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In vitro Biological Activity Testing of *Vitex doniana* Bark Extract and *virosa* Root Extract against *Taenia solium* Metacestodes

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ABSTRACT

Taenia solium, the pork tapeworm, is a foodborne parasite of global concern it affects more than fifty million people globally, with more than 80% of these being in the developing world. Infection of the brain with larval stages (metacestodes) leads to neurocysticercosis, a leading cause of epilepsy in endemic areas of the world where pig rearing is common. People in pig-rearing communities rely on local herbs for their primary health care. Exploring the use of these herbs as anthelminthics will give insights into the biological activity of locally available herbs and open up avenues for more research on local herbs. The objective of this study was to determine the in-vitro anthelminthic activity of locally used anti-parasitic herbs namely; *Vitex doniana* bark extract and *Flueggea virosa* root extract against *T. solium* metacestodes.

An experimental in-vitro study using intact *T. solium* metacestodes with full bladder walls was conducted to check for cysticidal activity of the locally available herbs of *V. doniana* bark extract and *F. virosa* outer root cover extract. Non-evagination of metacestodes at incubation, post-treatment was used as an indicator of the activity of the extract against the metacestode. Treatment with praziquantel was used as a positive control. The herbs (*V. doniana* and F. virosa) and drug extracts (Praziqauntel) were tested in five different concentrations.

Vitex doniana at concentration of 50µg/ml resulted in evagination of 64%, while *F. virosa* had 52% at the same concentration. *Vitex doniana* and *F. virosa* showed a dose dependent response, in that the higher the dose the higher the biological activity. This study showed an association between *T. solium* metacestodes response to *V. doniana* and praziquantel (Chi square = 29.63 versus tabulated value 3.84) indicating that there was an association between exposure of metacestodes to the treatment of *V. doniana* and Praziquantel. The same was observed with *F. virosa* and praziquantel (Chi square = 42.28).

Vitex doniana showed more biological activity than F. virosa, but both had at least biological activities above 50%. This shows that both herbs have potential to be anthelmintics against *T. solium* metacestodes and are recommended to be explored further in animal models for activity against the adult tapeworm.

KEYWORDS: *Taenia solium, Flueggea virosa, in-vitro, Vitex doniana, and biological activity*

INTRODUCTION

Taenia solium, the pork tapeworm, is a foodborne parasite of great global concern [1]. *Taenia solium* larva stage is the leading cause of preventable epilepsy in the world responsible for 30% of all epilepsy cases [2]. *Taenia solium* causes great public health and economic impact with 503,000 Disability Adjusted Life Years (DALYS) lost due to cysticercosis [3].

These factors show that there is an urgent need to control, eliminate and, if possible, eradicate this disease complex which was also recognised and re-confirmed by the World Health Assembly with the adoption of resolution WHA66.12 on May 23, 2013 [4]. In many developing countries, people are still dependent on various herbal treatments to cure worm infections [5]. For example, in Asia, Africa, and some parts of Latin America, the herbal

medicines, traditional treatments, and traditional practitioners constitute the main source of health care to treat various common ailments including intestinal worm infections[5]. Thus, these herbal medicines hold a great scope for not only new drug discoveries against parasitic diseases but also for further exploration for scientific evidence regarding the treatment and control of intestinal helminthiasis [5].

Local herbal medicines have been used in Zambia over generations by rural communities to treat different ailments including parasitic diseases [6]. *Vitex doniana* (Chikamba) bark powder and *Flueggea virosa* (Mulizya kalumbu) roots have been used to treat parasitic infections and are readily available to rural communities [7, 8]. *Vitex doniana* is an ethnomedicine with multiple uses [9, 10]. Its root extract is known to be antitrypanosomal and Anti-Plasmodial. [11]. In Southern Benin, *Vitex doniana* bark has been used for treatment of sterility, haemorrhoids, dysentery, upset liver, cough, diarrhoea and dermatosis [12]. The bark extract is also used to treat liver diseases and control bleeding after child birth in Kenya [13] and it is known to have anti-diarrhoeal properties [14]. *Vitex doniana* bark powder has been reportedly used for the treatment of internal parasites by the indigenous people of North Western province in Zambia (personal communication). *Flueggea virosa* is also another widely used ethnomedicine [15]. It is known to be trypanocidal and antimalarial [16]. It is also known to have anti-HIV activity [17]. Extracts from *Flueggea virosa* have anti-Hepatitis C activity [18].

