Phytochemical Study and Anthelmintic Activity of Nine Congolese Medicinal Plants

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors may use the following wordings for this section. Author MGRM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NLC and KAB managed the analyses of the study. Authors MBJA, BYA and OJM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Despite advances in hygiene and preventive medicine, parasitic diseases, particularly digestive parasitosis, remain a public health problem in tropical countries. Elaborate the ethnobotanical survey carried out in Brazzaville - Congo, 21 plants (divided into 20 families, 20 genera) were identified, among them 9 were selected for this study. These were: Ageratum conyzoides (L.) L., Rauvolfia mannii Stapf, Aloe buettneri A. Berger, Garcinia kola Heckel, Piper guineense Schumach & Thonn., Aframomum albviolaceum (Ridl.) K. Schum., Plagiostyles africana (Müll.Arg.) Prain, Morinda lucida Benth, Cogniauxia podoleana Baill.

Materials and Methods: The in vitro evaluation of the anthelmintic activity of the aqueous extracts of these 9 plants was determined at concentrations of 10, 25 and 50 mg/mL against Lumbricus

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1. INTRODUCTION

The parasites are very widespread in the world, but this presence is especially observed in the tropical zones. The number of subjects suffering from intestinal parasitosis is particularly high among African populations with low levels of economic and social organization, with poor hygienic conditions [1,2]. There are various modern drugs to fight against intestinal parasitosis. However, the difficult access to modern anthelmintics and, moreover, the phenomena of resistance developed by gastrointestinal parasites prompt African populations to have recourse to nature, to plants which are easily accessible and less expensive [3]. This is how ethnobotany and ethnopharmacology are working to identify reputedly biologically active plants whose modern research must specify the properties and seek new bioactive molecules [4,5]. The promotion of endogenous plant-based recipes and practices is now a scientific concern. Indeed, different techniques have been implemented to extract natural substances or active ingredients of plant origin [5-7]. Several plants which fight against intestinal parasites have been inventoried and studied in Congo [8,9]. With an aim of widening this repertory, the present study was identified 21 plants and 9 among them: Ageratum conyzoides (L.) L. (Asteraceae), Rauvolfia manii Stapf(Apocynaceae), Aloe buettneri A. Berger(Liliaceae), Garcinia kola Heckel(Clusiaceae), Piper guineense Schumach &Thon (Piperaceae), Aframomum alboviolaceum (Ridl.) K. Schum. (Zingiberaceae), Plagiochilus africana (Müll. Arg.) Prain(Euphorbiaceae), Morinda lucida Benth. (Rubiaceae), Cogniauxia podoleana Baill. (Cucurbitaceae) which have been the subject of a phytochemical study and an in vitro evaluation of the anthelmintic activity. The richness of secondary metabolites of plants leads to their use in the manufacture of phytomedics, in agriculture to control pests through plant protection effects, in cosmetics as perfume or additives, in the agri-food industry as an ingredient, food preservative [10-12]. Also, these plants are used in several preparations to remedy various diseases such as malaria, diarrhea, microbial and bacterial infections, skin troubles [5,4].

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Animal sample

Lumbricus terrestris Linn., commonly called earthworms, has been used as biological material to carry out vermicidal tests on [8,13]. They were collected in Brazzaville (Congo) near the river located at Diata (4 ° 16 ’21,747 ’South ; 15 ° 14 ’34,725 ” East) in the Makélékélé district. Their mass and their size varied from 0.4 to 3 g and 7-15 cm respectively.

2.1.2 Plants Material

Ten organs from nine plants Ageratum conyzoides (leafy stems), Aframomum alboviolaceum (Leafy stems), Aloe buettneri (Leaves), Cogniauxia podoleana (Roots and Leaves), Garcinia kola (Seeds), Morinda lucida (Roots barks), Plagiochilus africana (Roots barks), Piper guineense (Seeds), Rauvolfia manii (Roots) were harvested in July 2018 in the Mayanga nature reserve (4 ° 15’48.0954 ” South;
15° 14 '34, 3854 " East) in the morning and identified by the Systematic Botanist, Doctor Jean Marie MOUTSAMBOTE of the National Institute for Research in Exact and Natural Sciences (IRSEN). These organs were dried out of direct sunlight and then sprayed.

