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Phytochemical composition and *in vitro* antileishmanial activity of *Bidens pilosa*, *Tithonia diversifolia* and *Sonchus oleraceus* extracts

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Leishmaniasis is the second-largest parasitic killer in the world (after malaria). Currently, there are no effective vaccines or therapeutic interventions thus creating a gap for new alternative remedies in the management of the disease. Medicinal plants offer this attractive option as antileishmanial agents since they are relatively safe, locally available and affordable. The study assessed antileishmanial activity of methanol, hexane, dichloromethane and ethyl acetate extracts, in addition to the aqueous phase of *Bidens pilosa, Tithonia diversifolia* and *Sonchus oleraceus* on *Leishmania major* parasites as well as their phytochemical composition. The phytochemical screening of the plants showed presence of alkaloids, saponins, terpenoids, anthraquinones, glycosides, tannins and flavonoids. Cytotoxicity studies on Vero cells showed a low toxicity with IC_{50} more than 0.12 mg/ml. *L. major* viability was determined at various concentrations of the extracts and standard (Pentostam) using MTT reagent and results displayed as IC_{50}. The extracts from *S. oleraceus, B. pilosa,* and *T. diversifolia* displayed IC_{50} values from 0.03 to 4.89 mg/ml on *Leishmania* parasites. Among the three plants extracts, dichloromethane extract had the highest activity with IC50 0.03 mg/ml. As indicated, all the extracts demonstrated a great potential in inhibiting the growth of *Leishmania* promatigotes with low toxicity making them good candidates for compound isolation and drug manufacture.

Key words: Vero cells, MTT, cytotoxicity, promastigotes, *Sonchus oleraceus, Bidens pilosa, Tithonia diversifolia*

INTRODUCTION

Leishmaniasis refers to a broad spectrum disease brought by intracellular *Leishmania* parasites. The disease is transmitted by bites from infected female *Phlebotominae* sand fly (Mutoro et al., 2018b; De Souza et al., 2016) and results into various types ranging from self-recuperating lesions, muco-cutaneous and fatal

visceral form of the disease (Mutoro et al., 2018a). This parasite has been known to kill thousands and debilitates millions of people each year (De Queiroz et al., 2014). Sadly, it remains one of the most neglected diseases with new cases at seven hundred thousand to one million (700,000- 1,000,000) and twenty thousand to thirty thousand (20,000-30,000) annual deaths for the various forms of the disease (WHO, 2018). According to World Health Organization (WHO)'s report in 2017, Leishmaniasis is found in 88 countries; Latin America, Africa and other regions associated with low income. Majority of the new cases of the disease occurrence are found in South Sudan, Brazil, Sudan Somalia and Kenya. Burza et al. (2018), record that over 50% of the people suffering from the disease in India, Sudan and Brazil are children. According to Al-Hajj et al. (2018), Leishmaniasis remains a neglected tropical disease despite increased disease burden and death cases as there are no effective vaccines. Treatment of the disease has relied on pentavalent antimonials which have various drawbacks majorly the adverse side effects on users (Oryan and Akbari, 2016), high procurement cost (Gutierrez et al., 2016), long periods of administration (Clemente et al., 2018) and development of resistance by the parasites (Ponte-Sucre et al., 2017).

Though many experimental procedures have been investigated for *Leishmania* species, use of natural products remains stand-alone due to variety of chemical constituents present which work synergistically on multiple-target bringing about therapeutic effect (Bastos et al., 2016). This is collaborated by Mutoro et al. (2018b), in which the growth of *Leishmania major* parasites was inhibited by *Plumbago capensis* and *Solanum nigrum* extracts combined. Plants therefore may offer an alternative remedy as they are affordable, less toxic and easily available. The investigations of plants belonging to family of Leguminosae, Rosaceae, Aloaceae, Compositae, Myrtaceae, Barberidaceae, Boraginaceae, Lamiceae, Solaneceae, Juglandaceae, Asteraceae, Fabaceae etc have proven to be effective against Leishmaniasis (Da Silva et al., 2018; Bahmani et al., 2015; Serakta et al., 2013). Kinuthia et al. (2013) reported that *Moringa stenopetala, Callistemon citrinus* and *Allium sativum* plants are effective in elimination of *L. major*. More than twenty- six (26) Asteraceae plant families have demonstrated usefulness in allergies, burns; examples are *Giardia lamblia* and *Leishmania* species (Ivanescu et al., 2015).