The epidermis of the root of *Flueggea virosa* has also been reportedly widely used by the indigenous people of the Southern Province of Zambia to treat intestinal ailments. (personal communication), The purpose of this study was to investigate the in-vitro biological activity of these two herbal remedies against *T. solium* metacestodes in vitro in order to determine their usability as anthelmintics against the pork tapeworm.

MATERIALS AND METHODS

Study Design

The study was a laboratory based-experimental study conducted at the School of Veterinary Medicine of the University of Zambia.

Study Site

The herbs and the pig were prepared for the experiment at the School of Veterinary Medicine, University of Zambia where the laboratory based-experimental study was done.

Source of Herbs

Flueggea virosa was collected from the Rusangu area near Rusangu dam in Monze district of Southern province in Zambia, while *Vitex doniana* was collected from Chiteve village in Manyinga District in Northwestern Province.

Source of Cysticerci

A pig positive for cysticercosis on tongue inspection was purchased from Chibolya Small Livestock market in Lusaka District.

COLLECTION AND PREPARATION OF MATERIALS

Treatment preparations

Both *V. doniana* bark and *F. virosa* root epidermis was dried and ground to powder and then sieved. Six hundred milligrams of powder was added to 1000ml of distilled water and 99% ethanol at a ratio of 7:3 (7 parts of water and 3 parts of ethanol) and mixed; the concentration of the herb stock solution was 0.6g/l. The containers with the ground powder and ethanol were placed on a shaker/mixer and left there for at least 24 hours. The containers were then removed from the shaker and left to stand for 6 hours before undergoing filtration. The filtrate was then left to stand at room temperature before use. Twenty-five millilitres was drawn from the filtrate which had 30% ethanol and it was diluted with 300ml water to reduce the concentration of the alcohol to 2.5% for the serial dilution which would be used.

One tablet of praziquantel (600mg) was dissolved in 1000ml of distilled water and 99% ethanol at a ratio of 7:3 and mixed resulting in a concentration of 0.6g/l of the drug stock solution. The container with the tablet and solution was placed on a shaker/mixer and left there for 24 hours. The filtration process was done just as for the herb extracts and the serial dilution was done to the filtrate in order to reduce the alcohol percentage from 30% to 2.5%.

Collection of Metacestodes

The heavily infected pig was stunned using a captive bolt gun and then exsanguinated as described by [19]. The carcass was sliced into small pieces (Figure 2.1) to retrieve metacestodes with intact bladder walls as shown in Figure 2.2. The metacestodes were washed in phosphate buffered solution until there was no visible blood and then placed in petri dishes [20].

Culture Medium

The metacestodes were cultured in a medium made of 1:1 ratio of porcine bile (PB) and phosphate buffered solution (PBS). The bile was extracted from the pig during slaughter. A total of 16 petri dishes each containing 25 metacestodes were used and 20ml volume of culture medium and herb/drug solution were added to each petri dish.

Treatment

The anthelmintic/drug solutions were added at different concentrations to the petri dishes with the 16th one being used as negative control lacking any anthelmintic or drug. Each herb/drug solution was tested in five different concentrations specifically 50 µg/ml, 30µg/ml, 20µg/ml and 0.006µg/ml and 0.001µg/ml. One point seven millilitres (1.7ml) was drawn from 0.6g/l stock solution and 18.3ml culture medium was added to reach a volume of 20ml, to make the concentration of 50µg/ml. One ml was drawn from 0.6g/l stock solution and 19ml culture medium was added to reach a volume of 20ml, therefore making a concentration of 30µg/ml. Zero point seven millilitres (0.7ml) was drawn from 0.6g/l stock solution and 19.3ml culture medium was added to reach a volume of 20ml, hence making a concentration of 20µg/ml. A volume of 0.7ml was drawn from 0.6g/l stock solution and added to 19.3ml distilled water to reach a volume of 20ml, 6ul was drawn from 20µg/l stock solution and added to 19.9ml culture medium to reach a volume of 20ml, to make a concentration of 0.006µg/ml concentration. Zero point seven millilitres (0.7ml) was drawn from 0.6g/l stock solution and added to 19.3ml distilled water to reach a volume of 20ml. One µl was drawn from 20ug/ml stock solution and add 19.9ml culture medium to reach a volume of 20ml, to make a concentration of 0.001µ/ml concentration.

The petri dishes were then placed in a Yamator incubator at 37 degrees Celsius and later observed after six hours.

