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Phytochemical and *in vitro* bioactivity of *Entada africana* Guill & Perott (Fabaceae) stem bark methanol extracts on *Schistosoma haematobium* cercaria

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Abstract

Schistosomiasis, has attained socioeconomic and public health significance, especially in sub-Saharan Africa; ranking second after malaria. Potential resistance to niclosamide, resulted in search for alternative control measures that is cheap and accessible to most rural communities. This study was therefore aimed at screening the bioactive metabolites of Entada africana (E. africana), and larvicidal effects of the stem bark methanol extracts against Bulinus globosus (B. globosus) cercaria. Soxhlet extraction of the crude powder was done using methanol solvent. Qualitative phytochemical screening was carried out using standard procedures. Cercariae were challenged with different concentrations. Dechlorinated water with cercaria was used as control. Rate of mortality, LC50 values and Kaplan-Maier survivorship curve at 60 minutes of post exposure (PE) was assessed. Data were analyzed using GraphPad® Prism version 8.4. Screening revealed the presence of carbohydrates, tannins, saponins, and alkaloids among others. Saponins and carbohydrate was high in the stem bark and leaf extract respectively. At $p \leq 0.05$, there was significant difference in rate of mortality; LSD: 11.52, % interaction = 16.00, 95% CI (-2.65 to 9.06). At 50 minutes of PE, all parasites were dead. The LC50, valued at 46.00±6.93, and 100% mortality across all concentrations was at 90mg/l in 10 minutes of PE. Mortality was concentration and time dependent. Control showed motility throughout 60 minutes of PE. The in vitro evaluation of E. africana stem bark methanol extract was potent against the infective larval stage of S. haematobium, and showed high potential as an anticercarial agent.

Keywords: Cercaricidal, E. africana, Phytochemical S. haematobium Schistosomiasis.

Introduction

Schistosomiasis is a disease that is prevalent in most rural communities of Nigeria and other parts of the world, especially in the tropics and subtropical regions[1,2]. It is known to cause global morbidity and mortality with at least 243 million people that require treatment for *S. haematobium* infection[3]. Generally, over 700 million people are at risk of *Schistosoma* disease with 240 and 120 million people that are infected and asymptomatic respectively; mostly found in poor rural communities that often lack adequate water sanitation[2,4].

Majorly, high rate of infection is common in third world countries, who are the most vulnerable, largely due to ignorance of the disease and its transmission dynamics[2,5]. The disease has devastating effect worldwide with an estimated 3.5 million Disability-Adjusted Life Years (DALYs) as at 2019 for soil-transmitted helminths and schistosomiasis[6]. This often led to poor healthy life expectancy[7]. Six schistosome species (*S. haematobium*, *S. mansoni*, *S. japonicum*, *S. guineensis*, *S. intercalatum*, and *S. mekongi*) are the major cause of schistosomiasis infection[7].

Increase infection relates with the increase demand of domestic water; and expansion rate in dam construction for hydroelectricity and agricultural activities such as dry season irrigation[8].

Challenges associated with control of urinary schistosomiasis, include preference for flavor of stream water, and/or flowing stream water that is said to be "purer" and/or "life-giving," and also serve as opportunity for social interaction during washing; mostly among rural women and children[9,10]. Water sanitation, hygiene and health education – "WASH-related technology," should be part of an integrated control measures for schistosomiasis[11]. Cercaria has a lifespan of about 1-3 days; as such, penetration of hosts' skin has to be within this short period; and infective species tend to congregate at the water surface, which are locations likely to be frequented by potential hosts[12]. In some cases, cercaria could be found in untreated water supplied to swimming pools[8].

Control of schistosomiasis is largely by use of synthetic molluscicides such as niclosamide, or cultural removal of weeds in water-contact sites[13].

The use of synthetic chemical is known to have negative environmental effects, beside its high cost and unaffordability among the local and sometimes endemic communities[8]. Alternative compounds such as salicylanilide have also shown strong cercarial toxicity as well as causing fracturing of anterior extremity and tail joint as well as complete mortality; after a 1-hour period of exposure. At a low concentration of 0.625mg/l, 90% of the cercaria was motionless and often at the bottom of the dish[14].

