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EVALUATION OF *IN-VITRO* ANTHELMINTIC ACTIVITIES OF METHANOL LEAF EXTRACT OF *JATROPHA CURCAS* LINN ON THE EGG AND LARVAE OF *ASCARIS SUUM*

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Abstract: High rates of infections and re-infections of soil transmitted helminthes (STHs) is a public health challenge perhaps due to resistance to anthelmintics in STHs endemic areas. This public health challenge necessitates renewed efforts to discovering newer agents. This study evaluated the ovicidal and larvicidal activities of methanol leaf extract of *Jatropha curcas* against *Ascaris suum.* The plant's material was collected, pulverized and extracted by cold maceration using 70% methanol, and qualitative analyzed for phytochemicals. Graded concentrations (25, 50 and 100 mg/ml) of the extract were tested for *Ascaris suum* egg's hatch inhibition (EHI) and larvae development inhibition (LDI). Distilled water and 1 mg/ml albendazole served as negative and positive controls, respectively. The extract caused a dose-dependent EHIs of 82.57, 89.44 and 92.08% inhibitions at 25, 50 and 100 mg/ml, respectively. In addition, larvae development was inhibited by 91.72, 95.52 and 98.11% at 25, 50 and 100 mg/ml, respectively. The EHI and LDI of the extract at 100 mg/ml was not significantly different (p<0.05) relative to albendazole that produced EHI and LDI of 94.01 and 99.14%, respectively. The phytochemical detected were alkaloids, glycosides, flavonoids, saponins, steroids, and tannins. *Jatropha curcas* extract demonstrated excellent anthelmintic activity justifying its uses in ethno-medicine.

Keywords: Anthelmintic, *Ascaris suum*, *Jatropha curcas*, phytochemicals

1. Introduction

There had been public outcry at the high rate of infections and re-infections of soil transmitted helminthes (STHs) especially in the rural areas with Pre-school age and school age children being the most vulnerable groups (Rahimi et al., 2022). It is estimated that about 1.5 - 2 billion people were infected with STHs globally (WHO, 2022; WHO, 2020). This staggering high figure is not only disturbing; it poses great concern to global health and the economic status of many Nations. Programs to mitigate the burden in children and other vulnerable groups such as adolescent girls and pregnant women have however been put in place. These efforts include improved environmental and personal hygiene, health

education and advocacy for the use of preventive chemotherapy intervention in areas endemic for the transmission at regular interval (Kache et al., 2020). Sequel to these, various Governmental and Non-governmental Organisations embarked on mass deworming exercises of the vulnerable groups in their respective homes and schools, routinely, using any of albendazole, mebendazole, levamisole and pyrantel pamoate or albendazole/mebendazole combination therapy (WHO, 2017; Yarinbab and Darcha, 2019). Notwithstanding the initiatives for preventive chemotherapy intervention initiatives, soil-transmitted helminth (STH) infection is still a major public health problem especially in developing countries; both the prevalence and the intensity of infections remain high, and the intervention has been insufficient in controlling STH morbidity (Yarinbab and Darcha, 2019; Ame et al., 2022). The target population was also not adequately covered which might probably be due to limited availability of effective drugs. Additional factors militating against their successful treatment are the evolution of reduction in cure rate of the available effective drugs and high rate of re-infections, contributing greatly to the development of resistance by the parasites to the drugs (Ghazanfar et al., 2024; Agne et al., 2021; Aurelie et al., 2024). The imperative to investigate alternative treatment options or novel drugs is therefore not optional but a necessity to avert the possible unpleasant consequences of their infestation which are often associated with the disorders such as malnutrition, stunted growth in children coupled with intellectual impairment, cognitive deficits and diminished school attendance, while parasitized pregnant women are anaemic and their newborn have low birth weight (Nisa et al., 2022; Ojo et al., 2023).

