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In vitro Evaluation of the Anti-scavenging and Anthelmintic Activities of *Artocarpus heterophyllus* LAM Leaves (Moraceae) in the Democratic Republic of Congo

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Abstract: The extracts of *Arthocarpus heterophyllus* Lam. leaves were evaluated *in vitro* for their anthelmintic activity. *Benhamia rosea* and *B. itoleisis* were used as animal models and Albendazole as reference product (positive control). After calculating the yield, it appears that the ethanol extracts had given a better yield (0.70%) compared to the organic extracts. The result of the phytochemical screening by TLC (thin layer chromatography) showed the presence of phenolic compounds including anthocyanins, coumarins, anthraquinones, phenol acids and terpenoids. From this study, it appears that *A. heterophyllus* Lam. contains various secondary metabolites such as flavonoids (2.63±0.007mg EQ/100g MS), phenolic acids, coumarins, anthraquinones, terpenoids and anthocyanins (10.46±1.05 mg/100 MS) and total polyphenols (27.33±9.34 mg EAG/100 g MS). The organic/terpenic acids extract showed very high antioxidant activity against the ABTS radical (IC_{50}: 0.97 ± 0.13 µg/ml). The ethanolic and organic acid extracts from the leaves of this plant species have an anthelminthic activity, but this activity is dose dependent. However, at the lowest concentration (0.625 mg/mL), ethanolic extract showed better activity with a paralysis time of 67.3±1.8 minutes compared to 76±2.1 minutes for the organic extract. But the mortality rate at the lowest concentration was higher for organic extracts, at 62.7% compared to 33.3% for ethanol extracts. It is therefore desirable to test bioactive extracts on gastrointestinal parasites of farm animals in order to confirm the results obtained. Ongoing in-depth phytochemical studies will identify the chemical compound (s) and active principle (s) for the formulation of anthelminthic phytomedicine for managing pathologies due to helminthes in farm animals.

Keywords: Anthelminthic Activity, Earthworm, Phytomedicine, *Arthocarpus heterophyllus*

1. Introduction

Livestock is an important source of income, livelihoods, nutrition and food security, as well as resilience in much of Africa [1, 2]. Unfortunately, its mode of exploitation is dominated by a traditional system confronted with

pathologies due to digestive parasites which reduce the expression of animal productivity in general and that of small ruminants in particular [3, 4]. Parasitic infections by gastrointestinal helminthes are thus a source of great and constant concern for rural producers who do not have sufficient financial income to ensure good medical coverage [5-7]. Among these gastrointestinal parasites, widespread gastrointestinal strongylosis is frequently involved in significant economic losses on farms [4, 8]. Farmers use synthetic molecules called anthelmintics to control these parasitic diseases and therefore increase zootechnical performance [9-12].

However, repeated and/or abusive use of these products has led to the selection of helminthes strains with chemoresistance to the three main groups of antihelmintics (benzimidazoles, imidazothiazoles and macrocyclic lactones) [13-15]. The combination of these factors has stimulated farmers to seek alternatives or complementary solutions that can control animal parasitosis [16-18]. There are many reasons for this: easy access and low toxicity [8, 11].

In order to scientifically validate the traditional use of anthelmintic medicinal plants, a particular choice was made for *Artocarpus heterophyllus* Lam. on the assumption that this plant species would contain biologically active compounds with anthelmintic and anti-free radical properties. Indeed, in the defense mechanism against parasitic infections, the animal organism uses free radicals which are also toxic to its own cells in the event of overproduction [19].

2. Materials and Methods

2.1. Materials

2.1.1. Animal Material

Earthworms (*Benhamia rosea* and *B. itoleisis*) were used as an animal model for this study. This choice is justified by the fact that it is, on the one hand, close to helminthes, and on the other hand easily accessible [20].

Specimens of earthworms were collected along the Keni River, a few meters from the intersection of the By-pass road and the Kimwenza road in Makala commune. Their identification was made at the Laboratory of Natural Resources Management, Faculty of Agronomic Sciences of the University of Kinshasa.

