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Evaluation of Anti-helminthic Activities of Ethanolic Extracts of *Calotropis procera* and *Morinda lucida* on Adult *Fasciola gigantica*

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Abstract

Mainstay anti-fascioliasis drugs are faced with the threat of drug resistance, hence, the need for new alternative chemotherapy for the management of the disease. The present study was conducted to determine the anthelminthic efficacy of ethanolic leaf extract of *Calotropis procera* and *Morinda lucida* against the adults of *Fasciola gigantica*. The ethanolic extract of air-dried pulverized leaves of *C. procera* and *M. lucida* was tested on matured flukes at different concentrations; 10, 20, 40, 50 and 100 mg/ml. The anti-Fasciola efficacy of *M. lucida* and *C. procera* extract was both time and concentration dependent ($p < 0.05$). The lowest death time (i.e. highest flukicidal activity) 26.67±5.77 min and 40±0.00 min was recorded at 100 mg/ml extract concentration in *C. procera* and *M. lucida* respectively. Generally, the adult flukes' death time decreased with increase in the concentration of the plants' extracts. The LC_{50} and LC_{90} of *C. procera* and *M. lucida* within 110 min exposure time were 4.61 and 10.87 mg/ml, and 24 and 46.8 mg/ml respectively. The study showed that *C. procera* and *M. lucida* have anti-Fasciola activities. The activity of *C. procera*, however, was superior to that of *M. lucida*.

KEYWORDS
Fasciola species, *Morinda lucida*, *Calotropis procera*, Mortality, Lethal concentration.

INTRODUCTION

The zoonotic trematode infection known as fascioliasis is currently a major global public health concern (Mia *et al*., 2021), causing economic losses of over US$3 billion per year due to decreased milk and meat production resulting from a decrease in the population of ruminants (Elliott *et al*., 2015; Olsen *et al*., 2015). Although it is classified as a neglected tropical disease, human fascioliasis is now considered an emerging parasitic disease (WHO, 2021) and poses a significant health problem in numerous countries around the world (Lukambagire *et al*., 2015; Sah *et al*., 2018; Bekana *et al*., 2021; Periago *et al*., 2021). Fascioliasis is caused by two species of macroscopic trematodes with leaf-like shapes: *Fasciola gigantica* (found in the tropics) and *Fasciola hepatica* (found in subtropical regions). These parasites inhabit the liver and bile ducts of ruminant definitive hosts, but in the case of humans, they can serve as accidental hosts. These adult worms are the target of many anti-Fasciola drugs. The two mainstay drugs for the treatment of fascioliasis; albendazole (ABZ) and triclabendazole (TCBZ), are faced with challenges of fluke's resistance. TCBZ is selectively used against *Fasciola* spp. (liver fluke) and *Paragonimus* (lung fluke), and has shown excellent potency against the larval and adult forms of liver fluke (Calpovina *et al*., 1998; Sanabria *et al*., 2013). The activity of ABZ is however, limited to adult flukes. The development of drug-resistant liver fluke is attributed to intensive use of these drugs (Olaechea *et al*., 2011), and hence, new alternatives with potential for greater efficacy are required. The use of plant-based anti-helminths could offer an alternative to fluke control, given the great biodiversity of plants in the ecosystems (Alvarez-Mercado *et al*., 2015). The richness of plants in secondary metabolites offers the possibility of identifying plants with anti-fluke properties as some of these secondary metabolites were shown to possess anti-parasitic properties (Anthony *et al*., 2005). Extracts of the plants, *Lantana camara, Bocconia frutescens, Piper auritum, Artemisia mexicana and Cajanus cajan* showed anti-fluke potential (Alvarez-Mercado *et al*., 2015). The edible mushrooms, *Ganoderma applanatum* and *Cantharellus cibarius* have also been used against the immature form (miracidia) of *Fasciola* spp. (Nwofor *et al*., 2018).