Plants have commonly been considered to be possible alternative and important source of new drugs as large number of secondary metabolites that may serve as reservoir for novel drugs and therapeutic agents are continuously being discovered and extracted from them (Kalpesh and Priya, 2020). Apart from this, a good proportion of the population across the globe still adopt traditional methods of treatment, using herbal extracts, which have been claimed to produce beneficial responses especially in the developing nations (Amar and Arun, 2020). These have resulted in renewed interest and efforts toward screening medicinal herbal plants that are used locally as remedies for many diseases including helminthic infections (Hassen et al., 2022).

Four species of nematodes are collectively referred to as Soil transmitted Helminthic (STH): *Ascaris lumbricoides* (round worm), *Trichuris trichiura* (whipworm), and *Necator americanus* or *Ancylostoma duodenale* (hookworms) (Sumo et al., 2021). Amongst the STH infections ascariasis caused by *A. lumbriciodes* is the most prevalence (Addisu and Mebrate et al., 2020), infecting an estimated one sixth of human population globally, with the highest rate of infection occurring in Sub-Saharan Africa, America, China and East Asia (WHO, 2022). Gonzalez Quiroz et al. (2020) in their study to determine the prevalence and intensity of STH infections, nutritional status, and anemia in children, using multistage stratified probability sampling also reported that *A. lumbricoides* and *T. trichiura* were responsible for most of the infections.

A. lumbricoides and *A. suum* which infect human and pigs respectively are two parasitic nematodes which are closely related and bear significant morphological similarities. In a molecular and morphological study of *A. suum* in a human - pig contact scenario in north-eastern Brazil, concomitant infection has been demonstrated in human and pig, suggesting zoonotic transmission cycles (Bacelar et al., 2023). The analysis of research carried out on

pig farmers in Bali province, Indonesia using a molecular testing showed that ascariasis in human was also caused by *A. suum* indicating that *A. suum* was a zoonosis (Augustina et al., 2023). A comparison of the protein profile of their body wall and reproductive organs manifested considerable similarities in banding pattern which reflect their close genetic relationship (Alba et al., 2009). Additional experimental studies suggested the possibility of cross-infection, cross-breeding and hybridization between them (Silva et al., 2021; Easton et al., 2020). An *A. suum* was removed from a 75 years old Patient with a small enterotomy and identified based on morphological and molecular analysis, suggesting that *A. suum* can function as a relevant agent of human zoonosis. The pig ascaris was therefore suggested to be important source of human ascariasis in endemic area where both human and pig live closely (Romano et al., 2021). It is therefore imperative that any effort toward the total control and eradication of ascariasis in human must not ignore other factors such as the presence of pigs in such environments. The observed evidences of strong relationship and similarities have thus strengthened the justification of the routine usage of *A. suum* as experimental research surrogate for human form, *A. lumbricoides* (Deslyper et al., 2021).

Jatropha curcas (**Fig. 1**), commonly called physic nut or purging nut, belonging to the family of Euphorbaceae is a small branched tree that is widely. It is characterised by features such as succulent smooth grey bark which when cut or its leaf plucked, exudes whitish watery latex which causes brown stains that are difficult to remove. The latex dries on hard surface to form sticky paste. It is a drought resistant perennial plant. However, it sheds most of its leaves during dry season. When fully grown, it usually attains a height of 3 to 5 meters but may exceed this under optimal soil and climatic conditions (Kamal et al., 2011; Namrata et al., 2023). Although its natural habitat is reported to be South and Central America, South-east Asia, Africa, and India, the plant is currently distributed widely across the tropical regions (Riayatsyah et al., 2021). In Nigeria, it is cultivated in all regions, with greater prevalence in the central area of the country. It is called *Binidazugu* in Hausa, *Lapalapa funfun* in Yoruba, (Adebusuyi et al., 2021) *Olulu idu* in Ibo, *Gyedan* in Tiv, it is termed *Ochigbede* in Idoma and known as *Omangba* in Igede. The root, stem-bark, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa and the world for the treatment of diverse diseases (Igbinosa et al., 2011).