2.1.2. Plant Material

It consisted of leaves of the plant *Artocarpus heterophyllus* Lam, collected from the University of Kinshasa site, precisely on the students' plateau in the Commune of Lemba. It was then dried in the open air at a temperature of around 30 °C, in the total absence of the sun. After drying, the plant material was ground and sieved with a sieve to obtain a fine powder.

2.2 Methods

2.2.1. Phytochemical Studies

(i) Extraction

The dried and powdered plant material (10 g) was repeatedly extracted by cold percolation with 95% ethanol (EtOH) (100 mL x 2) for 48 hours. Fractions were filtered and concentrated to dryness under reduced pressure using a rotary evaporator. Organic/triterpenic acids were extracted as follow: the powdered material (40 g) were macerated with 100 mL of dichloromethane-methanol-NH_4OH (100:1:1; v/v/v) and then percolated with 300 mL of the same solvent mixture at room temperature. The extract was concentrated under reduced pressure until 100 mL (pH 10). The resulting solution was then mixed with 5% citric acid (v/v) to precipitate organic/triterpenic acids [21].

(ii) Thin Layer Chromatography (TLC) analysis

Phytochemical screening by Thin Layer Chromatography (TLC) was performed to detect the presence of secondary metabolites. Briefly, the TLC analysis of 10µl of solution for 10 mg/ml of aqueous extracts were performed in normal phase using Silicagel $60F_{254}$ plates (Merck) with Ethyl Acetate/Formic acid/Acetic acid/water (100: 11:11:27 v/v) as mobile phase. For organic extracts, butanone-2/toluene (4; 6; v/v) was used as mobile phase. Before and after adding specific reagents, the TLC plate observation was carried out using the visible light and underneath UV light (254 and 366 nm). Flavonoids and phenolic acids were revealed due to the presence of Neu reagent, quinones owing to Borntrager reagent (KOH 10%), alkaloids due to Dragendorff reagent; terpens and steroids were revealed by using sulphuric anisaldehyde and of antimony 20% (methylene dichloride) [22, 23].

(iii) Quantification of secondary metabolites

(a) Total polyphenols content

Total phenols content of yam tuber extracts was determined by spectrophotometry UV-Vis using Follin-Ciacalteu's method as described by Ngbolua & Djolu [24]. Briefly, the reaction mixture was made of 0.5 ml of methanol extract of each of the yams prepared at 1ml/ml, 5ml of distilled water and 0.5 of Follin Ciacalteu's reagent. Then 1ml of a saturated solution of Na_2CO_3 20% was added three minutes later. The mixture was shaken and then incubated at the ambient temperature far from the light for an hour. The absorbencies were read thru the spectrophotometer GENESYS 10S UV-Vis at 725 nm. Each dosage was performed as a triplicate. For the eight different dilutions of gallic acid standard solution (5 à 150 µg/ml), the same procedure was followed in order to the calibration straight line. For the control (blank), we followed the same procedure as well but the extract was replaced by methanol 80%. Results are expressed into mg equivalent of gallic acid per g of dry vegetal material using the following equation y= 0.0098x-0.0195 (R^2= 0.967).

(b) Flavonoids content

The content of flavonoids of yam extracts was determined by spectrophotometry UV-Vis as previously described by Ngbolua et *al*. [25]. The reaction mixture contained 1 ml of methanolic solution of each yam extract at 1mg/mL of concentration and 1mL of ACl_3 2% dissolved in methanol 80%. Then, the mixture is well homogenized and incubated

for an hour at the ambient temperature and in the dark. Absorbencies reading are carried out by using the spectrophotometer GENESYS 10S UV-Vis at 425 nm. Quercetin solution (50-200 mg/mL) was used as a standard. For the control (blank), the extract was replaced by methanol 80%. Results are expressed in mg equivalent of quercetin per g of dry vegetal material using the following equation y= 0.0232x + 0.1535 (R^2= 0.945).

(c) Anthocyanins

The samples were diluted with the mixture ethanol/water/HCl conc. (70:30:1; v/v/v) and the absorbance was measured at the wavelength of 540 nm. The anthocyanins content (expressed as malvidin-3-glucoside equivalent, M-3-GE) was calculated using the following relation: Anthocyanins =A_{540} *(10/0.6)*d (with A_{540}= maximum of absorption at 540 nm; d = dilution factor; 0.6 maximum of absorption of 10 mg/L of M-3-GE standard solution) [24, 25].