The findings by Shamsi et al. (2018) implicated *Matricaria chamomilla, Echinacea purpurea*, *Artemisia sieberi* and *Lavandula spica* to be effective against Leishmaniasis. In Kenya, *Warbugia ugandensis* has been used by healers in management of Leishmaniasis (Ngure et al., 2009). These findings offer hope that natural products with antiparasitic activity could aid as templates for the manufacture of antileishmanial drugs. In the current work, the activity of *B. pilosa, T. diversifolia*

and *S. oleraceus*; methanol, hexane, dichloromethane, ethyl acetate extracts and aqueous phase on *L. major* parasites was investigated. The plant has been used ethnobotanically to treat Leishmaniasis but has not been scientifically rationalized.

MATERIALS AND METHODS

Plant collection and extraction

Fresh aerial parts of *S. oleraceus* (Figure 1a), *T. diversifolia* (Figure 1b) and *B. pilosa* (Figure 1c) were collected from the Egerton University, Botanical garden (0°22'11.0"S, 35°55'58.0"E) with the help of a taxonomist for identification; they were assigned voucher number SK 214, SK 215 and SK 216 respectively . The botanical garden was chosen due to its richness in a wide variety of indigenous plant and its proximity to the laboratory where the analysis was performed. The aerial parts of *S. oleraceus, T. diversifolia* and *B. pilosa* were collected in September 2017 and processed as described by King'ondu et al. (2009).

Briefly, the plant parts were chopped into pieces, dried under a shade for 21 days and finally ground to powder in an electric mill. Approximately, 500 g of powdered plant was soaked in methanol overnight at room temperature. The filtrate obtained through Whatman No.1 filter paper was evaporated in rotary evaporator (BUCHI R-200) at 30-35°C under reduced pressure. The methanol extract was mixed with water and partitioned sequentially with hexane, dichloromethane and ethyl acetate to obtain their respective extracts after evaporation.

Phytochemical analysis

Phytochemical screening for alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids and anthraquinones was done employing the methods of Santhi and Sengottuvel (2016) and Verma et al. (2018).

Preparation of test compounds

Stock solutions of methanol, hexane, dichloromethane, ethyl acetate and aqueous phase of *B. pilosa*, *T. diversifolia* and *S. oleraceus* were made in 5 ml Schneider's *Drosophila* insect medium in readiness for assays. Each stock solution was filtered through 0.22 µm filter flasks in a laminar flow hood. Some plant extracts that did not dissolve in the medium were dissolved in 1% dimethyl Sulfoxide (DMSO) to prevent solvent carry-over. All the prepared plant extracts were stored at 4°C and retrieved later for use.

Cytotoxicity on Vero cells

The cytotoxicity assay on Vero cells was conducted by the 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) (Sigma®) protocol (Wabwoba et al., 2010). The quantity of insoluble formazan obtained from reduction of MTT reagent was proportional to number of viable cells. Vero cells were cultured and maintained in Minimum Essential Medium (MEM) (ATCC® 30-2003™) and supplemented with 10% Fetal bovine serum (FBS). Vero cells were harvested by trypsinization and pooled in 50 ml vials. A hundred microliters (100 µl) of the cell suspension was placed into two wells of rows A-H in a 96- flat-bottomed microtiter plate at a concentration

Figure 1. Images showing the leaves and flowers of a, b, c representing *S. oleraceus*, *T. diversifolia* and *B. pilosa* respectively.