Fig. 1. *Jatropha curcas* in its natural habitat taken at Area C staff residential Quarters of the Samaru campus, Ahmadu Bello University Zaria, Nigeria

The plant had been screened for its anti-inflammatory and antimicrobial activities. The anthelmintic activity of the plant was documented by Iwu (1993) and Sarabia et al. (2022). These folkloric claims of the plant's uses, to the best of our knowledge had not been validated scientifically. Therefore, the aim of this study was to evaluate the *in-vitro* anthelmintic properties of the methanol leaf extract of *J. curcas* on the eggs and larvae of *A. suum*.

2. Materials and methods

2.1. Chemicals

Studies Methanol, (Sigma Aldrich, USA), Potassium hydroxide, sulphuric acid, and albendazole used were of analytical grade. The extract and reagents were freshly prepared prior to each test by dissolving a known weight of each of them in a measured volume of distilled water. The required concentration was prepared by serial dilution.

2.2. Collection and authentication of plant materials

Jatropha curcas plant was collected from Area C staff residential Quarters of the Samaru campus, Ahmadu Bello University Zaria, Nigeria located on latitudes 11°15′ N -11°3′ N and longitudes 7°30′ E -7°45′ E and authenticated at Herbarium unit of Department of Botany, Ahmadu Bello University Zaria where the voucher number 1911 was assigned

2.3. Extraction of plants materials

The plant leaves were collected, cleaned, air-dried to constant weight and pulverized to a coarse powder using mortar and pestle. Two hundred and fifty grams (250 g) of powdered leaf was macerated in 2 litres of 70% methanol for 72 hours. The mixture was filtered using Whatman's No. 1 filter paper. The filtrate was concentrated to dryness by heating over a water bath at 50℃ to give solvent free extract used for the study.

2.4. Phytochemical analysis of the extracts

Phytochemical screening was carried out using standard tests of Trease and Evans (2002) for qualitative phytochemical constituents including alkaloids, cyanogenic glycosides, flavonoids, saponins and steroids.

2.5. Collection and authentication of the worms *Ascaris suum*

Live adult female *A. suum* was collected from the intestine of local pig which was slaughtered in piggery slaughter slab in Sabon Gari Zaria. The intestine containing the worms was collected in a beaker and taken to Helminthology laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, where it was opened and *A. suum* isolated. The worm was authenticated.

2.6. Removal and preparation of *Ascaris suum* eggs

The extraction of *A. suum* eggs from the worm for the investigation was conducted with the method outlined by Coles et al. (1992) and Suleiman et al. (2014). The worm collected was gently crushed in mortar, transferred into a beaker containing 0.5 M potassium hydroxide (KOH) solution and filtered after 30 minutes to obtain the eggs. The filtrate was centrifuged at 1,500 rpm for 3 minutes and supernatant decanted to recover the eggs. Distilled water was added to the egg sample, mixed, centrifuged and the water at the surface

decanted. The procedure was repeated three times. The eggs sample was further washed by centrifuging with embryonating fluid (0.1 M sulphuric acid) for another three times. A 0.1 ml of the egg sample was smeared on microscopic slide and observed under light microscope at x 40 magnification to obtain the egg counts. The number of eggs in 0.1 ml sample was used to estimate the eggs in the remaining egg sample and diluted with distilled water such that 0.2 ml of the sample contained 100 eggs.