Brimstone tree, also known as *Morinda lucida* Benth and belonging to the Rubiaceae family, is a tropical rainforest plant found in West Africa. It can either grow as an evergreen shrub or a small to medium-sized tree reaching a height of approximately 18-25 meters, characterized by often crooked or gnarled branches protruding from a stem covered with smooth and rough-forming irregular grey-brown patches at the bark. The plant produces white flowers with a narrow glabrous corolla-tube of about 2.5 cm, while its leaves are broad, ovate and tapering to the end, typically ranging in size from 7-15 cm long and 3.5-7.5 cm wide (Fakoya *et al*., 2014; Ayertey, 2016). *Morinda lucida* is a nutrient-rich plant and an abundant source of phytochemical constituents available throughout the year in Southwestern Nigeria. It contains high levels of vitamins A, E, and K, as well as various secondary metabolites such as alkaloids, tannins, saponins, flavonoids, and phenols (Adeleye *et al*., 2018).

Calotropis procera, a member of the Apocynaceae family, is a

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perennial evergreen shrub or small tree with large broad leaves that produce milky latex (Cavalcante *et al*., 2020). It can reach up to 5.4 m in height and is commonly known as Milkweed, Sodom's apple, rubber bush, or rubber tree (Morsy *et al*., 2016; Cavalcante *et al*., 2020). This plant is abundant in tropical regions of Asia and Africa, with Nigeria having widespread distribution, particularly in the northern regions (Akinloye *et al*., 2002). The leaves are broadly ovate-oblong, elliptic or obovate, acuminate, and opposite, decussate, and sub-sessile, with dimensions ranging from 10 to 20 cm in length and 4 to 10 cm in width. Studies suggest that *C. procera* has potential anthelmintic, antimicrobial, anticancer, anticoagulant, analgesic, anti-inflammatory, purgative, and antipyretic properties, and is used in the treatment of various ailments, including leprosy, leucoderma, liver, and abdomen (Jain *et al*., 1996; Bhaskar and Ajay, 2009; Meena *et al*., 2010; Nenaah, 2013).

While much has been reported on the antimalarial or antiplasmodial efficacy of *M. lucida* (Arise *et al*., 2013; Agbedahunsi *et al*., 2016; Afolabi and Abejide, 2020), little is known about its anti-helminthic potential. On the other hand, *C. procera* has been reported to show anti-helminthic properties against the trematode *Gastrothylax indicus* (Aggarwal *et al*., 2016), and the nematode *Hemonchus contortus* (Iqbal *et al*., 2005). To the best of our knowledge, there is currently no information on the anti-fluke potential of *M. lucida* and *C. procera*. So, this study aims to evaluate the potency of *M. lucida* and *C. procera* against adult *Fasciola gigantica in vitro*.

MATERIALS AND METHODS

Fresh leaves of *Calotropis procera* and *Morinda lucida* were collected from the University of Ibadan and Ologuneru area in Ibadan, Nigeria. A taxonomist at the Department of Botany, University of Ibadan identified the leaves, and voucher specimens (UIH-23073 and UIH-23074) were deposited at the herbarium of the same department. The leaves were washed, air-dried at room temperature for 21 days, ground into fine powder, and stored. To obtain the extracts, 80 g of *C. procera* and 140 g of *M. lucida* powdered leaves were macerated in 1 L and 1.5 L of 70% ethanol, respectively, using the cold maceration method as described by Nwofor *et al*. (2018). The mixtures were stirred periodically to ensure complete extraction and then strained through muslin cloth and Whatmann No. 1 filter paper. The extraction process was repeated two times with 70% ethanol, and the resulting extracts were concentrated using a rotary evaporator at 40 °C under reduced pressure. Finally, the extracts were weighed and stored in the refrigerator until their use.

Adult *Fasciola gigantica* were obtained from naturally infected cattle. The live worms were retrieved from freshly slaughtered cattle's infected liver at the central abattoir in Amosun, Ibadan, Oyo State, Nigeria. The collected parasites were then transported in bile to the Parasitology Laboratory, University of Ibadan, Ibadan, Oyo state, Nigeria. Bile obtained from the gall bladder of uninfected cattle was used as inoculating media for the *in vitro* maintenance of adult *F. gigantica*.