Therefore, there is need for alternative molluscicides and cercarial control that may cause less or no ecotoxicity and as well, easily degradable with less toxicity effect on non-target organisms[15,16]. Further, plant extracts such as ethyl acetate of *Glinus lotoides* has shown high measure of success by *in vitro* bioassay on both *S. mansoni*, and *S. haematobium*; resulting in less penetration of mice after the cercaria has been expose to sub-lethal dosage of the aqueous extracts[17]. Greater anticercarial activities has also been demonstrated in plants like *Ocimum americanum*, *Bridelia micrantha*, and *Chenopodium ambrosoides* at various degree and exposure time frame, with *O. americanum* hexane extract being the most bioactive against the cercaria[18].

The candidate drug, praziquantel (PZQ) that is use for the treatment of schistosomiasis is expensive and, there is looming resistance against PZQ[19]. There is a persistent challenge to control of the intermediate host and treatment of schistosomiasis. Thus, to justify the ethnobotanical and medicinal uses of *E. africana* as posited by Mvondo et al., Orwa et al., and Daben et al.[20,21,23]; we set to investigate on alternative control measures of *S. haematobium* cercarial infection that are cheap, easily accessible, and with potential of sustainability among the local communities.

Materials and Methods

Study locations: The research was carried out in the Post Graduate Laboratory of the Department of Chemistry, and Science Laboratory Technology, Faculty of Natural Sciences, University of Jos.

Location and collection of plant materials: Plant materials were located and collected at Dangshang and Rom, that is, sites A and B in Mushere-Central, Bokkos Local Government Area, of Plateau State. Geographical coordinates of site A were at $9^0$08'31.66 N and $09^0$04'55.09 E, at an elevation of 2963ft, eye altitude 23.62ft. While that of site B was at Latitude $9^0$08'27.70 N and Longitude $9^0$06'00.75 E, elevation 3298ft, and eye altitude 23.62ft all of Plateau State, Nigeria. The scientific name: *Entada africana* Guill & Perrott is as contained in the works of Yusuf & Abdullahi[21], Orwa et al'[22] and Daben et al.'[23].

Preparation of powdered materials: The powder was prepared from the stem bark of the plant according to El-Sherbini et al.[15]; after being washed thoroughly under clean running tap water, it was chopped into smaller pieces by use of a kitchen knife and shade-dried for about three weeks. These were then pulverized, using mortar and pestle in order to obtain the crude powder; and were further sieved using a wire mesh of 0.5mm sieve sizes. It is worthy of note that, the stem bark powder is known to cause nasal irritation, hence, investigators are advised to put on nose mask.

Soxhlet extraction and filtration of the solvent extract: This was according to Harborne[24], where three hundred grams (300g) of the powdered stem bark sample was put in a thimble. This was placed in a Soxhlet extractor of 1000cm^3 capacity. Seven hundred (700) cm^3 of methanol solvent was poured into the 1000cm^3 quick-fit flask and heated at 64.7^0C by reflux. This process continued for 8 hours until all the active components of the crude plant powder was extracted; indicative by clear appearance of the solvent at the end point of the thimble as it was being siphoned. The extract was then filtered by use of a Suction pump and a Buchman funnel and concentrated by use of a Rotary evaporator (R-205).

Phytochemical Screening: Qualitative phytochemical analysis of E. africana extract was carried out using appropriate methodologies[24]. The test for steroids/terpenes – Salkowski's test was according to Gul et al.[25].

Cercarial Bioactivity: The procedure for parasitological bioassay on *S. haematobium* cercariae was as described by Tucker et al.[26] and Al-Obaidi et al.'[27]. Investigators and workers as a matter of necessity gave due consideration to biohazard safety measures, by wearing of personal protection equipment, which began right from the collection of snail vectors from the wild or as maintained in the laboratory; with particular focus on feeding, hygiene and watching against possible contaminants.

Infected snails collected from the wild were exposed to direct sunlight, or place under an electric bulb 100W depending on the ambient weather condition. Ten (10) snails were placed in 200ml of water in a beaker and exposed under power of an electric bulb (100W) for 2 hours, at room temperature (29± 2^0C). High yields of cercaria were realized when infected snails were kept in the dark for 24 hours, before shedding. This sometimes led to mortality in the snail colony especially over multiple shedding periods, so patent snails were often continually added to the colony, as in the works of Tucker et al.[26].