2.7. *In vitro* egg hatch inhibition (EHI) assay

The protocols established by the World Association for the Advancement of Veterinary Parasitology (WAAVP) as documented by Coles et al. (1992) and Iqbal et al. (2004) was adopted for the *in vitro* egg hatch inhibition (EHI) assay. Hundred (100) freshly collected *A. suum* eggs contained in 0.2 ml of the egg sample were distributed in each of a flat-bottomed micro titration well and designated as groups 1, 2, 3, 4 and 5. The same volume, 0.2 ml of each concentration of the extract (25, 50 and 100 mg/ml) was added to mix with the eggs in well group 2, 3 and 4 respectively, while 0.2 ml each of the negative control, distilled water (DW) and 1 mg/ml albendazole (Alb), the positive control were added to eggs in well group 1 and group 5, respectively. The plates were incubated at 27 ℃ for 18 days for egg hatching. The embryonating fluid was intermittently dropped on to the culture to avoid desiccation. On the 19th day, a drop of 10% lugol's iodine solution was added to the culture to stop eggs from further hatching. All the unhatched eggs and the first stage larvae within the egg shell were counted in all the cultured well of the different treatment to assess the hatch inhibitory effects. The percentage inhibition of egg hatch was calculated for each concentration using the formulae (1) described by Suteky and Dwatmadji (2011) and Suleiman et al. (2014) as:

$$\% \text{ inhibition} = 100 \left(1 - \frac{X1}{X2}\right) \quad (1)$$

Where X1 = number of hatched eggs in extract and positive control micro titration well, X2 = number of hatched eggs in negative control well. The experiment was replicated 6 times.

2.8. *In vitro* larval development inhibition (LDI) assay

The technique described by Assis et al., (2003) was employed. Larvae obtained after 18 days of egg incubation were washed three times with distilled water. Subsequently, each well of a flat-bottom microtitration plate, labelled 1 through 5, received 0.2 ml of a distilled water sample containing 100 larvae. One hundred (100) µl of lyophilized penicillin-streptomycin was added to each well to control fungal growth (Okoli et al., 2016). Twenty (20) µl of nutritive medium of Earle's balance salt solution was added into each well (Igbal et al., 2004) after 48 hours of incubation at 27℃ to provide nutrients to the larvae. A volume of 0.2 ml of each of the graded concentrations (25, 50 and 100 mg/ml) of the extract were added to larvae in wells 2, 3 and 4 respectively, 24 hours after the addition of the nutrient medium. The negative control treatment (well 1) and positive control (well 5) were similarly mixed with 0.2 ml of distilled water and 1 mg/ml albendazole respectively. After seven days of incubation, one drop of 10% Lugol's iodine solution was added to each well. The larvae were categorised into distinct developmental stages (L1, L2, and L3), and the L3 larvae were enumerated. Data was presented as percentage inhibition of L3 using the formula (2) of Cavier (1973) as:

Percentage Inhibition = $\frac{N-n}{N}$ x 100% (2)

Where N = number of L3 in negative control well, n = number of L3 in extract and positive wells. The experiment was replicated 6 times.

2.9. Data analysis

Data were expressed as Mean ± standard Error of Mean and percentages where applicable. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test for multiple comparisons. The value $p< 0.05$ were considered significant.

3. Results

3.1. Phytochemical screening of the methanol leaf extract

Methanol extract of *J. curcas* tested positive for the presence of alkaloids, carbohydrates, cyanogenic glycosides, flavonoids, saponins, steroids, and tannins after a qualitative phytochemical screening (**Table 1**). Micrograph pictures representative of observations made in a larvae development inhibition assay carried out with or without methanol extract of *J. curcas* at 50 mg/ml or 100 mg/ml are presented in **figure 2**. The development of *A. suum* larvae, **figure 2 B and C**, show inhibited larvae or un-larvated egg with abnormal or distorted content with more significant inhibition at 100 mg/ml. However, a full larva emerges from untreated control as shown in **figure 2 D**.

3.2. *In vitro* egg hatch inhibition of methanol leaf extract of *Jatropha curcas* on *Ascaris suum*

The result showed that the extract exhibited concentration dependent egg hatch inhibition (EHI) of 82.57 and 89.44% at the doses of 25 and 50 mg/ml, respectively. Albendazole, the standard drug used as positive control 100 mg/ml of the extract showed percentage EHI of 94.01 and 92.08%, respectively (**Table 2**).