2.2.2. Radical Scavenging Activity

(i) DPPH radical scavenging capacity

In 100 ml of methanol 80% is dissolved the DPPH radical (3.2 mg) then the mixture is kept in a dark place for a hour. The absorbance is adjusted at 0.8 ± 0.05 after homogenization. In order to get the trial solutions of different concentrations at 0.5, 1, 2, 3, 4 and 5 mg/mL respectively, the extract (5 mg/mL) is diluted into methanol. Afterwards, 20 µL of extract are mixed with 1980 µL of DPPH radical in micro-tubes then incubated into darkness for 30 minutes. Different are read with spectrophotometer UV-Vis at 517 nm. DPPH radical inhibition percentage is determined by the following relation: % inhibition= [1-(Ax/Ac)] × 100. Where Ax is the absorbance of DPPH radical in presence of the extract and Ac is the absorbance of DPPH radical (Control) [26].

(ii) ABTS radical scavenging capacity

ABTS assay was based on the method of Re et al. and performed as reported by [27]. The extract (5 mg/mL) is diluted with methanol for the obtention of different trial solutions of respective concentrations at 0.5, 1, 2, 3, 4 and 5 mg/mL. Afterwards, 20 µL of extract are mixed with 1980 µL of ATBS radical in microtubes then incubated into darkness for 30 minutes. The decrease of absorbance at 734 nm was compared to the control and standard with a Spectrophotometer GENESYS 10S UV-Vis. The inhibition percentage of the radical and IC_{50} are determined by the above mentioned relation.

$ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity of extracts were expressed in IC_{50} values. Different values of IC_{50} for samples are determined using Graph Pad Prism version 6.0 Software.

2.2.3. Evaluation of Antihelminthic Activity

Anthelmintic activity was evaluated using the method of Guissou et *al*. [21]. Different concentrations of ethanolic extract and the dichloromethane fraction of *A. heterophyllus* were prepared. The working solution was prepared by diluting the mother solution in series (reason 2) to optimize concentrations ranging from 0.625 to 5 mg/ml. A solution of albendazole was prepared as a positive control under the same conditions as the extract, i.e. 5 mg of albendazole. Distilled water was used as a negative control.

The worms, previously washed, were divided into three batches containing three specimens per petri dish: In the first batch, there was a series of boxes, three per dilution, containing the extract in decreasing order of concentration, in which we had placed three worms per box. In the second batch, we dissolved the deworming agent (albendazole), proceeding in the same way as in the first batch. Finally, in the last batch, there was a petri dish containing distilled water, used as a negative control.

After the worms were exposed to the products, various parameters were then observed, namely: the behavior of worms, the time of paralysis and the mortality rate. This observation was made for 48 hours. After this time, the experiment was repeated three times in a row under the same conditions.

3. Results and Discussion

3.1. Extraction Yield of A. heterophyllus Lam Leaves

The figure 1 below gives the extraction yield of different extracts after 48-hours maceration.