Data Analysis

The results were analysed using Chi square test to test if there was an association between *T. solium* metacestodes responses to praziquantel, *Vitex doniana* and F. virosa, Table 3.2 shows the specific variables, characteristic, indicators and scale of measure. A contingency table was made that compared *Vitex doniana* and praziquantel in relation to *T. solium* metacestodes responses (evagination and non-evagination) and another was made for *F. virosa* too.

RESULTS

Treatments biological activity against metacestodes

The herb extracts of *V. doniana* and *F. virosa* and the drug, praziquantel were tested in vitro against *T. solium* metacestodes. Non evagination of metacestodes post treatment showed that the herbs/ drug had some biological activity, while evagination post treatment meant the herbs/ drug did not show biological activity. The summary of the biological activity of each herb are shown in Table 3.1 below.

***Vitex doniana* Biological Activity Against Metacestodes**

The biological activity of *Vitex doniana* against metacestodes from the lowest concentration to the highest ranged between 36% to 64%. The first two highest concentration biological activities of *Vitex doniana* against metacestodes were above 50% and the remaining three concentrations the biological activity was below 50% for the same herb extract. This shows that higher concentration doses of *V. doniana* showed more biological activity against *T. solium* metacestodes than lower concentration doses when tested.

***Flueggea virosa* Root Extract Biological activity against Metacestodes**

Flueggea virosa had the lowest biological activity against metacestodes for all concentrations as compared to V. doniana. The biological activity ranges for *F. virosa* from the lowest to the highest were 28% to 52%. The highest concentration is the only one had biological activity above 50% the other four had biological activity below 50%. This shows that *F. virosa* had a lower biological activity against *T. solium* metacestodes as compared to V. doniana.

Comparing negative and positive controls to *V. doniana* and F. virosa

Negative Control

The negative control (16th petri dish) had no anthelminthic drug or herb extract added and all the metacestodes evaginated indicating that the culture medium was suitable for growth of metacestodes.

Positive Control

Praziquantel biological activity for all concentrations tested ranged between 68% to 96%. The biological activity was dose dependent, the higher the concentration the higher the biological activity. Metacestode non evaginations were above 50% for all Praziquantel concentrations. This shows that praziquantel is a reliable positive control for in vitro testing. Praziquantel the positive control

was compared to the herb extracts using chi square. The test statistic for all concentrations were higher than the critical value which meant there was an association between exposure of metacestodes to the treatment of herb extracts and praziquantel and the expected outcome. A contingency table was made that compared the responses of *T. solium* metacestodes to treatments with praziquantel and V. doniana. The critical value (tabulated value) for 1 degree of freedom and 5% level of significance was 3.84; however, the test statistic found was 29.63 which were higher than the critical value. This shows that there was an association between exposure of metacestodes to *V. doniana* treatment. Another contingency table was made that compared the responses of *T. solium* metacestodes to treatment with *F. virosa* treatment and praziquantel. The test statistic was 42.28 which was higher than the critical value 3.84; indicating that there was an association between exposure of metacestodes to the treatment of *F. virosa* and Praziquantel. Table 4.2 below is a summary of V. doniana and F. virosa results of the Chi square test after contingency tables were drawn in comparison to praziquantel. All concentrations showed that there was an association between treatment of praziquantel and *V. doniana* and F. virosa.

DISCUSSION

The control of *Taenia solium* is partly dependent on the treatment of cysticercosis in pigs, which are the source of infection to humans leading to taeniasis. The use of ethnomedicines provides a potential source of local drugs that can be used. This study evaluated the biological activity of local herbs on *T. solium* metacestodes in-vitro. The herbs evaluated here included *Vitex doniana* which was tested against *Plasmodium falciparum* in a study done by [21].The suggested mechanism of action was that for symptomatic relief of malarial fever. The ecdysteroids from the stem bark which may be involved in reduction of prostaglandin E(1) & E(2) activity in the hypothalamus via modulation of the monoaminergic system [21]. *Vitex doniana* which has alkaloids, phenols, tannins and saponins [22] showed some biological activity against *T. solium* metacestodes.