3.3. Percentage inhibition of larvae development by methanol leaf extract of *Jatropha curcas* against *Ascaris suum*

The result showed that the extract exhibited concentration dependent larvae development inhibition (LDI) of 91.72 and 95.52% at 25 and 50 mg/ml, respectively. At 100 mg/ml, the LDI of the extract was 98.11% and statistically similar to 99.14% produced by albendazole, the standard drug used as positive control as shown in **table 3**.

Table 1. Preliminary phytochemical screening of the methanol leaf extract of *Jatropha curcas*

Phytochemical group	Observation
Alkaloids	+
Carbohydrates	+
Cyanogenic glycosides	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+

+ = present

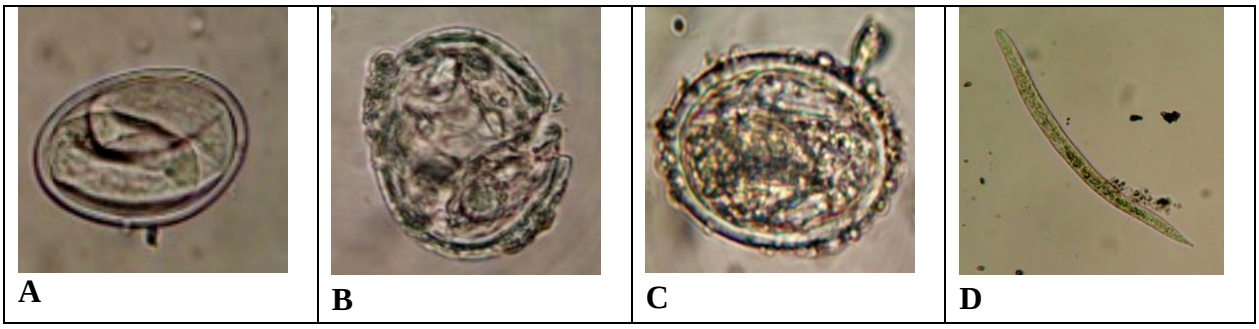

Fig. 2. Microphotograph of larvae development inhibition assay of *Ascaris suum* larvae (Magnification = *100): **A.** Freely developing larval stage of *A. suum* egg after 18 days incubation; **B.** Inhibited *A. suum* egg that failed to larvate after treatment with 50 mg/ml methanol leaf extract of *Jatropha curcas*; **C.** Inhibited *A. suum* egg that failed to larvate after treatment with 50 mg/ml methanol leaf extract of *J. curcas*; **D.** Fully larvated *A. suum* egg in the control treated with distilled water.

Table 2. *In vitro* egg hatch percentage inhibition of methanol leaf extract of *Jatropha curcas* on *Ascaris suum*

Concentration (mg/ml)	Means ± SEM	Percentage Inhibition (%)
DW(0.2 ml)	94.67 ± 0.62	0
25	16.50 ± 1.06[a,b]	82.57
50	10.00 ± 0.58[a,b]	89.44
100	7.50 ± 0.43[a]	92.08
Alb	5.67± 0.67[a]	94.01

Data presented as mean ± SEM; analysed using one way analysis of variance (ANOVA) followed by Bonferroni *post hoc test*; n = 6; [a] and [b] = significantly difference at $p < 0.05$ from DW and Alb. Respectively; DW = distilled water; Alb. = albendazole

Table 3. *In vitro* larvae development inhibition of methanol leaf extract of *Jatropha curcas* on *Ascaris suum* larvae

Concentration (mg/ml)	Means ± SEM	Percentage Inhibition (%)
DW	96.67 ± 0.42	0
25	8.00 ± 0.45[a,b]	91.72
50	4.33 ± 0.43[a,b]	95.52
100	1.83 ± 0.54[a]	98.11
Alb	0.83± 0.31[a]	99.14