2.2 Methods

2.2.1 Preparation of the aqueous extracts

According to the preparation methods collected from traditional healers, a mass of 30 g of powder from each organ was either macerated during 24 h for G. kola, P. africana, P. guineense or brought to a boil during 20 min for A. alboviolaceum, A. conyzoides, A. buettneri, C. podoleana, M. lucida, R. manii in 300 ml of distilled water. After filtration and concentration in a rotary evaporator (BUCHI Water bath B-480), the dry extracts obtained are put in the bottles for analysis.

Preparation of drug solutions: The extracts used to carry out the vermicidal tests were prepared by dissolving 0.2; 0.5 and 1 g of dry extract in 20 mL of distilled water.

2.2.2 Evaluation of the anthelminthic activity (Vermicide test)

The method of Ajayieoba [14] with slight modifications was used for carrying out the vermicidal tests of the aqueous extracts. A range of three concentrations (10, 25 and 50 mg/mL) has been prepared for each plant extract. Mebendazole (20 mg/mL) was used as the standard anthelmintic. Earthworms were placed in sets of five in petri dishes:

- **Lot 1**: negative control lot, has been treated with distilled water only;
- **Lot 2**: test lot, was treated with plant extracts at different concentrations;
- **Lot 3**: positive control lot, was treated with Mebendazole (20 mg/mL), standar drug.

The behavior (hypermobility and mortality) of the worms in each dish was observed during 24 h. The paralysis and lethality times (100%) were noted. The experiment is repeated three times.

2.2.3 Phytochemical screening by TLC

The TLC was determined with the extract macerated in organic solvents (1g in 100 mL) in order of increasing polarity (Hexane, Chloroform, Ethylacetate, Ethanol). Specifics developers were used to identify the chemical families present in plants extract [6,7,15,16] (Table 1).

2.2.4 Quantitative composition of phytochemical families

2.2.4.1 Total polyphenols

The determination of total polyphenols is made by UV-Visible spectrophotometry with the Folin-Ciocalteu reagent according to the method of Singleton [17]. Gallic acid, prepared under the same conditions as the plant samples, was used as the reference compound. The contents expressed in equivalent micrograms of gallic acid per gram of dry matter (µg EAG/g DM).

2.2.4.2 Total Flavonoids

The total flavonoids contents of the aqueous extracts are determined with aluminum trichloride (AlCl₃) according to the method of Swain [18,19]. Catechin prepared under the same conditions as the plant samples was used as the reference compound. The results obtained are expressed in micrograms of catechin equivalent per gram of dry matter (µg ECAT/g DM).

2.2.4.3 Condensed and hydrolyzable tannins

The condensed and hydrolyzable tannins are measured respectively by sulfuric vanillin and iron trichloride (FeCl₃) according to Swain [18,19].

2.2.5 Evaluation of antioxidant activity by spectrophotometry UV-Visible

For each extract, a range of concentrations (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL and 0.125 mg/mL) is prepared in absolute ethanol. 1 mL of these samples was added to 1.5 mL of ethanolic solution of DPPH radical (0.03 mg/mL). The mixture obtained is well agitated and left to incubate for 30 min at room temperature. The absorbance of the mixture is read at 517 nm with a UV-Visible spectrophotometer against a blank consisting of 1 ml of absolute ethanol (without extract) and 1.5 mL of DPPH solution. The reference positive control was ascorbic acid (vitamin C) [20].

The percentage reduction of the DPPH radical is given using the following formula:

\[ R(\%) = \frac{(A_b - A_e)}{A_b} \times 100 \]  (Eqn. 1)
R: percentage reduction of the DPPH radical; Ab: Absorbance of white; Ae: absorptance of the sample.

The concentrations necessary to reduce 50% (CR₅₀) of radical DPPH were given with the Excel software.