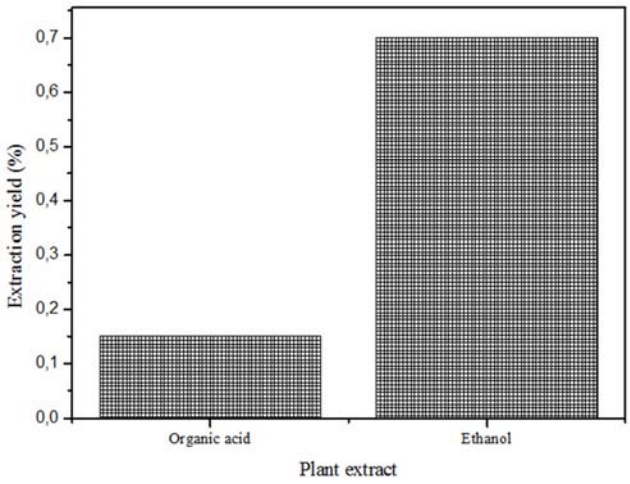


Figure 1. *Yield of extraction (%).*

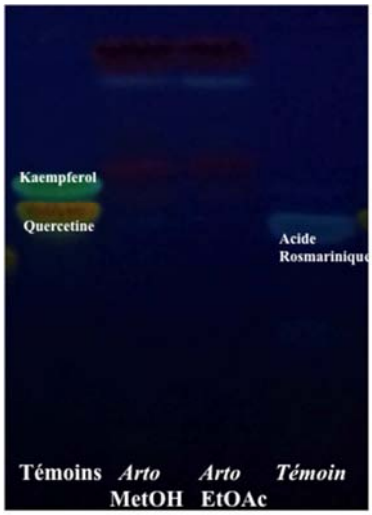
It can be seen from this figure that the yield obtained from the ethanol extract (polar) is better than that of organic acids (non-polar): 0.70% vs 0.15%. This also shows that the metabolites quantitatively important in the leaves of our plant are those that pass easily through polar solvents, especially polyphenols including anthocyanins, tannins, flavonoids, but also saponins and alkaloids.

3.2. Phytochemical Screening

The result of the phytochemical screening by TLC showed the presence of phenolic compounds (including flavonoids, anthocyanins, coumarins, anthraquinones and phenol acids) and terpenoids as revealed in figures 2 to 7.

Figures 2 and 7 give the chromatographic profile of

Artocarpus heterophyllus extracts.



Figures 2. *CCM chromatogram for phenolic acids from methanolic extracts (Arto MetOH) and ethyl acetate (Arto EtOAc) from Artocarpus heterophillus at 366 nm.*

Figure 3. *CCM chromatogram for flavonoids from methanolic extracts (Arto MetOH) and ethyl acetate (Arto EtOAc) from Artocarpus heterophillus at 366 nm.*

Phenolic acids give blue fluorescent spots with Neu reagent (Figure 2) and flavonoids from yellow spots (Figure 3).

By comparing these spots with those of the control, the blue fluorescent spots would correspond to chlorogenic acid and caffeic acid. Caffeic acid and chlorogenic acid are part of the polyphenol families [28]. Our results are similar to those found by Prakash et *al*. [29] who reported the presence of flavonoids in the leaves of *A. heterophyllus*. Saxena et *al* [30], who isolated flavonoids from the leaves of A. heterophyllus, also confirm these results.

The figures below indicate the presence of coumarins and anthraquinones.

Figure 4. *CCM chromatogram for coumarins of methanolic extract (Arto MetOH) and ethyl acetate (Arto EtOAc) from Artocarpus heterophilus at 366 nm.*

Figure 5. *CCM chromatogram for anthraquinones of methanolic extract (Arto MetOH) and ethyl acetate (Arto EtOAc) from Artocarpus heterophilus at 366 nm.*

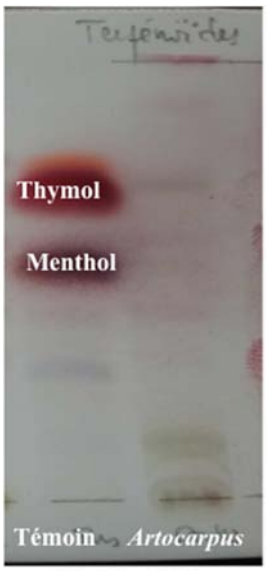
From these figures, coumarins appear as fluorescent blue spots with alcoholic KOH (Figure 4) while anthraquinones appear as red spots (Figure 5).

These results are consistent with those of Thapa et *al*. [31] and those of Sivagnanasundaram and Karunanayake [32] showing the presence of coumarins and anthraquinones in the leaves of *A. heterophyllus* Lam, respectively.

The figures 6 and 7 below provide information on the presence of anthocyanins and terpenoids.