With a small fish net, snails were removed from the beaker or plastic container and return to their aquarium. Contents were poured out of the beaker through a forty-seven (47-µm-pore-size) filtration screen apparatus in another clean beaker. The apparatus effectively traps snail feces and other debris, while allowing cercaria to pass through it. Freshly harvested cercaria was usually concentrated in a 50ml glass beaker and clearly labeled.

In order to establish the number of cercariae to be used in each concentration, the water containing the cercariae was gently swirls, not creating a vortex. This was to create a near homogenous mix and even distribution of cercaria. Two hundred (200) µl aliquots was pipetted into a counting dish and 2ml drops of iodine solution, added to kill and stain the cercariae. Intact cercariae in the dish were counted under a dissecting microscope. This process was duplicated to establish a near accurate assessment of the number of cercaria to be used; since it is difficult to maintain a homogeneous mix of live cercaria, because of their rapid movement especially when freshly harvested.

***In vitro* bioactivities of *E. africana* methanol extract on *B. globosus* cercaria**: Serial concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100mg/l) of the methanol extracts, were dispensed in small sterilized injection bottles containing about 20cercariae. Use of small injection bottles of about $5cm^3$ was to prevent overflow when a microtiter was used. The cercaricidal bioassays were done in triplicates. While 2ml of cercaria in 2ml of dechlorinated water (0.0mg/l), serve as control, similar to the methodology adopted by Victor et al.[18]. Each concentration was observed under a dissecting microscope for cercarial motility at time points (10, 20, 30, 40, 50, and 60) minutes of post exposure. Immobile or dead cercariae at 10, 20….60 minutes time points were counted and recorded. Death rate was confirmed randomly, by a gentle touch with a tiny pin.

Statistical Analysis: Data were analyzed using GraphPad® prism 8.4 version 2020; and SPSS software version 23.0 to determine the mean mortality of dead cercaria. The level of significance was determined at $p = 0.05$, where $p < 0.05$ was considered significant. Two-way ANOVA was used to determine the significant differences between the various treatment groups and sources of variations in interaction, concentration and residual actions of the extracts in the 60 minutes of PE. Kaplan-Meier survivorship curves by Etikan et al.[28] was used to compare the lapsed time or different time interval that resulted in cercarial mortality, exposed to the various concentrations of the *E. africana* methanolic extracts. Probit regression analysis was calculated by use of SPSS version 23.0, to determine the $LC_{50,}$ and LC_{90} values of the extracts on the test organisms.

Results and Discussion

Qualitative determination of primary and secondary metabolites of leaf and stem bark extracts of *E. africana*. The qualitative phytochemicals screening contained both primary and secondary metabolites in stem bark and leaf extract of *E. africana*. The primary metabolites found included carbohydrates, starch and volatile oils**.** Secondary metabolites were tannins, saponins, steroids/terpenes, alkaloids, flavonoids, cardiac glycoside, anthraquinones, resin, polysterol, phenols. There were high concentrations of saponins and carbohydrate in the stem bark and leaf extracts respectively (Table-1).

Table-1: Constituents of stem bark and leaf extract of *E. Africana*.

Parameter	Stem bark	Leaf
Tannins	+	+
Saponins	+++	++
Steroids/Terpenes	+	-
Alkaloids	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Anthraquinones	+	-
Resin	+	-
Polysterol	+	-
Phenols	+	+
Carbohydrates	++	+++
Starch	++	+
Volatile oils	+	+
Xanthoproteic	-	+

Key: +++ = High, ++ = Moderate, + = Present, - Not detected

Cercarial Bioassays: Methanolic stem bark extracts of *E. africana*, demonstrated cercaricidal bioactivity in a relatively short time of exposure. This is because, all cercaria appear very lethargic under microscopic examination after 20 minutes of post exposure (PE) at 20mg/l in all replicates. Meanwhile, the two (2ml) aliquots of cercaria, placed in dechlorinated water (control), remained active and motile during the 60-minute exposure period.