2.2.6 Data processing and analysis methods

Two data precesion software were used Microsoft Excel 2013 and STATISTICA 7.1. The vermicidal test of the extracts was represented by three variables, the concentration of the extracts, the paralysis and death of worms. The statistical analysis of the results of the vermicidal test, content of phytocompounds and the antioxidiant activity were carried out by using the software Excel 2013 and STATISTICA 7.1. The average value are accompanied by the standard error on the average (Average ± SEM).The difference between two values was considered as significant when p < 0.05 and p < 0.01. The Student-Newman-Keuls test was used to separate the averages obtained. The significance of the differences is appreciated at the 5% and 1% threshold. Also, STATISTICA 7.1 was used to show changes in the percentage reduction of DPPH based on concentrations, and the hierarchical ascending classification (CAH) to show plant organization by group according to the concentration needed to reduce 50% of DPPH (CR₅₀) range of values. This analysis synthesizes a large amount of data to extract the main factors that have caused variables to evolve simultaneously with their own relationships [21,22].

3. RESULTS AND DISCUSSION

3.1 Anthelmintic Activity

3.1.1 Paralysis time of Lumbricus terrestris Linn

The worms were suspected to be paralyzed when they can no longer move completely in the petri dish [8,13]. Thus, at the lowest concentrations of plant extracts (10 and 25 mg/mL), the paralysis of the worms occurred after a long time of contact with the samples. On the other hand, this time decreased at the concentration of 50 mg/mL for three plants, namely P. guineense (10.25 ± 0.89 min), followed by A. conyzoides (19.66 ± 0.81 min) and A. albiovaleaceum (35.67 ± 2.03 min). The standar drug, Mebendazole, showed a lower paralysis time (7.00 ± 0.28 min), as shown in Table 2. The six plants (P. africana, C. podoleana, C. podoleana, M. lucida, A. buettneri, G. kola, R. mannii) showed a longer paralysis time at all concentrations used (10, 25 and 50 mg/mL) compared to the standar drug Mebendazole (20 mg/mL) which caused paralysis of the worms after 5 min. The constant contact of the worms with extracts has led to hyper-mobility and a tendency to want to get out of the petri dish. The permeability of the worms caused their paralysis and some resolved over time before dying. This phenomenon has been observed on the same type of worms during works carried out by Ongoka in Congo and Guissou in Burkina [8,13]. The increasing order of paralysis time of worms by extracts at all concentrations was the same and presented as follows: P. guineense < A conyzoides. < A. albiovaleaceum < P. africana < M. lucida < C. podoleana < A. buettneri < G. kola < R. mannii (Table 2).

3.1.2 Mortality time of Lumbricus terrestris Linn

The death time of worms based on concentrations showed in Table 2 for each species and the standar drug Mebendazole. Earthworms are considered dead when they were stationary 5 min after having noted the death time. The shorter time of the total lethality time (100%) of worms proved that the extract exhibited a strong vermicidal activity [14]. The results obtained showed that the death time of worms depends on the concentration of plant extracts, so at 10 and 25 mg/mL this time looks longer than at 50 mg/mL. In comparison with the standar drug (Mebendazol), the aqueous extract of the seeds of P. guineense showed the best vermicidal activity against L. terrestris followed by the aqueous extracts of the leafy stems of A. conyzoides and A. albiovaleaceum. Analysis of these results points out that P. guineense (40 < 0.68 min) followed by A. conyzoides (62 ± 0.28 min) and A. albiovaleaceum (86 ± 3.21 min) showed total death of worms at low times at 50 mg/mL compared to the other 06 plants studied namely : P. africana (160 ± 0.96 min), C. podoleana leaves and roots (210 ± 3.08 min and 519 ± 3.23 min), M. lucida (175 ± 2.98 min), A. buettneri (270 ±7.21 min), G. kola (280 ± 2.88 min), R. mannii (325 ± 1.45 min). While at concentrations of 10 of 25 mg/mL, total worm mortality occurs at longer times as shown in Table 2 relative to the standar drug Mebendazol. The increasing order of death time of worms by extracts at all concentrations makes it possible to classify these species as follows: P.
studies revealed positive vemicide activity of Koorse et al., Adate et al., and Akter et al., and the worms model used is not that of humans. In the treatment of intestinal worms although the surveys conducted by Bouquet near traditional healers in Congo [5] indicated that fresh seeds of \textit{P. guineense}, \textit{A. alboviolaceum} and macerated \textit{P. africana} leaves have anthelmintic properties. The results obtained confirm the use of these plants by traditional medicine in the country in the treatment of intestinal worms although the worm model used is not that of humans. In addition, the work of Koné et al. carried out in Ivory Coast showed an anthelmintic activity of the leaves of \textit{A. alboviolaceum} and the fruits of \textit{G. kola} on the \textit{Haemonchus contortus} [23]. Also, Koorse et al., Adate et al., and Akter et al., studies revealed positive vemicide activity of leaf and fruit on the genus \textit{Piper} tested on strongyles in ruminants [24-26]. The results obtained seem to confirm the use of these plants by traditional healers in the Congo in the treatment of intestinal worms.

