Assessment of *Euphorbia retusa* and *Pulicaria undulata* activity against *Leishmania major* and *Toxoplasma gondii*

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### ABSTRACT

Leishmaniasis and toxoplasmosis are parasitic protozoal diseases that pose serious health concerns, especially for immunocompromised people. *Leishmania major* and *Toxoplasma gondii* are endemic in Saudi Arabia and are particularly common in the Qassim Region. The present work was conducted to evaluate the *in vitro* antileishmanial and antitoxoplasmal activity of methanolic extracts and phytochemical fractions from two plants, *Euphorbia retusa* and *Pulicaria undulata*, which are ethnobotanical agents used to treat parasitic infection. Whole *E. retusa* and *P. undulata* plants were extracted with methanol and fractionated using petroleum ether, chloroform, ethyl acetate, n-butanol, and water and then were tested *in vitro* against *L. major* promastigote and the amastigote stages of *T. gondii*; the cytotoxicity of the extracts was tested against Vero cell line. The methanolic extracts of *E. retusa* and *P. undulata* exhibited promising antitoxoplasmal activity against *T. gondii* with EC₅₀ values 5.6 and 12.7 μg mL⁻¹, respectively. The chloroform fraction of *P. undulata* was the most potent, exhibiting an EC₅₀ of 1.4 μg mL⁻¹ and SI value of 12.1. It was also the most active fraction against both *L. major* promastigotes and amastigotes, exhibiting an EC₅₀ of 3.9 and 3.8 μg mL⁻¹ and SI values 4.4 and 4.5, respectively. The chloroform fraction from *P. undulata* is a very good candidate for the isolation of active antitoxoplasmal and antileishmanial ingredients; therefore, further phytochemical analysis for active compound isolation is highly recommended.

**Keywords:** *Toxoplasma gondii, Leishmania major, In vitro, Euphorbia retusa, Pulicaria undulata.*

### INTRODUCTION

Both toxoplasmosis and leishmaniasis are major parasitic diseases of global importance. They are also highly endemic in Saudi Arabia, while their prevalence is characterized by regional variation (Alsammani, 2016; Rasheed *et al*., 2019). Leishmaniasis diseases show different types of clinical manifestations that etiologically occur from species of the genus *Leishmania*, which is contained in more than 20 species, and all of them are transmitted by infected female phlebotomine sandflies. There are three main types of leishmaniasis: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (ML), and VL and CL are the most common types recorded and are endemic from 56 and 59 countries, respectively, as of 2020 and based on the WHO Global Leishmaniasis program for 2018 (WHO, 2020).

CL is the common form in Saudi Arabia and reported in many parts of the country because of the distribution of vectors and presence of reservoir animals (Haouas *et al*., 2017; Al Nasr *et al*., 2019a). This disease has been an epidemic in Al Qassim Province many times during the last two decades (Rasheed *et al*., 2019).

In Saudi Arabia, CL is commonly caused by *L. major* species that become increasingly resistant to commonly used therapeutics (Croft & Coombs, 2003), which also have many side effects (Sundar *et al*., 2000). For this reason, medicinal plants that also have been traditionally used without any major side effects can be considered suitable candidates for further scientific investigation towards novel drug discoveries that may show better efficacy.

Toxoplasmosis is commonly caused by *Toxoplasma gondii*, which is an intracellular pathogenic globally distributed protozoon and known to be found in almost all warm-blooded animals (Peng *et al*., 2011; Alzaheb, 2018). This parasite infects approximately one third of the world’s population and is considered one of the most successful human parasites (Pappas *et al*., 2009). In fact, the Centers for Disease Control have prioritized *T. gondii* as one of the top “Five Neglected Parasitic Infections” due to the severity of...
illness, high incidence, and potential for prevention (Li et al., 2014). The infection can be fatal due to severe symptoms in immunocompromised people, and it can also be associated with abortions during pregnancy and highly serious congenital abnormalities (Khali et al., 2018).

Toxoplasmosis has been reported as an epidemic in Saudi Arabia several times, especially in the southwest and central parts of the country (Phillipson & Wright, 1991; Al Nasr et al., 2016).