***In vitro* bioactivities of *E. africana* methanol extract on *B. globosus* cercaria**: In 10 minutes of PE, the LC_{50} was valued at 46.00±6.93. About 90% mortality was recorded at 70mg/l after 10 minutes of exposure; and 100% mortality was at 90mg/l. Again, 100% mortality was feasible in about 30 minutes of exposure across all concentrations at 90-100mg/l. Two-way ANOVA, showed that there was a significant difference ($p \leq 0.0001$); LSD: 11.52, at 95% CI, and 16.00% total variation in the bioactivity of the methanolic extract of *E. africana* concentrations on the *S. haematobium* cercaria. Ranking across the rows of the concentrations of *E. africana* methanol extract at the 60-minute exposure time, revealed that values with the same superscript were not statistically different.

There was no significant variation ($p > 0.05$) in rate of mortality at these concentrations. At 60 minutes of PE, all concentrations resulted in 100% mortality. There was 100% mortality in 10 minutes of PE at 90mg/l – 100mg/l across all concentrations (Table-2).

Table-2: *In vitro* bioactivity of *E. africana* methanol extract on *S. haematobium* cercaria.

Conc. (mg/l)	Time in minutes					
	10	20	30	40	50	60
10	0.00±0.00[a]	23.33±20.82[a]	69.00±3.61[b]	96.00±1.73[b]	99.33±1.16[b]	100.00±0.00[b]
20	20.00±0.00[b]	40.00±17.32[c]	76.00±1.73[ab]	99.33±1.16[b]	100.0±0.00[b]	100.00±0.00[b]
30	31.67+10.41[c]	61.67±23.63[d]	85.33±4.62[b]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
40	**46.00±6.93**[d]	69.33±26.10[d]	95.00±5.00[bc]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
50	61.67±7.64[e]	81.33±22.05[e]	100.0±0.00[c]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
60	76.00±10.59[f]	90.00±17.32[f]	100.0±0.00[c]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
70	85.00±8.66[f]	96.00±6.93[f]	100.0±0.00[c]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
80	97.33±1.16[g]	98.33±2.89[f]	100.0±0.00[c]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
90	100.0±0.00[g]	100.0±0.00[f]	100.0±0.00[c]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
100	100.0±0.00[g]	100.0±0.00[f]	100.0±0.00[c]	100.0±0.00[b]	100.0±0.00[b]	100.0±0.00[b]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
LSD	11.52					

Results are expressed as mean values ± standard deviation from 3 replicates. There is no significant difference between averages with the same superscript. Superscript with different letters a, b, c, d, e, f and g are significantly different at $p < 0.05$ (Sidak' multiple comparison). While ab, bc indicates that true mean value lies between a & b and b & c.

Table-3: Probit analysis of the $LC_{50, 90}$ *in vitro* bioactivity of *E. africana* methanol extracts on cercaria of *S. haematobium*.

Time (minutes)	χ^2 (p >0.05)	LC_{50}	LC_{90}	Confidence limits	
				Lower	Upper
10	5.77	41.68	72.32	4.72	7.89
20	15.58	20.13	65.02	2.50	4.14
30	6.17	8.29	29.76	1.50	3.42
40	1.64	2.88	8.09	-0.25	4.47
50	0.11	3.06	7.62	-2.65	9.06

Probit analysis of the LC_{50} and LC_{90} bioactivity of *E. africana* methanol extracts on *S. haematobium* cercaria: There was no significant difference ($p > 0.05$) in the rate of mortality between the concentrations and period of exposure with the χ^2 values as reflected on Table-3 in 50 minutes of post exposure (PE). No survivorship was noticeable at the 60th minutes, hence the absence of probit regression data at 60 minutes of PE. With a comparable increase in time of exposure to the methanolic extract of *E. africana*, less extract was required to result in 50% and 90% mortality. At 10mg/l, the LC_{50} and LC_{90} were valued at 41.68 and 72.32, respectively, (95% CI = 4.72 to 7.89). Again, at 50mg/l, the LC_{50} and LC_{90} values were at 3.06 and 7.62, respectively, (95% CI = -2.65 to 9.06). With increase in time of exposure, less amount of the *E. africana* methanolic extract was required that would result in 50% and 90% mortality.