### 3.2 Preliminary Phytochemical Composition of Plant Extracts

The chromatographic profile of the extracts from the 9 anthelmintic plants investigated revealed the presence of five chemical families, namely sterols / terpenes, coumarins, flavonoids, tannins and alkaloids (chromatograms 1-4).

Observation of the chromatograms 1 and 3 of the extracts of the plants macerated in chloroform, obtained with eluting mixture Hexane / Chloroform / Ethyl acetate, after revelation with the Lieberman-Büchard reagent, KOH at 5%, respectively showed the presence of several spots of different colors (blue green, yellow, brown), compounds of the triterpene / sterol type at 365 nm UV and blue, yellow and green molecular imprints characteristic of coumarins [7,15].

**Table 1. Developers used and composed revealed into TLC**

| Developers                                      | Phytochemical families                  |
|------------------------------------------------|----------------------------------------|
| Liebermann-Burchard; KOH with 5%; NH\textsubscript{3} | Sterols and Terpenes, Coumarins        |
| AlCl\textsubscript{3} with 1%, NH\textsubscript{3}, FeCl\textsubscript{3} with 2%; Dragendorff | Flavonoids, Tannins, Alkaloids         |

**Fig. 1. Time of total mortality (100%) of worms as a function of concentrations**

Test extract: significant from normal control, * $P < 0.05$; ** $P < 0.01$

$\text{Mean} \pm \text{S.E.M} = \text{Mean values} \pm \text{Standard error of means of three experiments}$
Chromatogram 1: Sterols and Terpenes revealed under UV / 365 nm light in chloroform extracts

Chromatogram 2: Flavonoids revealed under UV / 365 nm light in hydroethanolic extracts

Chromatogram 3: Coumarins revealed under UV / 365 nm light in chloroform extracts

Chromatogram 4: Tannins revealed in visible form in ethyl acetate extracts

Developper reagent: Lieberman-Büchard
Eluting mixture: Chloroform / Ethyl acetate / Hexane (10/10/5 v/v/v)

Developper reagent: Neu
Eluting mixture: Ethyl acetate / Acetic acid / Water (8/1/1 v/v/v)

Developper reagent: KOH 5%
Eluting mixture: Chloroform / Ethyl acetate / Hexane (10/10/5 v/v/v)

Developper reagent: FeCl₃ 2%
Eluting mixture: Ethyl acetate / Methanol / Water / Hexane (11,9/1,6/1,4/3,5 v/v/v/v)

1: A. conyzoides (Leafy stems); 2: R. manii (Roots); 3: A. buettneri (leaves); 4: G. kola (Seeds); 5: P. guineense (Seeds); 6: A. alboviolaceum (leafy stems); 7: P. africana (trunk barks); 8: M. lucida (Roots barks); 9: C. podoleana (Leaves); 10: C. podoleana (Roots)

Fig. 2. Preliminary phytochemical composition of plant extracts
The extracts of the roots of R. mannii, C. podoleana, the seeds of G. kola, P. guineense, the leaves of A. buettneri, C. podoleana and the roots barks of M. lucida, revealed the co-presence of sterols, terpenes and coumarins. Some authors also proved the presence of terpenes, sterols, coumarins, flavonoids and tannins in the leaves of A. conyzoides, the seeds of P. guineense and the leaves of M. lucida noting the presence of alkaloids for A. conyzoides and their absence for M. lucida [27,28]. Also, the work of Munikishore et al., and Okunade, respectively, isolated two flavonoid-type molecules and determined the compounds present in the plant A. conyzoides [29,30].