Currently, there are few effective antitoxoplasmal drugs, but they have adverse side effects and require long courses of administration (Holzgrabe & Bechtold, 1999; Camacho et al., 2000). However, the previous investigation on natural products, mainly plants, provides an excellent chance to discover new molecules with potent efficacy to potentially provide drug candidates (Kayser et al., 2003; Al Nasr et al., 2019b).

Euphorbia retusa Frossk and Pulicaria undulata L. are among the herbaceous flora of the Qassim Region, which has previously been subjected to scientific studies for biological activities and phytochemistry. Euphorbiaceae is a family comprised of approximately 300 genera and 10,000 species, which are used in folk medicine to remove warts, for treating venous bites and trichiasis, and for wound infection (Mengiste et al., 2014). Phytochemically, E. retusa was visualized to afford a broad range of compounds that are mostly rich in carotenoids, fatty alcohol chains, sterols and essential fatty acids (Shaaban et al., 2018). Biological investigations have shown that Euphorbia species possess antitumoral activity (Duarte et al., 2006), antimicrobial activity (Murugan et al., 2007), inhibition of HIV-1 viral infection, and anti-hyperglycemic and hypolipidemic activities (Hezareh, 2005).

P. undulata belong to the family Asteraceae, which includes approximately 80 species that are widely distributed in Europe, Asia and Africa (Boumaraf et al., 2016). The plants of the genus Pulicaria have been used in traditional medicines to treat various ailments such as back pain, inflammation, menstrual cramps, intestinal disorders, dysentery, and diarrhea (Stavri et al., 2008; Liu et al., 2010). These plants possess various bioactivities such as cytotoxic, antipyreptic, antioxidant, antispasmodic, antimicrobial, anti-histaminic, analgesic, hepatoprotective, anti-inflammatory, and nephroprotective effects (Ezoubeiri et al., 2005; Yusufoglu, 2014; Foudah et al., 2015). P. undulata is an aromatic medicinal plant that possesses large varieties of chemical substances, these chemical substances include alkaloids, flavones, flavonoids, sesquiterpenes, lactones, diterpens, triterpenes, napthoquinones, anthocyanin, coumarin, isochafin, isocathecins, and others (Christaki et al., 2012; Chhetri et al., 2015). Although some Pulacarai and Euphobria species have shown antiparasitic activities (Mengiste et al., 2014; Mohamed et al., 2017; Fadel et al., 2018), this is the first investigation of P. undulata and E. retusa against L. major and T. gondii parasites.

MATERIALS AND METHODS

Plant material and preparation of extracts

Collection and processing of the plant materials

Whole plants of E. retusa and P. undulata were collected at December 2017 from Al Qassim region of Saudi Arabia, located in the center of the country (25° 32' 44" N, 43° 20' 40" E). Authenticated by Prof. Gamal E. Elghazali the major taxonomist from the College of Science and Arts in Ar Rass, Qassim University, Saudi Arabia, where the voucher specimens were deposited at the Department of Laboratory Sciences with reference numbers EURE041127111, PUUN041127112 respectively. The plant samples were washed by flushing with tap water dried under shade for 2 days, chopped to smaller pieces with a commercial blender and ground to fine powder using a Foss Tecator Cyclotec 1093 grinding mill. The fine powders were packed in airtight containers and stored at 4°C until ready for extraction (Al Nasr et al., 2019b).

Preparation of the crude extracts

Approximately 200 g powder from the whole plants of E. retusa and P. undulata were soaked in 2 L of analytical grade methanol at room temperature overnight and allowed to stay in a mechanical shaker for 16 h for extract formation followed by 1 h of decantation. The extracts were then filtered using cotton in a normal funnel. The collected filtrates were pooled and evaporated to dryness under vacuum using a rotary vacuum evaporator IKA V 10. For each 500 ml batch from the methanol filtrate, the water bath temperature was set to 50°C, pressure 300 mbar and rotation at 60 rpm. The final sticky sludge of the extracts was recovered using methanol allowed to dry in petri plates in a circulatory hot air oven at 40°C for 6 h. The final form after complete drying was sealed with parafilm and stored in a refrigerator at 4°C until ready for further processing (Al Nasr et al., 2019b).

