic extract of *A. sieberi* displayed the activity was stronger against HepG2, followed by MCF-7 and finally LoVo (Nasr *et al*., 2020). Further, exposure of the HUVEC cell line to ethanolic extract of A. sieberi (concentration-dependent) resulted in a significant decrease in the number of viable cells (Abdolmaleki *et al*., 2015). This may be a result of the presence of sesquiterpene lactones, which have previously been documented for this species (Abbas *et al*., 2012; Arab *et al*., 2006).

After 72 and 96 hours, the in vitro anticoccidial activity of ASLE against coccidial oocysts was evaluated and the results are shown in (Figures 4 - 7), respectively. The outcomes demonstrated that the ASLE prevented sporulation of coccidia oocysts at the highest concentration levels. After 72 and 96 hours of incubation, the highest dose (300 mg/mL) completely prevented the oocysts from sporulating (Figure 4,5). However, after 72 and 96 hours of incubation, respectively, sporulation increased in the negative controls and lowest concentration (50mg/mL) (Figures 3, 4). After coccidia were exposed to ASLE, it was found that the shell weakened, the oocyst burst at its weakest location, and the central cytoplasmic mass oocyst was destroyed, these results similar to what was reported by (Daiba *et al*., 2023). Oocysts subjected to negative controls and the lowest concentration (50mg/mL) showed normal sporulation. Our research demonstrated that ASLE had an impact on the development of coccidia spores; the extract significantly altered the morphology of coccidia oocysts. As shown by the abnormal sporocysts in oocysts subjected to higher concentrations, the ASLE would have harmed the oocyst shell wall, weakening it and damaging the core cytoplasmic mass (sporont) in the current study figure 8.

The results of this experiment demonstrated that the methanol leaf extract of ASLE has an in vitro anticoccidial effect on unsporulated oocysts of *E. papillata* in a concentration dependent manner, which is attributable to numerous bioactive phytochemical constituents studied (Abdulrahman *et al*., 2023; Salih *et al*., 2023). ASLE inhibited oocyst sporulation which was similar to what was reported by Fatemi *et al* (Fatemi *et al*., 2015). One of the most significant elements impacting the epidemiology of coccidiosis is oocyst sporulation. According to Fatemi *et al* (Fatemi *et al*., 2015), the petroleum ether and ethanol extract of *Artemisia annua* not only prevents oocyst sporulation but also alters the shape and size of specific morphological components. The mechanism is unknown, however at concentrations of 2 and 5 ppt, the plant extracts may pierce the oocyst membrane and harm the sporont.

It is also shown that the regularly used disinfectant formalin (5%) is the most efficient in inhibiting E. papillata oocyst sporulation, which agrees with Thagfan *et al*. (2020). Dettol™ and Phenol inhibited sporulation by 81.33%, 89.33% respectively, which is consistent with (Mai *et al* ., 2009) and Gadelhaq *et al*. (2018) that reported that the oocyst wall is impermeable to water-soluble component and resistant to proteolysis.

CONCLUSION

The use of herbal formulas containing the most potent alcoholic plant extracts is required to achieve the strongest anticoccidial effect. Statistical analysis revealed that the highest concentration levels of plant extracts were effective in inhibiting the sporulation of E. papillata oocysts as well as destroying them. It could be concluded that ASLE has cytotoxicity and anticoccidial efficacy in vitro. More research should be done to determine the in vivo effectiveness of ASLE where the results of these investigations indicate that herbal drugs have a substantial amount of promise for innovative medication development to treat parasite illnesses and that the derivatives of these plants are advantageous structures for drug synthesis and bioactivity optimization. This will inform ongoing studies geared toward the development of ASLE as a novel drug that can be used to manage coccidian diseases that affect animals.

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