The chromatographic profile 2 of the polar extracts (ethanol-water) after revelation, showed the presence of blue, green, yellow, orange spots, blue green and orange fluorescent spots, characteristics of flavonoids [7,15]. These compounds are less abundant in the extracts of P. africana trunk bark and the roots of C. podoleana.

The presence of tannins obtained after revelation with Iron trichloride, are only observed in the organs of A. conyzoides, G. kola, A. alboviolaceum, and M. lucida, C. podoleana (Chromatogram 4). Also, the studies of Dharajiya et al. revealed the presence of sterols, flavonoids, tannins, phenolic compounds and alkaloids in the Aloe genus [31]. The absence of alkaloids, terpenes and sterols as well as the presence of tannins, flavonoids, coumarins in the leaves of P. africana was proved by some authors [32]. Streaks of alkaloids are observed only in the roots of R. mannii and the leaves of C. podoleana. Two alkaloids, vincajamine and reserpine, have been isolated from the leaves and roots of R. mannii, respectively [33,34].

In addition, studies of Bosi et al. indicated the presence of alkaloids of the pyrrolizidine type in the whole plant of A. conyzoides [35]. Data from the literature have shown that the plant C. podoleana contains the purgative reserpine, the leaves and roots contain the alkaloids and terpenes-steroids [36]. The phytochemical screening of the seeds the species G. kola showed the presence of alkaloids, showed the presence of tannins, flavonoids, steroids, flavonoids on the one hand the absence of alkaloids on the other [37,38]. The absence of alkaloids in G. kola could be explained by the maturity stage of the harvested fruit, because studies of Morabandza et al. showed that the concentration of metabolites in the mesocarp of G. kola fruit increases with the stages of fruit maturity [39]. Though, the absence of these secondary metabolites in some plants studied, could be justified by influences of certain environmental factors of the place of harvest such as climate, soil composition and season of sample collection [40]. These compounds may be responsible for the vermicide effect of plants against earthworms Lumbricus terrestris. [41,42].

Table 2. Paralysis time (± E.S.M) of Lumbricus terrestris Linn

| Plants          | 10       | 25       | 50       |
|-----------------|----------|----------|----------|
| Pg              | 45.01±1.25** | 40.00±1.09* | 10.25±0.89* |
| Ac              | 71.67±2.36** | 73.39±2.97** | 19.66±0.81* |
| Aa              | 187.35±2.21* | 162.33±2.91* | 35.67±2.03* |
| Paf             | 244.00±7.05 | 143.52±1.28* | 44.66±2.60** |
| Cpf             | 289.36±0.89* | 61.66±6.00  | 53.33±0.89* |
| Cpr             | 304.35±2.19* | 215.33±1.20* | 73.35±2.96* |
| Ml              | 518.06±2.27** | 310.00±1.00* | 118±1.20*  |
| Ab              | 618.34±2.23** | 339±3.06**  | 59.53±2.96** |
| Gk              | 426±1.33* | 230±1.15*  | 157±3.21** |
| Rm              | 306.67±2.21** | 186.33±7.05 | 108.66±1.20* |
| Meb             | 20.33±0.89* | 7.00±0.12*  | -         |

Pg : P. guineense (Seeds) ; Ac : A. conyzoides (leafy stems) ; Aa : A. alboviolaceum (leafy stems) ; Paf : P. africana (Barks of trunk) ; Cpf : C. podoleana (Leaves) ; Cpr : C. podoleana (Roots) ; Ml : M. lucida (Root bark) ; Ab : A. buettneri (Leaves) ; Gk : G. kola (Seeds) ; Rm : R. mannii (Roots) ; Meb : Mebendazole Paralysis time (min); Concentration (mg/mL)

Test extract : significant from normal control, * P < 0.05; ** P < 0.01

Mean ± S.E.M = Mean values ± Standard error of means of three experiments
3.3 Quantitative Composition of Phytochemical Families (Contains total Polyphenols, Total Flavonoids and Hydrolysable and Condensed Tannins)