Data presented as mean ± SEM; Statistical analysis was carried out using one way analysis of variance (ANOVA), followed by Bonferroni post *hoc test*; n = 6; [a] and [b] = significantly difference at $p< 0.05$ from DW and Alb. Respectively; DW = distilled water; Alb. = albendazole

4. Discussions

The selection of the plant for the study was based on the reported folkloric use of the plant as an anthelmintic (Trease and Evans, 2002; Suleiman et al., 2014). The challenges posed by limited availability of synthetic anthemintic agents, the decrease in cure rate, the emerging resistance of helminthes to treatment and high cost of synthetic anthelmintic agents have necessitated the desires to research into alternate means, including the herbal plants to

supplement the exclusive use of available synthetic anthelmintic agents (Ahmed et al., 2023).

The study investigated *in vitro* anthelmintic effects of *J. curcas* against *A. suum* using the conventional drug, albendazole as standard. The *in vitro* screening of potential anthelmintic agents prior to their *in-vivo* testing has been shown to be rational and cost effective strategy since this minimizes the number of experimental animals necessary for development of new therapeutic agents (Belga et al., 2024).

The result of the investigation in which the extract produced statistically significant ($p< 0.05$) dose-dependent egg hatch inhibition (EHI) and larva development inhibition (LDI) activities indicated that the plant posses anthelmintic activities. The EHI of 82.57 and 89.44 at 25 and 50 mg/ml respectively indicated that at lower concentrations the plant has moderate activities while the LDI at all concentrations and EHI at 100 mg/ml had satisfies the recommendation of good and effective anthelmintic agents (Igbal et al., 2004; Suteky, 2011). Albendazole which is the standard drug showed higher anthelmintic activity, but not significantly different ($p<0.05$) compared to the extract at the highest test concentration of 100 mg/ml. The difference in activity profiles of albendazole and the extract is suggested to be due to the higher state of purity of albendazole relative to the extract.

The mechanism of anthelmintic activities of *J. curcas* extract is still not fully understood, however, previous studies have suggested that the mechanism of inhibition of egg hatching and larval development of different parasite were related to the inhibition of cell division and/or the formation and development of vital structures of the parasite (Gallardo et al., 1998). One of the mechanisms of uptake of the extract could also be by diffusion through the worm's eggshell or the cuticles of larvae (Hassen et al., 2022).

J. curcas extract has higher LDI compared to EHI and this could probably be due the greater exposure of larvae to the component of the extract than the eggs. The eggs have four protective layers of eggshell which provided it with supplementary mechanical strength (Wharton, 1980) which confers additional resistance on the eggs compared to larvae in which these extra protections were absent. This further suggested that the extract might have acted by diffusion through the egg shell and cuticle of the larvae. The preliminary phytochemical screening of the plant extracts used for this study which revealed the presence of tannins, alkaloids, glycosides and flavonoids supported the findings of Igbinosa et al. (2011). The anthelmintic activity of the extracts might be due to presence of these secondary metabolites, tannins, flavonoids and other bioactive compounds present in the extract (Tchetan et al., 2022; Tulasi et al., 2020).

Tannins were suggested to have interfered with energy generation of worms by uncoupling oxidative phosphorylation or binds to the free protein of the gastrointestinal tract of the worms which lead to their death (Mute, 2009). On the other hand, alkaloids were also suggested to be responsible for the anthelmintic activity of herbal plants (Kalpesh and Priya, 2020; Tirkey, 2019). Consequently, the anthelmintic properties of methanol leaf extract of *J. curcas* may potentially be attributed to the presence of these secondary metabolites, which could have interacted synergistically.

Conclusions

Methanol leaf extract of *Jatropha curcas* demonstrated anthelmintic activity against *Ascaris suum* comparable to albendazole, the standard drug. This work therefore validates the folkloric claims of anthelmintic properties

of the plant as the extract has shown excellent ovicidal and larvicidal activities against *A. suum* which was used as surrogate for human ascaris. The study therefore suggests that ***J. curcas*** could be a potential source of novel anthelmintic agent.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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