PS : Sillicagel 60F$_{254}$
PM : Acétate d'ethyl/Méthanol/Eau
(100:10: 40;v/v)
Détection : Vanilline phosphorique

Figures 6. *CCM chromatogram of anthocyanins from methanolic extract of Artocarpus heterophyllus in the visible.*



PS : Sillicagel 60F$_{254}$
PM : Toluène/ Acétate d'ethyle
(9:1;v/v)
Détection : Anisaldéhyde sulfurique

Figures 7. *CCM chromatogram of terpenoids from dichloromethane extract of Artocarpus heterophyllus in the visible.*

Concerning anthocyanins, Figure 6 shows spots that show the presence of these compounds in the plant, pink spots. Terpenoids are also present in the plant as shown in Figure 7, pink spots.

Our results confirm those obtained by Prakash et al. [29] and Sivagnanasundaram and Karunanayake [32] who also reported the presence of terpenoids in the leaves of *A. heterophyllus*.

3.3. Antihelminthic Activity

The figure 8 gives the morphology of untreated earthworm (*Benhamia rosea*) (a) and earthworm treated with 5 mg/mL of *A. heterophyllus* organic acids extract (b); 5 mg/mL of *A. heterophyllus* ethanolic extract (c) and 5 mg/mL of albendazole (d).

(a)

(b)

(c)

(d)

Figure 8. *Morphology of untreated earthworm (a) or earthworm treated with 5 mg/mL of (b) Organic acids extract of A. heterophyllus; (c) Ethanolic extract of A. heterophyllus; (d) Albendazole (positive control).*

Figure 8a reveals that the control sample contains living earthworm. Mixed together with drugs: organic acids extract (Figure 8b); ethanolic extract (Figure 8c) or albendazole (Figure 8d); the earthworms are died. This indicates that *A. heterophyllus* have anthelmintic properties.

Tables 1 and 2 below give the results of the average values of paralysis time of worms and mortality rate of earthworms.

Table 1. *Average values paralysis time of worms (in minutes).*

Dose (mg/mL)	Drugs			dH_2O
	Albendazole	OAE	EtOH	
5	18±2.8	37±1.4	28±4.2	0
2.5	20.5±6.4	62.3±4.8	44±5.6	0
1.5	42±3.5	44.75±3	56±2.8	0
0.625	45±9.9	76±2.1	67.3±1.8	0

Legend: OAE, organic acid extracts; EtOH, ethanolic extract; dH_2O, distillated water (negative control)

Table 2. *Rate of mortality.*

Drug	Dose (mg/mL)			
	5	2.5	1.5	0.625
Albendazole (Positive control)	33,3	55,6	66,7	66,7
Organic acid extract (OAE)	96,3	70,4	67,0	62,7
Ethanolic extract EtOH)	100	94,4	66,7	33,3
dH_2O (Negative control)	0	0	0	0

It is observed from these two tables that the ethanolic extract showed better anthelmintic activity compared to the organic acid extract. This implies that compounds with high deworming activity easily pass through polar solvent (ethanol) [28]. It should be noted that both extracts have a dose-dependent anthelmintic activity whose shortest paralysis time is observed in the ethanol extract. The positive control has a short paralysis time compared to our extracts (the mortality rate remains the lowest, however). This may be due to the concentration used. In reality, the positive control is composed of a single molecule that is well identified and whose family is well known [33]; whereas the extracts are composed of a mixture of bioactive compounds.

These results are consistent with those found in India by Shekhawat and Vijayvergia [34] and Yashaswini et *al*. [35] who found high anthelmintic activity of ethanolic extract from *A. heterophyllus* Lam seeds compared to aqueous extracts; in their studies, they used the species *Pheretima posthuma* (annelid) as a biological model in the evaluation of anthelmintic activity.

Similar findings were reported by Anbu et *al*. [36] who worked with the species *Pheretima posthuma* (one annelid) and *Ascaridia galli* (one nematode). They found a strong anthelmintic activity of the ethanol extract. On the other hand, a study conducted in the Republic of Congo by Ongoka et *al*. [20] on twenty (20) anthelmintic plants using vermicompost *Vermicus terrestris* as a biological system, showed that methanol extracts have better anthelmintic activity than aqueous extracts. Studies have shown that flavonoids are among the compounds involved in anthelmintic activity [37-39]. Thus, the vermicidal activity of *A. heterophyllus* Lam extracts observed in this study would likely be due to flavonoids or polyphenols in general.

The table below shows the phenolic compound content of the leaves of *A. heterophyllus* Lam.