3.3.1 Total polyphenols

The total polyphenol contents were expressed in equivalent micrograms of gallic acid per gram of dry matter according to the calibration curve for gallic acid (y = 0.0152x + 0.0045; R² = 0.9997). As shown in Fig. 3A, the *A. alboviolaceum* plants followed by *P. africana* and *C. podoleana* leaves showed high total polyphenol values 782.03 ± 0.87, 276.71 ± 1.19 and 241.61 ± 1.47 µgEAG / g DM respectively. The plants *A. conyzoides*, *C. podoleana* roots, *G. kola*, and *R. mannii* showed average total polyphenol contents between 157.61 ± 0.32 µg EAG / g DM and 116.88 ± 0.16 µg EAG / g DM. On the other hand, the plants *P. guineense*, *A. buettneri* and *M. lucida* had the low total polyphenol contents respectively 78.22 ± 3.94, 47.95 ± 0.55 and 17.33 ± 0.09 µg EAG / g DM.

3.3.2 Total flavonoids

The amounts of flavonoids were expressed in equivalent micrograms of catechin per gram of dry matter according to the standard curve for Catechin (y = 0.3706x - 0.0038; R² = 0.9998). The Graph 3A indicated that the flavonoid contents of plants were between 12.08 ± 2.07 and 44.57 ± 0.21 µg EC / gDM. Thus, *G. kola* and *A. alboviolaceum* showed high levels of flavonoids, respectively 44.57 ± 0.21 and 36.19 ± 0.57 µg EC / g DM. *C. podoleana*, *M. lucida*, *A. buettneri*, *A. conyzoides* plants showed average flavonoid contents between 32.25 ± 1.01 and 22.17 ± 1.55 µg EC / g DM. While the plants *R. mannii*, *P. africana* and *P. guineense* showed low levels of flavonoids namely 14.9 ± 0.15, 12.66 ± 0.11 and 12.08 ± 2.07 µg EC / g DM.

3.3.3 Condensed and hydrolysable tannins

The contents of condensed tannins have been indicated in graph 3B. The values obtained were between 278.72 ± 0.01 and 12.48 ± 0.08%. The high values were observed for the species *A. alboviolaceum*, *P. guineense* and *G. kola* (278.72 ± 0.01, 228.54 ± 0.01 and 227.68 ± 0.02 mg%). The average contents were observed with the plants *M. lucida*, *A. buettneri*, *A. conyzoides* *R. mannii* whose values were between 116.74 ± 0.08 and 25.48 ± 0.02%. On the other hand, the plants *C. podoleana* (leaves) *P. africana* and *C. podoleana* (roots) presented the low contents of condensed tannins namely 15.6 ± 0.57, 18.72 ± 0.11 and 12.48 ± 0.08%.

The hydrolyzable tannin contents have been indicated in graph 3B. The values obtained were between 46.20 ± 0.29 and 1.66 ± 0.07%. The high values were observed for the species *A. alboviolaceum*, *M. lucida*, *P. guineense* and *C. podoleana* leaves whose respective contents 46.20 ± 0.29, 40.399 ± 0.12, 39.70 ± 0.08 and 39.14 ± 0.17%. The plants *G. kola*, *A. conyzoides* and *A. buettneri* showed average contents whose values were between 31.12 ± 0.12 and 15.63 ± 0.06. Unlike *P. africana* plants, *R. mannii* and *C. podoleana* roots exhibited low levels of hydrolyzable tannins namely, 5.394 ± 0.05, 1.66 ± 0.07 and 0.97 ± 0.58%.

