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ARTÍCULO ORIGINAL

In-vitro antihelminthic activity of alcoholic extract from *Paullinia pinnata* Linn against *Ascaris suum.*

Actividad antihelmíntica *in-vitro* del extracto alcohólico de las hojas de *Paullinia pinnata* Linn contra *Ascaris suum*

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ABSTRACT

Introduction: Medicinal plants are used both for the treatment of certain conditions and to preserve health and vitality. According to estimates by the World Health Organization, 80% of the world population, excluding industrialized countries, rely on traditional medicine, particularly medicinal plants. Several parts of the plant *Paullinia pinnata* Linn have been used by many African tribes to treat a variety of conditions, including helminthiases.

Objective: Evaluate the antihelminthic effect of *P. pinnata* alcoholic leaf extract against *Ascaris suum.*

Methods: Dry leaves of *P. pinnata* were used to obtain the 23.33 % alcoholic extract, which then underwent phytochemical analysis. Thirty *Ascaris suum* worms were divided into 6 groups: 2 control, 1 negative and 1 positive, and 4 experimental. The experimental groups were exposed to different concentrations of the alcoholic extract (5, 10, 40 and 80 mg/ml). Goodwin's physiological solution was used as negative control, whereas the positive control was albendazole 40 mg/ml. Motility testing was performed to determine the antihelminthic activity of the plant, and the mortality rate was estimated. The chi-square test was applied, and a *p* value below 0.05 was considered to be significant.

Results: Saponins, alkaloids, flavonoids, tannins and triterpenoids were all found in the alcoholic extract of *P. pinnata*. The plant showed significant antihelminthic activity, and mortality rates were higher than those obtained with albendazole.

Conclusions: Saponins, alkaloids, flavonoids, tannins and triterpenoids may be responsible for the antihelminthic activity of the study alcoholic extract, which was greater than that of albendazole.

Key words: *Paullinia pinnata* Linn, albendazole, antihelminthic activity, motility, mortality rate.

RESUMEN

Introducción: Las plantas medicinales se utilizan tanto para el tratamiento de ciertas enfermedades como para conservar la salud y la vitalidad. La Organización Mundial de la Salud estima que el 80 % de la población mundial, excluyendo los países industrializados, dependen de la medicina tradicional, sobre todo de las plantas medicinales. Varias partes de la planta *Paullinia pinnata* Linn han sido usadas por muchas tribus africanas para el tratamiento de diversas enfermedades, incluyendo las helmintiasis.

Objetivo: Evaluar el efecto antihelmíntico del extracto alcohólico de las hojas de *P. pinnata* contra *Ascaris suum*.

Métodos: Se usaron hojas secas de *P. pinnata* para obtener el extracto alcohólico al 23,33 % y se hizo su estudio fitoquímico. Treinta lombrices *Ascaris suum* se dividieron en 6 grupos; 2 de control, uno negativo y uno positivo, y 4 experimentales. Los grupos experimentales fueron expuestos a diferentes concentraciones del extracto alcohólico de la planta (5; 10; 40 y 80 mg/mL). La solución fisiológica Goodwin se usó como control negativo mientras que el control positivo fue albendazol 40 mg/mL. Se realizó la prueba de motilidad para determinar la actividad antihelmíntica de la planta y se calculó el índice de mortalidad. Se aplicó la prueba de Chi al cuadrado y se consideró significativo el valor de *p* menor que 0,05.
Resultados: Se encontraron saponinas, alcaloides, flavonoides, taninos y triterpenoides en el extracto alcohólico de *P. pinnata*. La planta mostró significativa actividad antihelmíntica y los índices de mortalidad fueron mayores que los obtenidos con el albendazol.
Conclusiones: Saponinas, alcaloides, flavonoides, taninos y triterpenoides pueden ser responsables de la actividad antihelmíntica del extracto alcohólico de la planta, la cual fue superior a la del albendazol.

Palabras clave : *Paullinia pinnata* Linn; albendazol; actividad antihelmíntica; motilidad; índice de mortalidad.