Phytochemical fractionation of the plant crude extracts

The crude extracts were then suspended in distilled water and partitioned in different solvents on the basis of increasing polarity (using the solvent-solvent system of fractionation) and using a large separating funnel (5 L). The fraction yields for P. undulata methanolic crude included water (1500 mg), chloroform (700 mg), ethyl acetate (500 mg), n-butanol (1500 mg) and petroleum ether (Pet. Ether) (20 mg). For E. retusa, the yield was recorded as water (3500 mg), chloroform (500 mg), ethyl acetate (1000 mg), n-butanol (2000 mg) and Pet. ether (700 mg) (Han et al., 2007).

Maintenance of Parasites and bioassays

Mice and parasite maintenance

Male and female SW and BALB/c mice (6-8) were obtained from pharmaceutical college, King Saud University, Saudi Arabia and maintained in specific-pathogen-free facilities. The research was conducted according to the rules of National Committee Bioethics, the research project was approved by Dean of Scientific Research, Qassim University (number cosoa-bs-2019-2-2-1-5619).

Tachyzoites from the RH strain of T. gondii were obtained from Dr. Saeed El-Ashram (State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, 100193, China). proliferated in SW mice (6-8 weeks age), and cryopreserved in liquid nitrogen at concentrations of 6×10⁶ parasite/ml. Vero cells (ATCC® CCL81™, USA) were cultured in 75 cm² culture flasks in RPMI medium supplemented with 10% FBS (Sigma, Germany) and incubated at 37°C and 5% CO₂. T. gondii tachyzoites were maintained by serial passage in Vero cells grown in RPMI medium (Sigma, Germany) with 2% FBS (Jentsch et al., 2020).

The promastigote of L. major was isolated from a Saudi male patient in February 2016 and maintained at 26°C in Schneider’s Drosophila medium (Invitrogen, USA) supplemented with 10% FBS and antibiotics in a tissue culture flask with weekly transfers. The promastigotes were cryopreserved in liquid nitrogen at a concentration of 3×10⁶ parasite/ml. The virulence of L. major parasites was maintained by

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passage in female BALB/c mice by injecting hind footpads with 1×10⁶ stationary-phase promastigotes. After 8 weeks, *L. major* amastigotes were isolated from the mice. The isolated amastigotes were then transformed to promastigote forms by culturing at 26°C in Schneider’s medium supplemented with 10% FBS and antibiotics. For infection, amastigote-derived promastigotes with less than five passages in vitro were used (Jentzsch et al., 2020).

### Evaluation of the antitoxoplasmal activity of plant extracts/fractions

Serial passage of the Vero cell line was used for the cultivation of *T. gondii* tachyzoites. Vero cells were cultured using complete RPMI 1640 medium with heat-inactivated 10% FBS in a humidified 5% CO₂ atmosphere at 37°C. The 96-well plates (5×10³ cells/well in 200 μL RPMI 1640 medium) were used for the cultivation of the Vero cells and then incubated at 37°C and 5% CO₂ for one day followed by removal of the medium and washing the cells with phosphate buffer saline (PBS). Then, RPMI 1640 medium with 2% FBS containing tachyzoites of *T. gondii* were added at a ratio of 5 (parasites): 1 (Vero cells). After incubation at 37°C and 5% CO₂ for 5 h, the cells were washed with PBS and treated with RPMI 1640 medium containing extracts/fractions (dissolved in DMSO) (100, 33, 11, 3.7, 1.2, 0.4, 0.14 and 0.04 μg mL⁻¹), whereas RPMI 1640 medium containing DMSO (1%) without extracts/fractions or with atovaquone (ATO) (100, 33, 11, 3.7, 1.2, 0.4, 0.14 and 0.04 μg mL⁻¹) were treated as the negative and positive control, respectively.

After incubation at 37°C and 5% CO₂ for 72 h, the cells were stained with 1% toluidine blue after washing with PBS and fixation in 10% formalin. The cells were examined under an inverted photomicroscope to determine the infection index (number of cells infected from 200 cells tested) of *T. gondii*. The following equation was used for the calculation of the observed inhibition:

\[
\text{Inhibition} \% = \frac{(I \text{ Control} - I \text{ Experimental})}{I \text{ Control}} \times 100
\]

where “I Control” refers to the infection index of untreated cells and “I Experimental” refers to the infection index of cells treated with test compounds.

Then, the effects of the test extracts/fractions on parasite growth were expressed as EC₅₀ (concentration that displayed 50% inhibition of parasites) values. The obtained EC₅₀ values resulted from three independent experiments (Jentzsch et al., 2020).