Five (5) adult flukes were placed in 10 ml of different concentrations; 10, 20, 40, 50, and 100 mg/ml of *C. procera* and *M. lucida* ethanolic extracts in Petri-dishes. The choice of these concentrations was based on a report on anthelminthic activity of latex of *C. procera* which adopted 5–100 mg/ml as the working concentrations (Shivkar and Kumar, 2003). Albendazole (ABZ) dissolved in bile (5 and 10 mg/ml) served as the positive control, whereas the negative control comprised only bile. Each concentration and control were tested in triplicate. Each set up was observed at a regular interval of 10 min for a period of 2 h (120 min). The viability of adult *Fasciola* was scored using the following scale (based on motility): 0, normal motility; 1, lethargy (reduced activities); 2, not moving (very weak and will only move when pricked with pin); 3, prick no movement but there is peristaltic movement; 4, no movement (Vanda *et al*., 2020). The number of motile and immotile flukes were recorded for each concentration. Immotile flukes were recorded as dead and then the percentage mortality was calculated for each concentration using the formula:
Mortality (%) = [Number of dead flukes / Total number of flukes per treatment group] x 100

Statistical analysis

The statistical software SPSS version 20 (IBM Corp., Armonk, N.Y., USA) was used to analyze the data. Mean values and standard errors were computed from the replicated data, and significance between treatment groups was determined through analysis of variance (ANOVA). Tukey's test was employed to make multiple comparisons between the various treatment groups. A statistically significant result was considered when $P<0.05$. Additionally, the LC_{50} and LC_{90} of the plant extracts were determined using Finney's Probit analysis.

RESULTS

The *in vitro* anti-Fasciola activity of *C. procera* is shown in Table 1. The results showed a gradual increase in percentage of mortality over time and with increase in concentration. A 100% mortality was observed at all concentrations, 10, 20, 40, 50 and 100 mg/ml but at varying exposure time of 120, 100, 90, 60 and 30 min respectively. The lowest percentage mortality (6.67%) was observed in extract concentration 10 mg/ml at 40 min exposure time. There was no mortality in 10, 20 and 40 mg/ml concentrations of *C. procera* at 10, 20 and 30 min. of exposure time respectively. Also, 50 mg/ml extract concentration showed no fluke mortality after 10 and 20 min. of exposure time. The 60 – 100% mortality recorded in 100 mg/ml within 10–50 min. exposure time was significantly higher than other treatments and control groups (positive and negative) ($p < 0.05$). Percentage fluke mortality in the positive control (ABZ) ranged between 20-100% within 60–120 min exposure time in 10 mg/ml ABZ, and 13.33 –46.67% within 100–120 min exposure time in 5 mg/ml ABZ. No mortality was observed in the negative control. The variation in mortality of the adult flukes was both concentration and time-dependent ($p < 0.05$).

The *in vitro* anti-Fasciola activity of *M. lucida* is presented in Table 2. Similarly, the anti-Fasciola efficacy of *M. lucida* extract was time and concentration dependent ($p < 0.05$). A 100% mortality was observed at concentrations 40, 50 and 100 mg/ml at varying exposure time of 120, 70 and 40 min respectively. The lowest percentage mortality (6.67%) was recorded in 10 mg/ml and 100 mg/ml at 110 and 10 min. of exposure time respectively. At 10 and 20 min. of exposure time, only *M. lucida* in 100 mg/ml extract concentration showed mortality of 6.67% and 40 % respectively, there was no death recorded in other concentrations (10, 20, 40 and 50 mg/ml). There was no mortality recorded in 10 mg/ml and 20 mg/ml concentration of *M. lucida* within 10 – 100 min and 10 – 70 min exposure time.

Table 3 presents the mean mortality time of *Fasciola* spp. in treatments and control groups. The mean mortality time reduced with increase in concentration of both extracts. The mean mortality time in adult flukes exposed to 10 mg/ml of ABZ was lower