INTRODUCTION

Herbs and herbal products have always been the main form of medicines in many countries of the world and presently, they are becoming popular throughout the developed world as people strive to stay healthy in the face of chronic stress and pollution as well as to treat illness with medicines that work in concert with the body's own defenses.[1]

The world's population is largely dependent on herbal medicines for treatment of several illnesses and a vast number of plant materials are used worldwide.[2] The major drawback in promoting the use of medicinal plants is the lack of standardization as well as the confusion in the identification of the plant and their substitutes or adulterants. To ensure reproducible quality of herbal plants, authentication is invaluable.[3]

Helminthic infections continue to be the major health hazard to people, especially those living in tropical developing countries and livestock. It is estimated that 60-80 % of the world's population is infected by intestinal helminthes such as Ascaris, hookworms, Trichuris, Enterobius, Strongyloides and tapeworms, and most of them live in remote rural areas in those developing tropical countries.[4]

Intestinal helminthes infections are attracting attention with regards to their direct contribution to morbidity and effects towards other important infections such as malaria, tuberculosis and HIV/AIDS.[5]

The helminthic infections are, however, frequently under diagnosed because many cases are asymptomatic; moreover, diagnostic methods lack sensitivity and without appropriate therapy, the infection may persist lifelong or even become life-threatening in cases of immunodeficiency since no public health strategies for controlling these diseases are active at global level.[6,7]

During the past few decades, despite numerous advances made in understanding the mode of transmission and treatment of parasites, there are still no efficient products to control helminthes and the indiscriminate use of some drugs has generated several cases of resistance. [8] Furthermore, it has been recognized recently that antihelminthic substances having considerable toxicity to human beings are present in foods derived from livestock posing a serious threat to human health.[9]

Paullinia pinnata Linn specie, known as sweet gum, which belongs to Sapindaceae family,[10] is used in the treatment of helminthiasis in livestock in some parts of Uganda, where human and veterinary services are still very poor; being compounded by many people living in rural

areas several kilometers away from health centers. This has resulted in a large proportion of the population relying on traditional methods of treatment, using herbal extracts, which have been claimed to produce beneficial responses. The natural remedies are not only more readily available and accepted but could also be cheaper, if their efficacy is scientifically validated.

Besides the above mentioned, the low income of the people living in rural areas of tropical developing countries like Uganda with high population and poor public health strategies make it difficult to access the current conventional antihelminthic drugs, leading to high prevalence of the helminthic infections.[11] Considering the use of *P. pinnata*, in some rural areas of Uganda, for the treatment of helminthiasis, the purpose of this study is to assess the antihelminthic effect of its ethanolic leaf extract.

METHODS

The antihelminthic activity of *P. pinnata* ethanolic leaf extract was assessed by determining the motility test and the mortality index in *Ascaris suum*.

Plant material

The fresh plant leaves were collected from Fortportal district, Western Uganda, taken to the Faculty of Science, Department of Biology for identification by the botanist Dr. Eunice Olet and it was given a voucher number: JOANITAH NYASINGE 001. *Preparation of P. pinnata ethanolic leaf extract*

The solvent used for the extraction was ethanol to ensure a high percentage yield. One kilogram of fresh leaves was cleaned and dried under shade for two weeks, to preserve the potency of the active constituents. The dried leaves were pulverized into coarse particles using an electric blender to achieve a surface area for optimal drug extraction.

Soxhlet extraction was used to prepare the ethanolic leaf extract where one litre of 70 % ethanol was added to a round bottom flask attached to the soxhlet apparatus and a condenser on an isomantle. The pulverized coarse powder was then loaded into the thimble inside the extractor. The side arm was lagged with glass wool and the solvent heated to evaporation using the isomantle. The condensed vapors were allowed to drip into the reservoir containing the thimble and the cycle was left to run for 24 h. After the process, the resultant solution was poured into a clean dry metallic container and the ethanol was evaporated off using a hot air oven at 40°C at reduced pressure to leave only the desired plant extract material.[12]

A sticky solid extract was obtained at a percentage yield of 23,33 % w/w.