The phytochemical composition and the variation of phytocompound content in these plants could justify the variation of the anthelmintic activity of these plants. Indeed, the *A. alboviolaceum* plant exhibited high levels of total polyphenols (782.03 ± 0.87 µg EAG / g DM), of total flavonoids (36.19 ± 0.57 µg EC / g DM), of condensed tannins (278.72 ± 0.01%) and hydrolyzable tannins (46.20 ± 0.29%). Thus, the *G. kola* with contents of total polyphenols (116.88 ± 0.16µg EAG / g DM), total flavonoids (44.57 ± 0.21µg EC / g DM), condensed tannins (227.68 ± 0.02%) and hydrolyzable tannins (31.12 ± 0.12%). While the *P. guineense* showed high contents of condensed tannins (228.54 ± 0.01%) compared to the contents of total polyphenols (78.22 ± 3.94 µg EAG / g DM), total flavonoids (12.08 ± 2.07 µg EC / g DM) and hydrolyzable tannins (39.70 ± 0.08%) as well as *M. lucida* with contents of condensed tannins (116.74 ± 0.08%), polyphenols (17.33 ± 0.09µgEAG / g DM), flavonoids (30.01 ± 2.30 µg EC / g DM) and hydrolyzable tannins (40.399 ± 0.1%). Other plants (*A. conyzoides*, *A. buettneri*, *C. podoleana*, *M. lucida*, *P. guineense*, *R. mannii*) presented only high contents of total polyphenols compared to the other quantified phytocompounds (total flavonoids, hydrolyzable tannins and condensed tannins). The phytochemical composition of these extracts could justify the observed vermicidal activity.

3.4 Antioxidant Activity by Spectrophotometry UV-Visible

The significant reduction of the DPPH radical by the plant extracts was shown in Figs. 4 and 5. Thus, the extracts of *A. alboviolaceum* (92.35%) followed by *P. africana* (86.9%) and *A. buettneri*
(80.5%) showed significant antioxidant activity at 0.5 mg / mL compared to the other six plants although it was moderately low compared to ascorbic acid (96.23%). In addition, the leafy stems of A. alboviolaceum exhibited similar efficacy (92.35%) to that of the standard compound ascorbic acid (Fig. 4). The CR$_{50}$ value is the concentration of extract reducing 50% of the DPPH radical, and values of CR$_{50}$ have been presented in Table 3 for each species. The lower value of CR$_{50}$ determines the greatest antioxidant activity. Thus, compared to ascorbic acid (CR$_{50}$ = 0.015 mg / mL), the plants A. alboviolaceum (CR$_{50}$ = 0.098 mg / mL), A. buettneri (CR$_{50}$ = 0.10 mg / mL) showed the lowest values of CR$_{50}$; while M. lucida (CR$_{50}$ = 6.11 mg / mL), C. podoleana (CR$_{50}$ = 8.13 mg / mL) have the highest values of CR$_{50}$.

Among the 07 plants studied, 03 of them (A. buettneri, A. alboviolaceum, P. africana) showed good antioxidant activity by the DPPH radical test. The CR$_{50}$ values varied between 0.098 and 0.19 mg / mL and that of ascorbic acid 0.015 mg / mL. In addition, the ascending hierarchical classification (CAH) was carried out to assess the results of the antioxidant activity of plant extracts in comparison with ascorbic acid. Fig. 5 has shown that the antioxidant potential of the extracts allows these plants to be subdivided into three distinct groups, the G$_1$ group, whose plant extracts have a significant antioxidant potential with CR50 values between 0.096 and 0.10 mg / ml: A. buettneri (CR$_{50}$ = 0.10 mg / mL), A. alboviolaceum (CR$_{50}$ = 0.098 mg / mL) and P. africana (CR$_{50}$ = 0.109 mg / mL). The G$_2$ group of plant extracts with low antioxidant potential with CR$_{50}$ values between 1.09 and 1.85 mg / mL: C. podoleana (CR$_{50}$ = 1.09 mg / mL), R. mannii (CR$_{50}$ = 1.23 mg / mL) and A. conyzoides (CR$_{50}$ = 1.85 mg / mL) finally the G$_3$ group showing plants with very low antioxidant potential compared to the reference molecule with CR$_{50}$ values between 3.284 and 8.13 mg / ml: P. guineense (CR$_{50}$ = 3.284 mg / mL), M. lucida (CR$_{50}$ = 6.109 mg / mL) and C. podoleana with CR$_{50}$= 8.13 mg / mL. Thus, the results obtained made it possible to conclude that the plants of the G$_1$ group (A. alboviolaceum, A. buettneri, P. africana) had a better antioxidant potential. The levels of polyphenols obtained in the 09 plants could be the cause of this variation in antioxidant potential from one plant to another.

**Fig. 3.** Contains polyphenols, flavonoids and hydrolysable and condensed tannins
Fig. 4. Evolution of the percentage of reduction of the various plants according to the concentration

![Graph showing the evolution of percentage reduction for various plants at different concentrations.]