Flueggea virosa root epidermis has concentrations of alkaloids which have been suggested to have anti-hepatitis, anti-HIV, anti-trypanosomial, and anti-malarial activity [17] [23]. The root was reported to treat testicular inflammation, frigidity, sterility, heavy menstruation, rheumatism, and arthritis. Root powder taken in water is reported to treat liver, bile, kidney, urinary and venereal diseases, upper respiratory tract infections, ranging from cough to tuberculosis, and to treat abdominal complaints, including stomach-ache, dysentery, intestinal worms and schistosomiasis [23]. In Tanzania, root decoction is used to treat epilepsy, convulsions, and rectal and uterine prolapsed. The Tharaka people of Kenya used to treat malaria [24]. In Nhema communal area, in Zimbabwe and in south-central Zimbabwe the extract is drunk as pneumonia medicine and it is also drunk before sexual intercourse as a contraceptive. The dried root powder is used as snake antidote and applied on wounds [25]. *Flueggea virosa* showed some biological activity against *T. solium* metacestodes but its biological activity was lower than that of V. doniana. *Flueggea virosa* root extract has been reported to show some biological activity in the treatment of protozoans and platyhelminthes, further research is encouraged in protozoans and platyhelminthes that its use as an anthelminthic can be concluded. The root extract has only one of the phytochemicals which are alkaloids as compared to *V. doniana* which has alkaloids, phenols, tannins and saponins so this may be the reason *Flueggea virosa* had lower biological activity against *T. solium* metacestodes than V. doniana.

The concentrations for the herb extracts and reference drug used in this study were lower than those used by [26] but the reference drugs used were both dose dependent despite the different concentrations. The same reference drug praziquantel that was used for this study was also used by [6]. who were testing herb extracts on *Hymenolepis diminuta* which is a cestode. According to a study done by [20], praziquantel was the most effective drug and had a sensitivity of 50% at concentration 0.006 ± 0.001µg/ml. In this study, praziquantel showed a dose dependent action, the higher the dose the more biological activity.

Albendazole the drug of choice for neurocysticercosis could not be used in this study because of its low solubility that limits its bioavailability, given the duration of when the *T. solium* metastodes effectiveness was only six hours, and albendazole requires a longer time to be effective [27] The exposure time of the herb extract to the parasite in the current study was lower than in the other studies, which lasted more than a day [26].

Herbal remedies have been tested on *T. solium* and they cure patients who had the infection. Pumpkin seed are a good example of herbal remedies what have been used to treat taeniasis. A study reported the case of someone who took pumpkin seeds and was cured [28]. The person was living in northwest Sichuan, China, was confirmed to be a taeniasis carrier of *T. solium* was treated with pumpkin seeds combined with Areca nut extract in

October 2009. All 20 tapeworms except one without scolex were expelled under good conditions. She was free of secondary cysticercosis within one year follow up [28]. In Taiwan, 32 patients with taeniasis due to T. saginata were successfully treated with the mixture of boiled areca nuts and pumpkin seeds. A total of 48 worms including 42 scolices were recovered from 29 cases. Side-effects were observed in 4 cases including 3 with complaints of dizziness, tinnitus, nausea and vomiting, and one with coma and abdominal pain. Mixtures of 75-150 g areca nuts and 50-100 g pumpkin seeds were judged effective and safe [29]. In this study, it was implicated that treatment with Areca nut extract and pumpkin seeds might represent a good cure against taeniasis. In China, the individual effect of pumpkin seed and areca nut was studied and a positive result in a community of patients with taeniasis was observed. The synergistic effects of these two have shown complete elimination of tapeworm in a mean time of 2 hours [30].

Another study reported the strong anthelmintic activity of the essential oil of Artemisia pallens Wall against *Pheritima posthuma* (earth worm), *T. solium* (tape worm) and *Ascaris lumbricoides* (round worm). The helminthes were found to be more susceptible to the oil than to piperazine phosphate at similar concentration. *Artemisia pallens* was ascribed to possess anthelmintic and stomachic properties in indigenous system of medicine. The screening not only confirmed the correct usage of the plant by the rurals but also enhanced the creditability of ethnobotanical explorations [31].

The antiparasitic activity of the drupe extracts of *Melia azedarach* (Meliaceae) growing in Argentina was tested against a tapeworm and an earthworm, showed to be better against tapeworms than the standard piperazine phosphate, which is used in the treatment of Cestoda infections. It is worth to mention that the drupe extracts were comparatively more active than piperazine phosphate against T. solium. The antiparasitic activity against this tapeworm was better than that of piperazine phosphate (80 min at 0.1 %, and 56 min at 0.2%) at drupe extract concentrations of 0.1 % and 0.2 % (mean death values of 52 and 32 min, respectively) [32].