Phytochemical screening

Phytochemical analysis was performed on 23,33 % ethanol extract using some chemical reactions (tests) to identify, predominantly by color change or precipitated formations, the presence of secondary metabolites: saponins (foam), alkaloids (Dragendorff), tannins (ferric chloride: $FeCl_3$), triterpenoids (Libermann-Buchard), flavonoids (Shinoda).[13]

Animals (Worms)

Ascaris suum, a close relative of *Ascaris lumbricoides* was the animal model used as recommended by previous researches conducted.[14]

The live worms (*Ascaris suum*) used in the study were identified and collected from a pig abattoir in Kamukuzi, Mbarara and another in Kabagarame, Bushenyi. The worms were obtained from the small intestines of freshly slaughtered pigs, after manually straining the contents to collect the worms to be immediately placed into a thermos flask containing freshly prepared Goodwin's solution at 37°C and then transported to Mbarara University of Science and Technology Pharmacy Laboratory for further identification, by the laboratory technician Mr. Nkwangu David and the Pharmacology lecturer Mr. Oloro Joseph, and later the susceptibility assay experiments to be conducted.

Antihelminthic Susceptibility testing

The *in-vitro* susceptibility assay was performed using 30 *Ascaris suum* live worms from freshly slaughtered pigs. The worms were divided into six groups of five worms each; four experimental groups, one negative and one positive control.

Goodwin's physiologic solution was used as the solvent to prepare a series of concentrations 5; 10; 40 and 80 mg/mL from the dried ethanolic leaf extract and this same physiologic solution was used as a negative control. Using the same procedure, a 40 mg/mL positive control of the available standard drug albendazole was also prepared.[15] All the dilutions were made twice and the average of the pair was used as the test solution as a quality control measure.

Five live worms were introduced into the different flasks containing the different extract concentrations, the positive control and negative control experiments at the same time. The flasks, beakers and their contents were then immersed in a water bath and maintained at 37^{o}C throughout the experiment. The worms were monitored and observed for motility, paralysis and mortality every 12 h for 48 h in accordance with the following criteria:[16]

1. Motility (alive) was noted with complete sinusoidal movement of the worms.

2. Paralysis was noted when there is minimal or no motility observed with a slight pin prick method.

3. Death was recorded only when worms have completely lost motility by either vigorous shaking or dipping them in warm water at 50^{o}C. Mortality Index was calculated as the number of dead worms divided by the total number of worms per conical flask.[17]

Statistical analysis

The results obtained from the phytochemical screening were tabulated and deductions made. The antihelminthic activity of the different concentrations of the plant extract and the standard at the different time intervals was tabulated and represented using a line graph with the help of SPSS software. The antihelminthic activity of the plant extract and the standard drug albendazole was compared using a Two-way between subjects, ANOVA followed by a Chi-square test and a *p*-value less than 0,05 was considered significant.

RESULTS

Phytochemistry

The phytochemical screening of the *P. pinnata* ethanolic leaf extract found a positive reaction to alkaloids, tannins, triterpenoids, and flavonoids (table 1).

Table 1. Phytochemical constituents of *P. pinnata* ethanolic leaf extract

Constituent	Results
Alkaloids	+++
Tannins	+++
Triterpenoids	+++
Flavonoids	+++
Saponins	+

Low (+), Moderate (++), High (+++)

Motility test

The number of worms motile, paralyzed and dead in the negative control, positive control as well as in the different concentrations of the plant extract at different times is shown in table 2.

Table 2. Antihelminthic activity of *P. pinnata* Linn ethanolic leaf extract

Concentration (mg/mL)	Time (Hours)	Number of worms motile	Number of worms paralyzed	Number of worms dead
100 mL of Goodwin's solution (Negative control)	0	5	0	0
	12	5	0	0
	24	5	0	0
	36	5	0	0
	48	4	1	0
5 of the extract	0	5	0	0
	12	4	1	0
	24	4	0	1
	36	3	1	1
	48	1	1	3
10 of the extract	0	5	0	0
	12	4	1	0
	24	2	2	1
	36	1	2	2
	48	0	1	4
40 of the extract	0	5	0	0
	12	1	2	2
	24	0	1	4
	36	0	0	5
	48	0	0	5
80 of the extract	0	5	0	0
	12	1	1	3
	24	0	1	4
	36	0	0	5
	48	0	0	5
40 of albendazole (Positive control)	0	5	0	0
	12	2	1	2
	24	1	1	3
	36	1	0	4
	48	0	1	4

The worms in the negative control (Goodwin's solution) were stable all throughout the experiment but in the study plant extract generally exhibited dose dependent paralysis, ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death.