### Activity of the crude extracts/fractions against *L. major* promastigotes

Promastigotes from logarithmic-phase cultures in phenol red-free RPMI 1640 medium with 10% FBS were suspended on 96-well plates to yield 10⁵ cells mL⁻¹ (200 μL/well), and a hemocytometer was used for parasite counting. The plant extract/fraction and reference drug Amphotericin B (AmB) were added to obtain the final concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14 and 0.04 μg mL⁻¹). The negative control wells contained cultures with DMSO without extract/fraction, while AmB was used as a positive control with the same extract concentrations. The plates were incubated at 72 h to evaluate the anti-proliferative effect. The number of viable promastigotes were assessed by the colorimetric method using tetrazolium dye (MTT) and by measuring the reduction of the MTT component into an insoluble formazan product. This colored product is solubilized by adding a detergent solution to lyse the cells. The samples were analyzed by using an ELISA reader at 540 nm. The assay was performed in three independent experiments (Koko et al., 2020).

### Toxicological evaluation of plant extracts/fractions in vitro using the MTT assay

The MTT assay was conducted for cytotoxicity evaluation of extracts/fractions. Briefly, Vero cells were cultured in 96-well plates (5×10³ cells/well/200 μL) for 24 h in RPMI 1640 medium containing 10% FBS and 5% CO₂ at 37°C. Cells were washed with PBS and treated with extracts/fractions for 72 h at varying concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14 and 0.04 μg mL⁻¹) in the medium with 10% FBS. As a negative control, the cells were treated with the medium in 10% FBS. Thereafter, the supernatant was removed and 50 μL of RPMI 1640 containing 14 μL MTT (5 mg mL⁻¹) was added and incubated for 4 h. Afterwards, the supernatant was removed and 200 μL DMSO was added to dissolve the formazan. The FLUOstar OPTIMA spectrophotometer was applied for colorimetric analysis (λ = 540 nm). Cytotoxic effects were expressed by CC₅₀ values (the concentration that displayed a 50% reduction in viable cells). The obtained CC₅₀ values resulted from three independent experiments (Koko et al., 2020).

### Statistical analysis

EC₅₀ and CC₅₀ values were calculated by a linear regression equation using Microsoft excel. The SI (selectivity index) was calculated by dividing CC₅₀ over EC₅₀ for the inhibition result of each parasite. For comparing the differences between control negative means and the means of extracts/fractions student T test was used for the level of significant at P < 0.01.

### RESULTS

**Antitoxoplasmal activity**

Table 1 indicates that the methanol extract of both *P. undulata* and *E. retusa* has good antitoxoplasmal activity with an EC₅₀ of 12.7 and 5.6 μg mL⁻¹, respectively and significantly different (P ≤ 0.01). In the case of their fractions, the chloroform fraction showed the best activities with an EC₅₀...
of 1.4 and 8.7 μg mL⁻¹, respectively significantly difference (P < 0.01). The activities of \textit{P. undulata} fractions increased with the polarity increasing from Pet. ether to chloroform fractions and then decreasing from chloroform to n-butanol by increasing polarity. The fractions of \textit{E. retusa} showed the same behavior, but the activity was not strong in comparison to the activity of \textit{P. undulata}. The chloroform fraction of \textit{P. undulata} showed a very strong SI of 12.1. The reference drug ATO gave EC₅₀ 0.1 μg mL⁻¹ and SI 93.3.

Antileishmanial amastigotes activity
Table 3 also revealed that chloroform fractions are still the most active for both \textit{P. undulata} and \textit{E. retusa} plants against \textit{L. major} amastigotes with an EC₅₀ of 3.8 and 18.5 μg mL⁻¹, respectively significantly difference (P < 0.01). The ethyl acetate fractions of \textit{P. undulata} also revealed antileishmanial activity against \textit{L. major} promastigotes with an EC₅₀ of 4.9 significantly difference (P < 0.01), while all other fractions of \textit{E. retusa} do not have any obvious activities against \textit{L. major} amastigotes. The reference drug AmB revealed EC₅₀ 0.46 μg mL⁻¹ and SI 16.09.