of 1×10^6 cells per ml of the culture medium per well and incubated at 37 °C in 5% CO_2 to facilitate plating. MEM was gently aspirated off and 200 µl of concentration (10 mg/ml) of the extract*s* was placed into two wells before dilutions of 2.0×10^3, 1.0×10^3, 5.0×10^2, 2.5×10^2, 1.25×10^2, and 6.25×10^1 were carried out. The tests were carried out in duplicate. The Vero cells and test extracts were then incubated at 37°C for 48 h in 5% CO_2. Ten micro-litres (10 µl) of MTT was added to each well and further incubated for 3 h until a purple precipitate (formazan) was observed. The medium with MTT was aspirated off and the formazan solubilized with 100 µL of DMSO left to settle for 10 min. The absorbance reading for each well was measured using a micro-titer plate reader at wavelength of 562 nm. The number of viable cells was calculated at each concentration, according to Wang et al. (2006)'s formula: [(A/B) × 100], where A was the absorbance value for treated cells and B was the absorbance value for untreated cells. The extracts were considered cytotoxic if IC_{50} was less than 30 µg/ml ($IC_{50} \leq 30$ µg/ml) and non-cytotoxic if otherwise as stated by Sudha and Masilamani (2012).

Parasite preparation

The *L. major* strain (IDUB/KE/94=NLB-144) was sourced from Institute of Primate Research (IPR), Kenya. The parasites were grown to stationary phase at 25°C in Schneider's *Drosophila* insect medium supplemented with 20% heat inactivated fetal bovine serum (FBS)-HYCLONE[®] USA), 100 µg/ml penicillin and 500 U/ml streptomycin (Hendricks and Wright, 1979), and 250 µg/ml 5-fluorocytosine arabinoside (Kimber et al., 1981). The stationary-phase meta-cyclic stage promastigote were obtained by centrifugation at 1500 × g and 4°C for 15 min. The meta-cyclic promastigote was used for analysis.

Anti-promastigote assays

The *in vitro* tests were done according to Mikus and Steverding (2000), using Alamar Blue bioassay. *L. major* meta-cyclic promastigote at the log phase (1×10^6 parasites per ml) of the culture medium was grown in a flat bottomed 24-well plate at 25°C for 48 h. Aliquots (50 µl) of the promastigotes were transferred into two wells of rows A-H in a 96-flat bottomed microtiter plate and further incubated for 24 h at 27°C. Two hundred microliters (200 µl) of (10 mg/ml) the plant extracts were added before serial dilutions of 10×10^3, 5×10^3, 2.5×10^2, 1.25×10^2, and 6.25×10^1 were done. The control wells contained *L. major* promastigotes in medium whereas the blank wells had the medium alone and positive control contained pentostam (Glaxo Operations (UK) Limited, Barnard Castle, UK) at concentration of 100 ug/ml. Each crude extract was tested in duplicate. After incubation for 24 h, the plates were removed and 10 µl of MTT was added into each well and further incubated for 4 h period. The medium and MTT were aspirated off, then 100 µl of Dimethyl sulfoxide (DMSO) was added and the plates shaken for 5 min. Optical densities readings were measured at 562 nm using micro titer reader. A decrease in fluorescence representing inhibition was deduced as percent of control culture fluorescence. Optical densities reading was used to generate 50% inhibitory concentration (IC_{50}) for the different plants using the Alamar blue bioassay program.

Data analysis

The IC_{50} values were determined using Alamar blue program V2. The data presented for activity were analyzed using SAS system. All the statistical comparisons were done with Ryan –

Table 1. Phytochemical composition of *B. pilosa, T. diversifolia* and *S. oleraceus* extracts.