At a dose level of 5 mg/mL, death was first observed in one worm after 24 h. At 36 h one worm was found paralyzed. After 48 h was observed paralysis in one worm and death in two more for a total of three death worms, at this time only one motile worm was observed.

At an increased dose of 10 mg/mL, one worm was found paralyzed at 12 h which progressed to death after 24 h and other two were found paralyzed. At 36 h one motile worm became paralyzed while another one remained like that progressing only one to death. After 48 h the observed motile worm progressed to paralysis and the two paralyzed progressed to death, so at this time four worms were observed dead and one paralyzed.

At a dose level of 40 mg/mL, two worms were paralyzed and two dead at 12 h. After 24 h, the motile worm was found paralyzed and two more worms died for a total of four worms dead by this time of the experiment. At 36 h the paralyzed worm died making the total number of worms of the experiment dead. At 80 mg/mL, one worm was paralyzed and three dead at 12 h, however at 24 h the same trend as that of 40mg/mL was observed.

With the dose of albendazole (positive control) used in the experiment, one worm was paralyzed and two dead after 12 h; at 24 h one more worm died and another one became paralyzed; at 36 h of the experiment another worm died to have four dead worms and only one motile; at 48 h the remaining motile worm became paralyzed.

Mortality Index

Table 3 shows the mortality indexes obtained at each plant extract concentration and that of the standard. The mortality index at a concentration of 5 mg/mL of the extract was slightly lower than albendazole with a value of 60 at 48 h. The same mortality index of albendazole was reached at 48 h with 10 mg/mL of the plant extract. At 40 mg/mL of ethanolic leaf extract, the mortality index was equal to albendazole at 12 h. From 24 h up to 48 h the mortality index of the plant extract was higher than that of the positive control for both 40 and 80 mg/mL.

Table 3. Mortality Index at different plant extract concentrations and albendazole

Concentration (mg/mL)	Mortality Index (%)			
	12 hrs	24 hrs	36 hrs	48 hrs
5 of extract	-	20	20	60
10 of extract	-	20	40	80
40 of extract	40	80	100	100
80 of extract	60	80	100	100
40 of albendazole	40	60	80	80

Comparison between *P. pinnata* ethanolic extract and albendazole taking into account number of dead worms.

Chart 1 shows the superiority of the plant extract over the standard drug. At 12 hours both albendazole and the study plant killed the same number of worms (two). With increasing time, the number of dead worms by the plant extract was more than that by albendazole since the plant extract was able to kill four worms while albendazole only three at 24 h of the experiment. At 36 h all the worms (five) were found dead in the plant extract while albendazole only killed four of them.

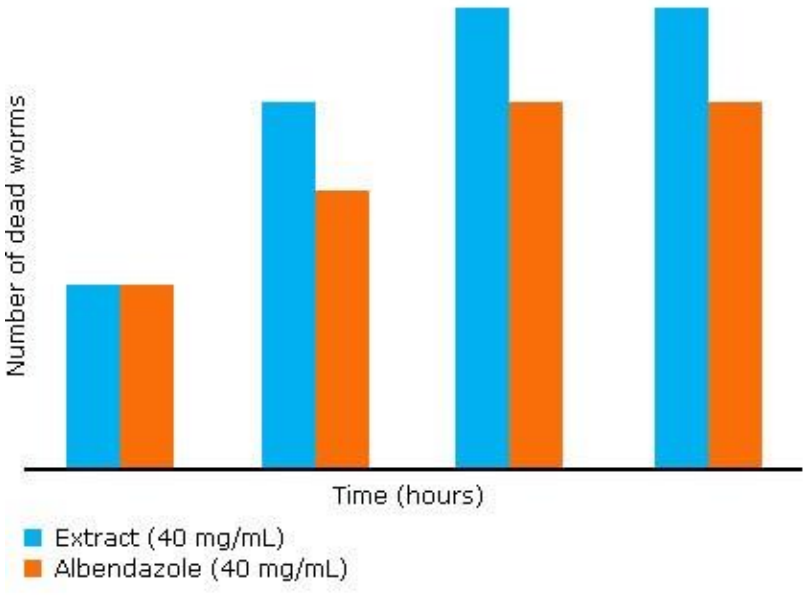

Chart 1. Dead worms at 40 mg/ml of the extract and albendazole.