Table 3. *Phenolic compounds content of the leaves of A. heterophyllus Lam.*

Secondary metabolites	Concentration (mg/g)
Total Polyphenol (mgEAG/100g)	27.330 ± 9.340
Flavonoids (mgQE/100g)	2.630 ± 0.007
Anthocyanins (mgEC/100g)	10.460 ± 1.050

Legend: GAE/100g Equivalent of gallic acid (GAE) per g of dry extract; QE/g Equivalent of quercetin (QE) per g of dry extract and CE Equivalent of Cathechin.

It is deduced from the Table 3 that there is a high concentration of total polyphenols, followed by anthocyanins and a low content of flavonoids. These results corroborate those found by Loizzo et *al*. [40], who reported a high concentration of total polyphenols in the leaves of *A. heterophyllus* Lam. Studies by Sreeletha et *al*. [41] supported also the abundant presence of polyphenols and flavonoids in *A. heterophyllus*. Studies have shown that not only extrinsic factors (such as geographic and climatic factors), genetic factors, but also the degree of maturation of the plant and the duration of storage has a strong influence on the content of polyphenols [42].

Table 4 gives the antioxidant activity of organic acid extracts of *A. heterophyllus* Lam.

Table 4. *IC_{50} values (µg/ml) of the organic acid extract for ABTS and DPPH° tests.*

Extract	IC_{50} (µg/ml)	
	Radical ABTS	Radical DPPH
organic acid	0,97 ± 0,13	-

Legend: (-): No activity in the dose range of 20 to 100µg/mL.

Organic (terpenic) acid extract showed very high antioxidant activity with the ABTS radical while the same extract has not displayed any activity in the presence of DPPH radical. This phenomenon is explained by the reaction mechanism. DPPH radical reacts only with hydrophilic

compounds while ABTS reacts with both hydrophilic and lipophilic compounds. Terpenic acids being lipophilic do not react with DPPH radical [22]. A lower IC_{50} value of *A. heterophyllus* indicates a higher antioxidant activity.

Recent findings revealed that the anthelmintic compounds with imidazothiazole based-core like Albendazole have the property to react with nicotinic receptor of the parasitic worms and thus imitate the action of acetylcholine. This fixing induces the permeability change of the post-synaptic membrane causing a muscular contraction, followed of the spastic paralysis and finally, the death of the worms [43]. Natural products such phenolic compounds were also reported to have the capacity to bind to the exoskeleton proteins of cuticule or parasite enzymes and therefore affect the physical and biochemical properties of parasitic worms like the nutrition or reproduction [44, 45]. The presence of such naturally occurring bioactive compounds in Jackfruit (*A. heterophyllus*) eaves (as herein demonstrated) revealed that this plant species constitutes a good candidate for the formulation of an anthelmintic medicine to fight both parasitic worms of the gastrointestinal tract and free radicals produced in the animal body.

To our knowledge, this is the first time that the antiradical activity of the organic acids of the leaves of *A. heterophyllus* Lam. and the anthelmintic properties of these leaves have been reported.

4. Conclusion and Suggestions

In this study, the aim was to determine the qualitative and quantitative chemical composition, to extract organic acids and to evaluate the anthelminthic and antioxidant activities of *A. heterophyllus* Lam, in order to contribute to the valorization of the plant used in ethnoveterinary medicine in DR Congo. Results revealed that *A. heterophyllus* contains various secondary metabolites such as flavonoids, phenolic acids, coumarins, anthraquinones, terpenoids and anthocyanins. The organic/terpenic acids extract displayed interesting antioxidant activity against the ABTS radical (IC_{50}: 0.97 ± 0.13 µg/ml). However, at the dose of 0.625 mg/mL, ethanolic extract showed better activity with a paralysis time of 67.3±1.8 minutes compared to 76±2.1 minutes for the organic extract.

It is therefore desirable to test bioactive extracts on gastrointestinal parasites of farm animals to confirm the results obtained. Ongoing in-depth phytochemical studies will identify the chemical compound (s) and active principle (s) for the formulation of anthelminthic phytomedicine for managing pathologies due to helminthes in farm animals.

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