Table 1. Anti-Fasciola activity of ethanolic leaf extract of *C. procera*

Conc. (mg/ml)	Mean mortality (± S.E) with time (min)											
	10	20	30	40	50	60	70	80	90	100	110	120
10	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	6.67±6.67^{ab}	13.33±6.67^{a}	26.67±17.64^{abc}	33.33±17.64^{ab}	66.67±6.67^{c}	66.67±6.67^{b}	66.67±6.67^{b}	86.67±6.67^{c}	100.00±0.00^{c}
20	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	26.67±13.33^{ab}	33.33±17.64^{ab}	53.33±17.64^{bcd}	66.67±17.64^{bc}	86.67±6.67^{d}	93.33±6.67^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}
40	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	33.33±17.64^{bc}	53.33±17.64^{bc}	66.67±24.04^{cde}	73.33±17.64^{c}	93.33±6.67^{de}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}
50	0.00±0.00^{a}	0.00±0.00^{a}	26.67±13.33^{b}	60.00±11.55^{c}	88.67±6.67^{de}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}
100	60.0±0.00^{b}	86.67±6.67^{b}	100.00±0.00^{c}	100±0.00^{d}	100±0.00^{d}	100±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}
5 (ABZ_1)	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}	0.00±0.00a	0.00±0.00^{a}	0.00±0.00^{a}	13.33±6.67^{a}	33.33±17.64^{b}	46.67±6.67^{b}
10 (ABZ_2)	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}	20.00±11.55^{ab}	26.67±13.33^{a}	40.00±0.00^{b}	66.67±6.67^{b}	80.00±11.55^{b}	100.00±0.00^{c}	100.00±0.00^{c}
Neg. 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}	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}

Note: Similar alphabetic superscripts denote no significant difference while different superscripts denote significant difference. Significant differences were compared across various concentrations and time of adult exposure to *C. procera*. S.E = standard error

Table 2. Anti-Fasciola activity of ethanolic leaf extract of *M. lucida*.

Conc. (mg/ml)	Mean % Mortality (± S.E) with time (min)											
	10	20	30	40	50	60	70	80	90	100	110	120
10	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}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	6.67±6.67^{a}	26.67±6.67^{b}
20	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}	13.33±6.67^{b}	20.00±0.00^{b}	26.67±6.67^{b}	33.33±6.67^{b}	33.33±6.67^{b}	46.67±.67^{c}
40	0.00±0.00^{a}	0.00±0.00^{a}	13.33±6.67^{b}	26.67±13.33^{b}	33.33±6.67^{b}	40.00±11.55^{c}	40.00±11.55^{c}	40.00±11.55^{b}	53.33±6.67^{c}	66.67±6.67^{c}	73.33±6.67^{c}	100.00±0.00^{d}
50	0.00±0.00^{a}	0.00±0.00^{a}	20.00±0.00^{b}	26.67±6.67^{b}	73.33±6.67^{c}	88.67±6.67^{de}	100.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{e}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
100	6.67±6.67^{b}	40.00±11.55^{b}	66.67±6.67^{c}	100.00±0.00^{c}	100±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{e}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
5 (ABZ_1)	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}	0.00±0.00a	0.00±0.00^{a}	0.00±0.00^{a}	13.33±6.67^{a}	33.33±17.64^{b}	46.67±6.67^{c}
10 (ABZ_2)	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}	20.00±11.55^{b}	26.67±13.33^{bc}	40.00±0.00^{b}	66.67±6.67^{d}	80.00±11.55^{c}	100.00±0.00^{d}	100.00±0.00^{d}
Neg. 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}	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}

Note: Similar alphabetic superscripts denote no significant difference while different superscripts denote significant difference. Significant differences were compared across various concentrations and time of adult exposure to *M. lucida*. S.E = standard error.

(106.0±5.77 min) than that of *C. procera* extract (116.67±5.77 min) exposed to similar concentration (p >0.05). The mean mortality time in adult *Fasciola* spp. was significantly higher in 40 mg/ml (110±0.00 min) and 100 mg/ml (40.0±0.00 min) *M. lucida* extract compared with 73.33±15.28 min and 26.67±5.77 min recorded for the same extract concentrations respectively (p <0.05).

The lethal concentrations (LC_{50} and LC_{90}) of *C. procera* and *M. lucida* extracts are presented in Table 4. The lethal concentration reduced with time. The results showed that LC_{50} for *C. procera* extract reduced from 93 mg/ml to 4.61 mg/ml within 10 to 110 min. Similarly, for LC_{90}, the concentration drastically reduced from 132 mg/ml to 11 mg/ml. The LC_{50} and LC_{90} (within 10 min exposure time) was 93 mg/ml and 132 mg/ml in *C. procera* as against 510 mg/ml and 1764 mg/ml in *M. lucida*. The overall mean LC_{50} for *C. procera* was found to be lesser (34.18 mg/ml) than that of *M. lucida* (84.48 mg/ml). The LC_{90} for *C. procera* was also lower (65.41 mg/ml) than that of *M. lucida* (141.39 mg/ml).