Survivorship effect of *E. africana* methanol extract on cercaria of *S. haematobium*: The Kaplan-Meier survival curve (Figure 1) indicated cercarial potency of *E. africana* methanolic stem bark extract, with substantial increase in rate of mortality with different concentrations in the 60 minutes of exposure. The parasites were weakened and appeared sluggish under microscopic examination at lower concentrations of 10mg/l of all the replicates.

Methanolic extract of *E. africana* showed increased mortality with corresponding increase in concentration and time. There was normal motility and viability without significant morphological changes like tail loss in the cercariae for the 60 minutes of post exposure in the control, exposed to dechlorinated water only (0.0 mg/l). There was 50% mortality (LC_{50}) at 40mg/l. At about 50 minutes of exposure, all concentrations resulted in 100% mortality.

Discussion: The qualitative screening revealed the presence of primary and secondary metabolites, such as carbohydrates, starch, resins, tannins, saponins, among others. These bioactive compounds found, are similar to the findings of Yusuf & Abdullahi[21] and Kwaji et al.[29], who worked on antibacterial–antioxidant and phytochemical & pharmacological actions of *Entada africana* respectively. Again, high presence of saponins proves its effectiveness and efficacy for cercaricidal properties, which is in consonance with World Health Organization (WHO) report of the scientific working group on plant molluscicide that molluscicidal plants, usually would have high content of saponins[30]. More so, absence of cyanogenic glycoside because of the release of poisonous cyanic acid as posited by Harborne[24] makes it safe for any potential formulations and/or treatments against cercarial infections or the juvenile and adult stages of the *S. haematobium* parasite; which could complement the continuous use of praziquantel. This in a way, would justify the several ethnomedicinal and ethnopharmacological values of the plant's parts, especially in West Africa; and possible posology of its medicinal values such as, treatment of diarrhoeal diseases and suppression of inflamed cells as posited by some authorities such as Mvondo[20], Yusuf & Abdullahi[21], Orwa et al.[22] and Mu'azu & Usman[32]. Again, high quantity of saponins as discovered in this investigation is common to many indigenous plants of Nigeria[33]; and other parts of the world[25]. This is indicative of the bio-potential value of *E. africana* as cercaricidal and/or antischistosomal agent.

The cercarial bioassay and mortality profile followed a time and dose-dependent mortality pattern. The higher the concentration, the higher the rate of mortality; and the longer the exposure time, the higher the mortality rate. This study's findings are similar to those of He et al.[14], Kiros et al.[17] and Bagalwa et al.[34], who used Solamargine, *Glinus lotoides* fruits, and Salicylanilide, respectively. Even though solamargine and salicylanilide are plant-based synthetic compounds, but are more effective and environmentally friendly than the conventional used of niclosamide, against the snail vectors that curtail the transmission of the schistosomes' parasites. According to Bagalwa et al.[34], exposing the cercaria to 0.01mg/mL concentration, resulted in 100% mortality after 10 minutes. However, the LC_{50} valued at 0.0025mg/mL concentration was considerably lower in 60 minutes of exposure, which relates to the findings of this study. Similarly, Abo-Zeid & Shohayeb[35] used alkaloids, saponins, and volatile oils of *Nigella sativa* against *S. mansoni* cercaria that resulted in 100% mortality in 35 minutes of exposure; this relates closely, to the 30 minutes of exposure of this study. Even though Abo-Zeid&Shohayeb[35] achieved a hundred percent mortality in both extracts in 24 hours of exposure, it opposes the 100% mortality, after 50 minutes of exposure in this investigation, which proves the efficacy of *E. africana* extract as a potential cercaricidal agent. Additionally, Victor et al.[18] equally discovered that methanolic extract had strong and effective anticercarial potency; by use of crude extracts of *Ocimum americanum.* This extract had the strongest cercaricidal activity at a considerably lower concentration of 30µg/mL and at a LT_{50} in 53.85 minutes, comparable to the 2.88mg/l in 40 minutes of exposure of this study.