Variable	Alkaloids	Glycosides	Saponins	Tannin	Flavonoids	Terpenoids	Anthraquinones
B. pilosa MeOH Hexane DCM EtOAc							
Aqueous phase							
	+	+	+	+	+	+	+
	+	-	+	-	-	+	-
	+	+	-	+	+	+	+
	+	+	-	+	+	+	+
	-	-	+	+	+	-	+
T. diversifolia MeOH Hexane DCM EtOAc							
Aqueous phase							
	+	+	+	+	+	+	+
	+	-	+	-	-	+	-
	+	+	-	+	+	+	+
	+	+	-	+	+	+	+
	-	-	+	+	+	-	+
S. oleraceus MeOH Hexane DCM EtOAc							
Aqueous phase							
	+	+	+	+	+	+	+
	+	-	-	-	-	+	-
	+	+	-	+	+	+	+
	+	+	+	+	+	+	+
	-	-	+	+	+	-	+

+ indicates present and – absent: MeOH-methanol, DCM-dichloromethane, EtOAc-ethyl acetate.

Einof-Gabriel-Wels multiple ranges tests and assigned at P≤ 0.05 level of significance.

RESULTS

Phytochemical tests

Phytochemical profiling of *S. oleraceus* (Figure **1**a), *T. diversifolia* (Figure 1b) and *B. pilosa* (Figure 1c) extracts showed that alkaloids, saponins, flavonoids, glycosides, tannins, terpenoids and anthraquinones were present. The tests were based on color change and observation of precipitation and scored as + for presence and – for absence as shown in Table 1.

Antileishmanial activity of plants extracts

The extracts from *S. oleraceus, B. pilosa,* and *T. diversifolia* on *Leishmania* parasites displayed IC_{50} values from 0.03 to 4.89 mg/ml (Table 2). The extracts showed a concentration dependent activity which reciprocated to a high activity (a low IC_{50}) of the extracts. *S. oleraceus*; methanol, hexane, dichloromethane extracts showed significant anti-leishmanial activity (0.04, 0.03, 0.03 and 0.03 mg/ml, respectively) in comparison to ethyl acetate and aqueous phase (2.88 and 4.04 mg/ml, respectively). *B. pilosa*; methanol, dichloromethane and ethyl acetate extracts had significant activity (0.05, 0.03 and 0.08 mg/ml, respectively) when compared to hexane extract and aqueous phase (0.59 and 4.89 mg/ml, respectively). Whereas, *T. diversifolia* methanol, hexane, dichloromethane, ethyl acetate extracts and aqueous phase have significant antileishmanbial activity (0.03, 0.03, 0.03, 0.08 and 0.03 mg/ml, respectively). Across plants extracts, dichloromethane registered the highest activity (0.03 mg/ml). The standard drug, pentostam at 0.1 mg/ml showed an IC_{50} value of 0.00028 mg /ml as shown in Table 2.

Cytotoxic effects of extracts

Extracts from the *S. oleraceus, B. pilosa* and *T. diversifolia* on Vero cells elicited IC_{50} from 0.12-5.38 mg/ml (Table 3). Criteria for scoring the cytotoxic effects on Vero cells was based on IC_{50} values from the National Cancer Institute (NCI) for extracts (Sudha and Masilamani, 2012): IC_{50} ≤ 0.03 mg/ml = cytotoxic, IC_{50} 0.031-1.0 mg/ml = moderately cytotoxic, IC_{50} 1.001-3.0

Table 2. The IC_{50} values representing the extracts activity on *L. major* promastigotes. The initial dose of the extracts was 10 mg/ml while standard drug was 0.1 mg/ml before dilution.