Dixit and Varma reported that the oils of the rhizomes of *Hedychium coronarium* (Zingiberaceae) and *H. spicatum* (Zingiberaceae) possessed better anthelmintic activity than piperazine phosphate against earthworms and tapeworms [33]. Later studies showed root oil *Hedychium coronarium* (Zingiberaceae) and *H. spicatum* (Zingiberaceae) had better effects than synthetic anti-helminthic drugs like piperazine phosphate against tapeworms [33]. Oil of *Gardenia lucida* (Rubiaceae), Cyperusrotundus (Cyperaceae), *Inula racemosa* (Compositae), *Psitacia integrrima* (Anacardiaceae), *Litsea chinensis* (Lauraceae) and *Randia dumetorum* (Rubiaceae) also showed good effects on tapeworms. In another study, oil plants, such as *Artemisia pallens* (Compositae), *Eupatorium triplinerve* (Compositae), *Capillipedium foetidum* (Poaceae) and the grass of *Cymbopogon martini* (Poaceae) showed a strong impact on *Ascaris* and *T. solium* [34].

A toxicological study was performed on rats to assess the toxicity of *Glinus lotoides* which is traditionally used to treat taeniasis/tapeworm infections in Ethopia [35]. This study showed that there was no toxicity associated with *Glinus lotoides* extracts and it could be further evaluated for clinical trials [36].

Thus, It can be noted that medicinal herbs can be used and tested to cure many infectious diseases, including helminthic diseases. It is significant to note that herbal medicine might be of fewer side effects than chemotherapy as has been reported in literature more or less [34]. Further, these herbs can be sourced locally in communities where they are needed.

CONCLUSION

Vitex doniana and *Flueggea virosa* both showed some anthelminthic activity against *T. solium* metacestodes. Both *F. virosa* and *V. doniana* are recommended for further testing in vivo studies, using hamsters infected with the adult tapeworm. The use of the herbs as anthelminthic can be concluded and later used as cheaper anthelminthic for the local people if they are efficient. The herbs are also recommended for testing in vivo in infected pigs to test if the herbs will have any biological activity against *T. solium* metacestodes. *Flueggea virosa* may be tested further in protozoan parasites as studies have shown anthelminthic activity in them.

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Conflict of interest

There were no conflicts of interest.

Figure 2.1 Muscle of pig infected with metacestodes

Figure 2.2: Metacestodes after retrieval in phosphate buffered solution

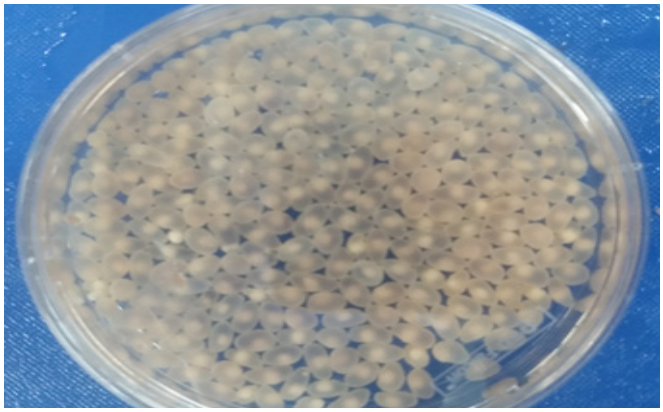

Table 3.1 Treatment and biological activity of Praziquantel, V. doniana and F. virosa against T. solium metacestodes.

Treatment	Concentrations	Number of metacestodes evaginated	Number of non evaginated metacestodes	% of evagination
V. doniana	50µg/ml	9	16	64
	30µg/ml	11	14	56
	20µg/ml	13	12	48
	0.006µg/ml	15	10	40
	0.001µg/ml	16	9	36
F. virosa	50µg/ml	12	13	52
	30µg/ml	13	12	48
	20µg/ml	14	11	44
	0.006µg/ml	16	9	36
	0.001µg/ml	18	7	28
Negative control		25	0	0

Table 3.2: Comparison in sensitivity treatment of positive control, Praziquantel to V. doniana and F. virosa using Chi-square

Concentration µg/ml	*V.doniana Observed statistic*	*x^2 Tabulated Statistic*	*F. virosa x^2 Observed statistic*	*Tabulated x^2 x^2 statistic*
50	8	3.84	12.57796	3.84
30	6.349296	3.84	9.191176	3.84
20	5.55556	3.84	6.876061	3.84
0.006	6.65	3.84	8.116883	3.84
0.001	5.12	3.84	8.1012821	3.84

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