The statistical analysis results obtained at 40 mg/mL of the extract and the standard drug albendazole are shown in table 4.

Table 4. Statistical analysis for 40 mg/mL of the plant extract and albendazole

	Number of paralyzed worms	Number of dead worms
Chi-Square	7.800[a]	12.400[b]
Degrees of freedom	2	4
Asymptotic Significance	0.020*	0.030*

* Significant difference

There was significant difference in both paralysis and death of the worms subjected to the plant extract and the standard drug (p=0,02) and (p= 0,03) respectively on the scale of 5 %.

DISCUSSION

The screened *P. pinnata* ethanolic extract contained alkaloids, tannins, triterpenoids and flavonoids in high concentrations, which match with those components reported by different researches.[18-20]

The anthelmintic drugs are relatively insoluble in water and partially soluble in most organic solvents and this has an impact on their bioavailability in body tissues. Whereas efficacy may be high on gastrointestinal helminths, their limited absorption and rapid metabolism means that high and/or prolonged doses are required for effective treatment of human systemic infections.[21]

One of the predominant effects of anthelmintic drugs like piperazine, praziquantel and ivermectin on the helminthes is to cause a flaccid paralysis by affecting neurosynapses and motion of worms that result in expulsion of the parasite by peristalsis. [22,23] Albendazole acts by blocking glucose uptake and depletion of its glycogen stores thus decreasing ATP formation; it binds to free protein in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of parasite leading to gradual loss of intracellular microtubules in the cells of the worm through inhibition of tubulin polymerization.[15] Albendazole is also known to cause slow immobilization (paralysis) and death of the parasites in feaces.[22,23]

The study plant extract not only demonstr ated that can cause the worms paralysis but also, as albendazole, caused death in the first 12 h meaning that the plant must contain a compound or a group of compounds responsible for the antihelminthic activity.

After a deep search in the accessed bibliography, researches evaluating the antihelminthic activity of *P. pinnata* were not found, however some studies[18,24-28] have reported antihelminthic activity of some of the metabolites identified in this plant.

A study conducted by *Dasgupta* and collaborators showed that tannins and alkaloids derived from plants exhibit antihelminthic activity, as they interfere with energy generation in the parasites by uncoupling oxidative phosphorylation.[18]

Tannins have also been found to bind to free proteins in the gastrointestinal tract of the parasites or glycoproteins on the cuticle of these parasites thus causing their death as described in a study conducted by *Athnasiadou* and collaborators.[24]

According to *Andrew* and collaborators, condensed tannins or a combination of both the tannins and other phenolic compounds from diverse plant sources have been found to be

responsible for antihelminthic activity.[25] A study conducted by *Yadav* established that flavonoids and triterpenoids inhibit the actions of certain parasitic enzymes causing decrease in glycogen and ATP leading to their death.[26] It has been reported also the effect of alkaloids on the parasites' Central Nervous System which leads to paralysis of the parasites.[27,28]

The presence of alkaloids, tannins, triterpenoids and flavonoids found in high quantities during the phytochemical screening may be responsible for the antihelminthic activity of the *P. pinnata* extract.

Some studies conducted to compare plant extracts with albendazole have shown powerful antihelminthic activity as more effective action has been reported when compared to albendazole[15,29,30] showing that medicinal plants like *P. pinnata* can be promising sources of antihelminthic products. Taking into account our findings it can be concluded that saponins, alkaloids, tannins, triterpenoids and flavonoids present in the plant under study may be responsible for the significant antihelminthic activity of the *P. pinnata* ethanolic extract, which was higher than that of albendazole.

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