DISCUSSION

One of the problems related to helminth infections including fascioliasis is the development of resistance to anthelminthic drugs, bringing about a critical need for the development of new anthelminthic agents. As a result of this set-back in the use anthelminthic drugs, new approaches such as the use of medicinal plants could serve as effective alternative for the control of fascioliasis. Medicinal plants such as *Eugenia uniflora, Psidium guajava, Harpagophytum procumbens, Stryphnodendron adstringens* (Marques *et al*., 2020), *Curcuma aeruginosa* (Vanda *et al*., 2020), *Zingiber officinale* (Ghafar *et al*., 2021), and *Moringa oleifera* (Hegazi *et al*., 2018), have exhibited anti-Fasciola efficacy.

In the present study, the anthelminthic effect of different concentrations of ethanolic leaf extracts of *C. procera* and *M. lucida* were evaluated on the adults of pathogenic liver fluke *Fasciola* spp. Results were compared with the drug, albendazole (ABZ) at concentrations 5 and 10 mg/ml. Our study revealed superior activity of ABZ compared with the ethanolic extract of *M. lucida*, but inferior activity compared with the extract of *C. procera*. The superior anti-Fasciola activity of *C. procera* over *M. lucida* was also supported by its > 2 folds lower LC_{50} compared to that of *M. lucida*. The concentration and time dependency of the treatments on adult flukes' mortality corroborates previous findings (Singh *et al*., 2015; Avinash *et al*., 2017; Hegazi *et al*., 2018; Nwofor *et al*., 2018; Ghafar *et al*., 2021). This study showed that the ethanolic leaf extract of *C. procera* and *M. lucida* exhibited adulticidal activities against adult *Fasciola* and higher concentrations are more effective than lower concentrations. The increase in percentage motility with an increase in the concentration of extracts could be due to a high rate of receptor occupancy i.e. the ability of the extracts at higher concentration to occupy all of the worm's receptors with their active ingredients leading to the possible breakage of flukes' tegument, disruption of the flukes' reproductive organs and villi desquamation as observed by Vanda *et al*. (2020) after treating adult *Fasciola gigantica* with *Curcuma aeruginosa* Roxb extract *in vitro*.

The anthelmintic activities of *C. procera* and *M. lucida* are supported by previous studies (Shivkar and Kamar, 2003; Iqbal *et al*., 2005; Aggarwal and Bagai, 2014; Murti *et al*., 2015; Singh *et al*., 2015; Aggarwal *et al*., 2016; Apenteng *et al*., 2017; Cavalcante *et al*., 2020; Yusuf *et al*., 2021). The anthelminthic activities of *M. lucida* and *C. procera* against adult Indian earthworms (Pheretima posthuma) have been reported (Apenteng *et al*., 2017; Yusuf *et al*., 2021). Aggarwal *et al*. (2016) reported the anthelmintic potential of *C. procera* (flowers) against the trematode *Gastrothylax indicus*. Cavalcante *et al*. (2020) reported the anthelminthic potential of *C. procera* against the parasite *Haemonchus contortus*. There are more reports on the anthelminthic potential of *C.*

Table 3. Mean mortality time of *Fasciola* spp. in treatment and control group.

Treatments	Death time (min) (Mean±SE)				
	10 mg/ml	20 mg/ml	40 mg/ml	50 mg/ml	100 mg/ml
C. procera	116.67±5.77	86.67±15.28	73.33±15.28	56.67±5.77	26.67±5.77
M. lucida	NM	NM	110±0.00	66.67±5.77	40±0.00
ABZ	106±5.77	ND	ND	ND	ND
P-value	0.23	ND	0.01	0.10	0.02

Note: NM – No complete mortality occurred within the 120 min exposure time; ND – not determined. P <0.05 were considered significant. SE- standard error

Table 4. Lethal concentrations of ethanolic extracts of *Calotropis procera* and *Morinda lucida* on adult *Fasciola* spp.