S/N	Antileishmanial activity IC50 (mg/ml)		
	Test extracts	***S. oleraceus***	***B. pilosa***	***T. diversifolia***
1	Methanol	0.04[c]	0.05d	0.03[b]
2	Hexane	0.03[c]	4.89[a]	0.03[b]
3	Dichloromethane	0.03[c]	0.03e	0.03[b]
4	Ethyl acetate	2.88[b]	0.08[c]	0.08[a]
5	Aqueous phase	4.04[a]	0.59[b]	0.03[b]
6	Standard drug, Pentostam	0.00028[d]	0.00028[f]	0.00028[c]

IC_{50} = Inhibitory concentration 50%. Within the column, IC_{50} values sharing letter(s) that are the same are significantly indifferent in activity (P≤ 0.05, Ryan-Einof-Gabriel-Wels Multiple Range test).

mg/ml = mildly cytotoxic, IC_{50} > 3.001 mg/ml = non cytotoxic. *S. oleraceus*: methanol, hexane and dichloromethane showed moderate toxicity (0.53, 0.59 and 0.76 mg/ml, respectively), while ethyl acetate and aqueous phase showed mild toxicity (1.44 and 2.04 mg/ml, respectively). *B. pilosa*; methanol, dichloromethane and ethyl acetate elicited moderate toxicity (0.50, 0.50 and 0.15 mg/ml respectively). While aqueous phase had mild toxicity (1.57 mg/ml) and hexane was non cytotoxic (5.38 mg/ml). Whereas *T. diversifolia*; methanol, hexane and dichloromethane, ethyl acetate and aqueous phase elicited moderate toxicity (0.37, 0.12, 0.29, 0.50 and 0.49 mg/ml respectively). The standard drug, pentostam exhibited IC_{50} values at 0.17 mg/ml (moderately toxic).

DISCUSSION

Phytochemical analysis

Qualitative phytochemical analysis of *B. pilosa, S. oleraceus* and *T. diversifolia* extracts revealed some differences in the constituents tested. The plants contained flavonoids, terpenoids, tannins, saponins, glycosides, alkaloids and anthraquinones. Presence of these phytonutrients could be linked to the activity displayed by the plants. The alkaloids present form the main constituents in herbs and used as remedy for Leishmaniasis (Ashok et al., 2015). For instance, harmalin from *Peganum harmala* has been shown to cure Leishmaniasis by intercalating DNA and interfering with aromatic amino acids metabolism (Di Giorgio et al., 2004; Chan-Bacab et al., 2001). According to Lage et al. (2015), flavonoids have shown significant *in vitro* activity against *Leishmania* parasites by altering the parasite ultrastructure and mitochondria functions. They cause depolarization of membrane potential as well as do not interfere with release of free radicals by macrophages. Moreover, flavonoids and terpenoids from Asteraceae have been reported to poses antileishmanial activity (Sujongya et al., 2018). Whereas the glycoside interferes

with unusual type IB topoisomerase enzyme of *L. donovani* causing *in vitro* and *in vivo* death (Saha et al., 2013). Anthraquinones were present in the extracts and have shown selective activity on *L. major* parasites (Chan-Bacab et al., 2001). The chemical constituents present suggests the importance of these plants and therefore supports basis for ethnobotanical use as well as in chemical synthesis of drugs used in modern medicine.

Cytotoxic effects on Vero cells

The plants displayed mild to moderate cytotoxicity on Vero cells. Generally, an increase in concentration of the extracts resulted in more toxicity on *L. major* parasites thus inhibiting their growth. Although pentavalent antimonials are preferred line for Leishmaniasis treatment, they have major draw backs on users (Monzote, 2009) and the current study confirmed that pentostam exhibited moderate toxicity. *B. pilosa* aqueous phase elicited mild toxicity while methanol, dichloromethane and ethyl acetate extracts showed moderate cytotoxic effects with a low IC_{50} value for activity. The cytotoxic effects of the plant's extracts could be responsible for antileishmanial property (Mans et al., 2016). The findings of Alvarez et al. (1996) demonstrated *B. pilosa* leaves to have significant carcinogenic action on colon cancer (HCT-15). This compliments our study in which the extracts showed moderate toxicity (Table 3). Whereas the hexane extract was non cytotoxic on Vero cells and studies by Shandukani et al. (2018) confirmed the plant to have no effect on cell lines after treatment exposure. *T. diversifolia* extracts had moderate cytotoxicity with high leishmanicidal activity (Low IC_{50} values). Studies by De Toledo et al. (2014) showed that presence of sesquiterpene lactones, major class of compounds present in *T. diversifolia* correlated with the cytotoxic effect on Vero cells. The plant's cytotoxicity has been facilitated by addition reaction of thiol moieties present in the carbonyls of lactone structures (Guillermo et al., 2016;