However, Tekwu et al.[36], who used *Rauwolfia vomitoria* ethanolic stem bark and root extract for a significantly longer period of nearly 2 hours, with LC_{50} valued at 207.4 and 61.18g/mL for the stem bark and root extracts, respectively; were found to be higher and contrary to the findings of this investigation both in terms of exposure period which is 1 hour in this investigation; and LC_{50} and LC_{90}, valued at 3.06 and 7.62 respectively, at 50 minutes of exposure, with none surviving up to the maximum exposure period of 60 minutes. Notwithstanding, there was a considerable decrease in cercarial activity after one hour of exposure; as opposed to the hundred percent mortality of cercaria, in less than 60 minutes of exposure of this study. Furthermore, the weakened stage of cercariae at 20mg/l within 20 minutes of exposure, implies that the cercaria may not be active enough to penetrate the host's skin in 20 minutes of post exposure. As a result, this study discovered a near 100% mortality of *S. haematobium* cercariae after 40 minutes of exposure. This outcome is in consonant to that of Michael et al.[37], who used *Entada leptostachya*, and also observed sluggish cercarial movement and a fifty percent lethal time (LT_{50})at 4.25 minutes, even though at a much higher concentration of 80mg/l, than the findings of this investigation. This showed that methanolic extract of *E. africana* have greater effectiveness and biopotency, particularly in having a LC_{50} valued at 41.68mg/l in 10 minutes of post exposure, compared to the 80mg/l of *E. leptostachya* even though at an earlier time of 4.25 minutes. Related to this, was the work of He et al.[14], who used salicylanilide and reported that at 1.250mg/l, total mortality of *S. mansoni* cercaria was feasible after 60 minutes of exposure, while at 0.625mg/l; over 90% of the cercaria were motionless.

Consequently, the pronounced bioactive effects of *E. africana* methanol extract may be attributed to the presence of saponins and other secondary metabolites. The frothing ability in saponins, may be said to have affected surface tension and cause rapid oxygen depletion, thereby resulting in high cercaricidal mortality within a relatively short time of exposure. This agrees with the positions of Bagalwa et al.[34], and Sadek et al.[38], where they used methanol extract of *Solanum nigrum* & *Callistemon citrinus*; and *Solanum syzybrilifolium* on *S. mansoni* cercaria respectively. Furthermore, the findings supported the view that increased cercarial mortality may be connected to the parasites' tiny sizes of 0.17-0.19mm as reported byAppleton & Miranda[39]; in their work on *Azadirachta indica*. While the use of methanolic extract appears to be gaining popularity over other solvent extracts; Armah et al.[40]on the hand, discovered ethyl acetate fractions to have most bioactive propertyas well as potent cercaricidal property, with a fifty percent inhibition concentration (IC_{50}) valued at 1.53+0.02µg/mL, than methanol and other solvent extracts.

The implications of this scientific effort include the possibility of controlling or eliminating cercaria, the parasite's infective stage, as well as any other developmental stage of the parasite such as, the miracidial stage. Moreover, at about 10 minutes of exposure in this investigation, the extract had already shown more than fifty percent mortality effect on the cercaria at a relatively low concentration of 40mg/l. Based on these promising results, *E. africana* methanolic extract was found to be effective against the cercaria; as the synthetic (niclosamide) molluscicide against the snail vectors, in terms of exposure time. This potent a high chance of plant anticercarial agent, if additional work such as sub acute determination of the extract, particularly on non-target organisms, is carried out; which may be more advantageous than just focusing on the control of the snail intermediate host.

Conclusion

The outcome of this study, showed that methanolic stem bark extract of *E. africana* has high cercaricidal properties against infective stage of *S. haematobium* parasites. This provides potential for anthelminthic efficacy against urinary schistosomiasis. Additionally, it may be a good lead for alternative or additional antischistosomal drug that may target the parasites' juvenile stages of development like the schistosomules; in place of praziquantel, which is only effective against the adult schistosomes. Further investigation can be carried out on isolation and characterization of the *E. africana* stem bark extract. This would help obtain the isolates that can provide information on the bioactive compounds necessary to combat the parasite, possibly at a much lower concentration; which may lead to possible elimination of the cercarial or larval stage of the schistosomes' parasites.

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