Extract Time (min)	*C. procera*					*M. lucida*				
	LC_{50} (mg/ml)	LC_{90} (mg/ml)	Regression	$\chi 2$	Sig.	LC_{50} (mg/ml)	LC_{90} (mg/ml)	Regression	$\chi 2$	Sig.
10	93	132	y = 8.46x + (-16.66)	1.2	0.75	510	1764	y = 2.38x + (-6.46)	2.24	0.52
20	89	126	y = 8.53x + (-16.64)	1.78	0.62	113.4	195	y = 5.48x + (-11.21)	3.49	0.32
30	67	102	y = 7.07x + (-12.92)	11.35	0.01	78	158	y = 4.20x + (-7.95)	1.23	0.75
40	38	101	y = 3.03x + (-4.78)	31.85	0	54	80	y = 7.48x + (-12.94)	17.34	0.00
50	29	82	y = 2.84x + (-4.15)	15.15	0.02	44	58.6	y = 10.15x + (-16.65)	0.18	0.98
60	19.4	63.5	y = 2.48x + (-3.19)	12.85	0.05	38.5	51	y = 10.32x + (-16.36)	25.45	0
70	15	44.6	y = 2.69x + (-3.16)	28.97	0	34.4	56	y = 6.01x + (-9.23)	52.48	0
80	6.7	23.6	y = 2.34x + (-1.94)	5.5	0.14	33	57.6	y = 5.28x + (-8.01)	59.77	0
90	7.86	17.24	y = 3.93x + (-3.52)	0.61	0.89	29.7	53	y = 5.10x + (-7.51)	42.69	0
100	6.4	16.7	y = 2.98x + (-2.40)	10.18	0.17	26.8	47.5	y = 5.16x + (-7.37)	28.17	0
110	4.61	10.87	y = 3.44x + (-2.28)	1.61	0.66	24	46.8	y = 4.46x + (-6.17)	17.99	0
120	-	-	-	-	-	16	32	y = 4.36x + (-5.28)	26.76	0
Mean	34.18	65.41				83.48	216.63			
S.E.	10.12	13.76				39.55	141.39			

procera than there are for *M. lucida*. Although this study showed that both *C. procera* and *M. lucida* have adulticidal potential, the leaf extract of *C. procera* was found to have a superior adulticidal activity. Similar observation was recorded in the study of Apenteng *et al*. (2017) on *M. lucida* and that of Yusuf *et al*. (2021) and Murti *et al*. (2015) on *C. procera*. The study by Apenteng *et al*. (2017) showed that the ethanolic stem bark extract of *M. lucida* at concentrations 10 and 20 mg/ml was found to have a mean death time of approximately 97 min and 85 min, respectively, while the study carried out by Yusuf *et al*. (2021) showed the ethanolic stem bark extract of *C. procera* at concentrations 10 and 20 mg/ml to have mean death time of approximately 30 min and 25 min, respectively. Murti *et al*. (2015) reported that the ethanolic leaf extract of *C. procera* at concentrations 10 and 20 mg/ml had a death time of approximately 31 min and 14 min respectively. This present study showed the mean death time at concentrations 10 and 20 mg/ml of *C. procera* leaf extract to be 116.67 min and 86.67 min respectively while at that same concentration of *M. lucida* complete mortality was not recorded within 120 min exposure time although paralysis was observed. The discrepancy observed in the mean death time recorded in our study compared to the aforementioned studies could be attributed to the different plants' parts used. Different parts in a plant may produce quite different compounds, in addition to the diverse chemical structures and physicochemical properties of the bioactive phytochemicals (Sarajlija *et al*., 2012). The difference in the test organisms could also be responsible for the differences observed in the plants' efficacy. *Fasciola* spp. possess metabolic teguments which are protective in nature and could confer resistance against drugs, earthworms, on the other hand, lack such features and hence, the recorded increase in efficacy in terms of shorter mortality time.

CONCLUSION

This present study demonstrated a better approach for developing anthelminthic agents in a safe, and cost-effective way by using ethanolic leaf extract of *C. procera* and *M. lucida* which are medicinal plants. The data generated from this present study demonstrated the promising anti-Fasciola activity of the ethanolic extracts of *C. procera* and *M. lucida*. The problem of anthelminthic drug resistance is not new, therefore, results like those presented in this study may help in the development of new anthelminthic agents, thus, making the control of fascioliasis more effective. However, further investigation into the cytotoxic potential of these extracts is necessary to determine the risk they might pose to the host's health and ensure a relatively safe use of these medicinal plants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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