Table 3. The IC $_{50}$ values showing the effects of *S. oleraceus*, *B. pilosa*, *T. diversifolia* and control on Vero cells. Extracts concentration and control drug initially was 10 mg/ml before serial dilution.

Variables	Cytotoxicity (IC$_{50}$, mg/ml)		
	S. oleraceus	*B. pilosa*	*T. diversifolia*
Methanol	0.53	0.50	0.37
Hexane	0.59	5.38	0.12
Dichloromethane	0.76	0.50	0.29
Ethyl acetate	1.44	0.15	0.50
Aqueous phase	2.04	1.57	0.49
Standard drug;			
Pentostam	0.17	0.17	0.17

IC$_{50}$ = Inhibitory concentration 50%, IC$_{50}$ ≤ 0.03 mg/ml = cytotoxic, IC$_{50}$ 0.031-1.0 mg/ml = moderately cytotoxic, IC$_{50}$ 1.001-3.0 mg/ml = mildly cytotoxic, IC$_{50}$ > 3.001 mg/ml = non cytotoxic (Modified national cancer institute-NCI).

Ivanescu et al., 2015). Therefore, the cytotoxic compounds present could be responsible for *in vitro* antileishmanial activity while causing low Vero cell viability. Zhao et al. (2012) affirmed that most of the compounds derived from *T. diversifolia* were nontoxic to differentiated 3T3-L1 adipocytes at the concentration tested. *S. oleraceus*; methanol, hexane, dichloromethane had moderate toxicity while ethyl acetate extracts and aqueous phase were mildly toxic. Interestingly, among the five extracts of *S. oleraceus*, toxicity reduced as polarity of solvent increased from hexane to aqueous phase. Moreover, studies by Mc Dowell et al. (2011) of *S. oleraceus* extracts on HepG2 cells at 100 mg/ml showed to be non-toxic. This was indicator that *S. oleraceus* had more polar compounds therefore least cytotoxic effects on Vero cells which corresponded to low antileishmanial activity.

Anti-leishmanial activity

Majority of world population depend solely on herbs for their remedies against numerous infectious diseases (WHO, 2017). Therefore, the advent of synthetic drugs led to neglect of natural products as source of antimicrobials (Ezatpour et al., 2015). However, the management of Leishmaniasis using these drugs have posed challenges such as adverse side effects on users, cost, requirement for long periods of administration and parasite resistance. Moreover, there are no effective vaccines available to cure the disease (Al-Hajj et al., 2018). In this work, antileishmanial activities of *B. pilosa*, *T. diversifolia* and *S. oleraceus*; methanol, hexane, dichloromethane, ethyl acetate extracts and aqueous phase was investigated on *L. major*. Consequently, the results showed the plants had significant antileishmanial activity and inhibited the growth of *L. major* parasites. The flavonoids and phenolic compounds present in *B. pilosa* confer the plant with pharmacological activity (Cortés-Rojas et

al., 2013). Interestingly, the plant has been used traditionally to cure illness as pain, diabetes, edema and inflammation (Lima et al., 2011). Other studies conducted have implicated *B. pilosa*, 1-phenyl-hepta-1,3,5-triyne to have antimalarial activity and cancer inhibitor (Kumari et al., 2009). The presence of flavonoids, abundant class of compounds in *B. pilosa* (Lima et al., 2011), promotes formation of cleavable complex as well as induces apoptosis through arrest of G1 phase (Mittra et al., 2000). This in turn inhibits growth of *Leishmania* parasites and subsequent low IC$_{50}$ values.