\textbf{DISCUSSION}

This study indicates that \textit{P. undulata} and \textit{E. retusa} methanolic extracts have good antitoxoplasmal activity (their EC₅₀ was 12.7 and 5.6 μg mL⁻¹) from primary screening, especially \textit{E. retusa}, but after fractionation of the extracts the chloroform fraction of \textit{P. undulata} was the best among all examined fractions with an EC₅₀ of 1.4 μg mL⁻¹. This activity started from the Pet. ether fraction (EC₅₀ 7.3 μg mL⁻¹) and then increased

\begin{table}[h]
\centering
\caption{Antitoxoplasmal activity of \textit{P. undulata} and \textit{E. retusa} methanolic extracts and their fractions against the \textit{T. gondii} RH strain in vitro}
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Plant} & \textbf{Crude Extract and Fraction} & \textbf{Yield %} & \textbf{Antitoxoplasmal} & \textbf{CC₅₀ of Vero cells SI = CC₅₀/EC₅₀} \\
 & & & \textbf{EC₅₀ at μg mL⁻¹} & \textbf{at μg mL⁻¹} & \\
\hline
\textit{P. undulata} & Methanol & 5 & 12.7 ± 3.1* & 35.4 ± 6.6 & 2.8 \\
 & Pet. ether & 0.1 & 7.3 ± 2.8* & 27.6 ± 6.4 & 3.8 \\
 & Chloroform & 0.35 & 1.4 ± 0.4* & 17 ± 3.9* & 12.1 \\
 & Ethyl Acetate & 0.2 & 10.8 ± 2.6* & 10.4 ± 2.3* & 1.0 \\
 & n-Butanol & 0.75 & 54.7 ± 11.2 & 33.9 ± 7.5 & 0.6 \\
 & Water & 0.75 & 29.8 ± 5.3 & 6.1 ± 1.7* & 0.2 \\
\hline
\textit{E. retusa} & Methanol & 6 & 5.6 ± 1.9* & 7.6 ± 2.1* & 1.4 \\
 & Pet. ether & 0.35 & 35.1 ± 6.7 & 24.9 ± 5.9 & 0.7 \\
 & Chloroform & 0.1 & 8.7 ± 2.3* & 17.4 ± 4.3* & 2.0 \\
 & Ethyl Acetate & 0.5 & 24.3 ± 4.6 & 29.3 ± 6.3 & 1.2 \\
 & n-Butanol & 1 & 58.9 ± 12.4 & 22.9 ± 5.4 & 0.4 \\
 & Water & 1.5 & 68.3 ± 14.9 & 29.2 ± 6.6 & 0.4 \\
\hline
ATO & & 0.1 ± 0.02 & 9.3 ± 2.08 & 93.3 & 3.3 \\
\hline
\end{tabular}
\footnotetext{* P < 0.01. The results mentioned in mean ± S.D.}
\end{table}

\begin{table}[h]
\centering
\caption{Antileishmanial activity of \textit{P. undulata} and \textit{E. retusa} methanolic extracts and their fractions against \textit{L. major} promastigotes in vitro}
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Plant} & \textbf{Crude Extract and Fraction} & \textbf{Antipromastigote EC₅₀ at μg mL⁻¹} & \textbf{CC₅₀ of Vero cells at μg mL⁻¹} & \textbf{SI = CC₅₀/EC₅₀} \\
 & & \textbf{at μg mL⁻¹} & \textbf{at μg mL⁻¹} & \\
\hline
\textit{P. undulata} & Methanol & 6.3 ± 1.9* & 35.4 ± 6.6 & 5.6 \\
 & Pet. Ether & 7.3 ± 2.7* & 27.6 ± 6.4 & 3.8 \\
 & Chloroform & 3.9 ± 0.8* & 17 ± 3.9* & 4.4 \\
 & Ethyl Acetate & 10.2 ± 2.6* & 10.4 ± 2.3* & 1 \\
 & n-Butanol & 31.6 ± 4.5 & 33.9 ± 7.5 & 1.1 \\
 & Water & > 100 & 6.1 ± 1.7* & – \\
\hline
\textit{E. retusa} & Methanol & 22.0 ± 5.2 & 7.6 ± 2.1* & 0.3 \\
 & Pet. Ether & 82.1 ± 14.0 & 24.9 ± 5.9 & 0.3 \\
 & Chloroform & 11.2 ± 3.7* & 17.4 ± 4.3* & 1.6 \\
 & Ethyl Acetate & 73.6 ± 9.6 & 29.3 ± 6.3 & 0.4 \\
 & n-Butanol & 89.1 ± 16.0 & 22.9 ± 5.4 & 0.3 \\
 & Water & > 100 & 29.2 ± 6.6 & – \\
\hline
AmB & & 0.78 ± 0.09 & 7.4 ± 2.64 & 9.49 \\
\hline
\end{tabular}
\footnotetext{* P < 0.01. The results mentioned in mean ± S.D.}
\end{table}
and reached the peak at chloroform (EC_{50} 1.4 μg mL^{-1}) and then started to decrease in the ethyl acetate fraction (EC_{50} 10.8 μg mL^{-1}). These results indicate the presence of active compound(s) eluted in the chloroform fraction. *P. undulata* is very rich in secondary metabolites, such as alkaloids, groups of terpenoids and flavonoids, all of them can be eluted in the chloroform fraction (Christaki et al., 2012; Chhetri et al., 2015). Previous research found low polar compounds with potent antitoxoplasmal activity such as terpenes (Khalid et al., 2001) or alkaloids (Wei et al., 2012; Maria et al., 2013). The previous research supports and agrees with our results.