According to Al-Hajj et al. (2018), flavonoids inhibit DNA topoisomerase I activity of *L. donovani* enzyme responsible for relegation step in parasite growth. In *Leishmania donovani* parasites, presence of flavonoids causes reversal of multi-drug resistance through binding to glycoprotein like transporter and inhibits synthesis of heat shock proteins responsible for evasion of host immunity (Hamarsheh et al., 2017). Therefore, a lower IC$_{50}$ for activity, a better antileishmanial activity for *B. pilosa*; hexane, dichloromethane, ethyl acetate extracts and aqueous phase. According to Makwali et al. (2012), presence of saponins in the *B. pilosa* extracts complemented the activity of the flavonoids by acting on the parasite membrane inducing a drop in membrane potential. Our study was further complemented by Delmas et al. (2000), in which alpha-Hederin and other saponins isolated had antileishmanial activity. Therefore, in contrast to a single therapy, the various bioactive compounds present in the extract led to a synergy (Makwali et al., 2012) as it brought concept of structure-function approach in killing the parasites. Moreover, studies by Mutoro et al. (2018b) demonstrated that combinational approach in treatment reduces toxicity, lowers parasite resistance and optimizes drug efficacies.

T. diversifolia extracts showed activity against *Leishmania* parasites, in which dichloromethane showed strongest antileishmanial activity. The report herein was corroborated by De Toledo et al. (2014), in which

dichloromethane extract from *T. diversifolia* had strong activity against *Leishmania* parasites. Notably, the dichloromethane extract becomes concentrated with large quantities of sesquiterpenes compounds from *T. diversifolia* glandular where they are manufactured and stored during successive partitioning (De Toledo et al., 2014). Additionally, *T. diversifolia* dichloromethane treated promastigotes lost distinct traits such as change in body morphology and loss of motility apparatus. Studies by Cortes-Selva et al. (2005), demonstrated the ability of sesquiterpenes to reverse multidrug resistance in Multi-Drug Resistance *Leishmania tropica* lines.

S. oleraceus has been used by different cultures to avert various diseases such as diarrhea, pneumonia and hepatitis (McDowell et al., 2011). Our study showed activity against *Leishmania* parasites thus validating the use of plant. Both dichloromethane and hexane extract had the highest activity. This could be attributed to polyphenols and flavonoids present (Table 1). Our study is in agreement with Jimoh et al. (2011) who showed that phenols were responsible for pharmacological activity.

On the other hand, activity decreased from ethyl acetate to aqueous phase, an indicator that bioactive compounds in *S. oleraceus* mainly remained in hexane and dichloromethane extracts, thus significant antileishmanial activity. The findings by Jimoh et al. (2011) revealed *S. oleraceus* methanol to have high flavonoid contents compared to aqueous phase. Moreover, the ethyl acetate extract registered a low activity. This could be as a result of low concentration of bioactive constituents. In summary, the activity of the various extracts on *Leishmania* parasites could be linked to the cell biology, where the chemical composition of the cell comprises ether lipids (LPGs, GPI-anchored metalloprotease, gp63, etc) which gives the parasite ability to evade host immunity and virulence (Weingartner, 2012). The availability of chemical constituents in the three plants leads to attachment of the corresponding groups in the parasite cell membrane. This attachment causes disruption of the *Leishmania* cell membrane and leakage of parasite contents leading to eventual death.

Conclusion

The findings of our study demonstrated that *B. pilosa*, *T. diversifolia and S. oleraceus*; methanol, hexane, dichloromethane, ethyl acetate extracts and aqueous phase had antileishmanial activity against *L. major* parasite*s*. Therefore, with these promising results, the plants can be explored as alternative source of drugs for the management of Leishmaniasis as they are easily accessible and safe. Moreover, the results obtained provide basis for scientific rationalization of *B. pilosa*, *T. diversifolia* and *S. oleraceus* for use in pharmaceutical

drug developments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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