The chloroform fraction of *P. undulata* showed the most antileishmanial activity against both *L. major* promastigotes and amastigotes with EC_{50} of 3.9 and 3.8 μg mL^{-1}, respectively. These results indicate the presence of an active ingredient(s) in the chloroform fraction. In a previous study, an active indole alkaloid against *L. amazoniasis* was isolated from the chloroform fraction of *Peschiera australis* (Jan et al., 2001). Much research has indicated the presence of active antileishmanial compounds in the chloroform fraction of methanolic extracts (Krishna et al., 2007). These results also agree and support our findings.

The cytotoxicity evaluation against the Vero cell line indicates the safety of the chloroform fraction against *T. gondii*, *L. major* promastigotes and amastigotes due to SI values of 12.1, 4.4 and 4.5, respectively.

Although the crude methanolic extract of *E. retusa* provided very good activity against *T. gondii* with an EC_{50} of 5.6 μg mL^{-1}, all the other fractions revealed less activity. That may indicate a synergistic active ingredient distributed in different polarities. This type of relationship between fractions and compounds was previously detected (Sharma et al., 2003).

In the present work the investigated reference drugs against both *T. gondii* and *L. major* promastigotes and amastigotes were gave EC_{50} values more strong in comparison to the used all plant extracts and fractions, that is due to their purity, while plant extracts and fractions contain multi number of compounds after isolation of the active ingredients we can expect more strong and potent results, those can be compared directly with the standard reference drugs.

### Table 3. Antileishmanial activity of *P. undulata* and *E. retusa* methanolic extracts and their fractions against *L. major* amastigotes in vitro

<table>
<thead>
<tr>
<th>Plant</th>
<th>Crude Extract and Fraction</th>
<th>Antiamastigote Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Antiamastigote EC_{50} at μg mL^{-1}</td>
</tr>
<tr>
<td><em>P. undulata</em></td>
<td>Methanol</td>
<td>35.4 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>Pet. Ether</td>
<td>25.2 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>3.8 ± 0.7*</td>
</tr>
<tr>
<td></td>
<td>Ethyl Acetate</td>
<td>4.9 ± 1.1*</td>
</tr>
<tr>
<td></td>
<td>n-Butanol</td>
<td>72.6 ± 10.2</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>29.5 ± 5.2</td>
</tr>
<tr>
<td><em>E. retusa</em></td>
<td>Methanol</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Pet. Ether</td>
<td>59.4 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>18.5 ± 4.0*</td>
</tr>
<tr>
<td></td>
<td>Ethyl Acetate</td>
<td>31.1 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>n-Butanol</td>
<td>61.1 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>65.8 ± 8.9</td>
</tr>
<tr>
<td>AmbB</td>
<td>0.46 ± 0.07</td>
<td>7.4 ± 2.64</td>
</tr>
</tbody>
</table>

* P < 0.01. The results mentioned in mean ± S.D.

### CONCLUSION

In conclusion, the chloroform fraction of *P. undulata* is a very good candidate for isolation of antileishmanial and antitoxoplasmal drugs. Therefore, the further phytochemical analysis of active ingredient isolation is highly recommended.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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