Fig. 5. Dendrogram of reduction concentrations of DPPH of plants extracts and vitamin C

Table 3. CR$_{50}$ of the aqueous extracts of the selected plants and the vitamin C

| Plants        | CR$_{50}$ | Ac     | Ab      | Rm    | Gk     | Pg     | Aa     | Paf    | Mi     | Cpr    | Cpf    | vit C |
|---------------|----------|--------|---------|-------|--------|--------|--------|--------|--------|--------|--------|-------|
| A. conyzoides | 1.85     | 0.096  | 1.233   | 0.759 | 3.28   | 0.098  | 0.191  | 6.109  | 8.125  | 1.09   | 0.015  |       |
| R. mannii     |          |        |         |       |        |        |        |        |        |        |        |       |
| A. buettneri  |          |        |         |       |        |        |        |        |        |        |        |       |
| A. guineense  |          |        |         |       |        |        |        |        |        |        |        |       |
| P. guineense  |          |        |         |       |        |        |        |        |        |        |        |       |
| A. alboviolaceum |      |        |         |       |        |        |        |        |        |        |        |       |
| P. africana   |          |        |         |       |        |        |        |        |        |        |        |       |
| M. lucida     |          |        |         |       |        |        |        |        |        |        |        |       |
| C. podoleana  |          |        |         |       |        |        |        |        |        |        |        |       |
| V. C          |          |        |         |       |        |        |        |        |        |        |        |       |

Ac (A. conyzoides); Rm (R. mannii); Ab (A. buettneri); Gk (G. kola); Pg (P. guineense); Aa (A. alboviolaceum); Paf (P. africana); Mi (M. lucida); CP (C. podoleana); Vit C (Vitamin C)
3.5 Correlation between the Contents (Total Phenols, Flavonoids, Tannins) and the CR$_{50}$

Correlations between the proportioned total compound (total polyphenols, flavonoids total, tannins condensed and hydrolyzables) and the concentration of reduction at 50% of radical DPPH have been established (Fig. 6). The coefficients were respectively $R^2 = 0.046$ and $R^2 = 0.014$ for total polyphenols and tannins hydrolyzables. The results proved, there is no correlation between the contents total polyphenols, tannins hydrolyzables with the concentration of reduction of DPPH radical for the majority of the species. In addition, this correlation remains still low between the total flavonoids, the tannins condensed with the concentrations of reduction which have coefficients about $R^2 = 0.0833$ and $R^2 = 0.0935$. The presence of one of these families of compounds could in part confer antioxidant activity in plants, and the presence of all of these families in plants could amplify this antioxidant potential of plants. Bouyahya's study proved the implication of the content of phytocomponents, mainly flavonoids, in the antioxidant potential [43]. In addition, flavonoids and phenols are recognized for their antioxidant power, also, the antioxidant activity determined although low could derive from the presence and synergy of all these phytocompounds in the selected plants [44,45,46].

Fig. 6. Correlation between CR$_{50}$ and contents of compounds polyphenolic
4. CONCLUSION

In order to contribute to the promotion of traditional medicine, precisely in the treatment of intestinal parasitosis, phytochemical screening, evaluation of antioxidant and anthelmintic activities of aqueous extracts from the organs of the 9 plants listed, have been carried out. Phytochemical screening made it possible to highlight 3 phytochemical families, namely alkaloids, phenolic compounds (coumarins, flavonoids, and tannins), steroids and terpenes. The contents of total polyphenols and condensed tannins are quite high compared to those of total flavonoids and hydrolysable tannins. The anti-radical test by spectrophotometry with regard to DPPH radical, revealed the significant manifestation of the antioxidant activity of the 9 plants studied, and this, in comparison with vitamin C. With regard to the standard drug Mebendazole, the aqueous extract of seeds of P. guineense, A. conyzoides and A. albiovaleceum exhibited the best vermicidal activity against L. terrestris. would justify the use of said plants in the treatment against intestinal worms. The results of anthelmintic activity in vitro obtained cannot be the same to those obtained in vivo. Thus, the determination and identification of the chemical structures of these anthelmintic phytocompounds would be necessary to complete this work and be able to carry out in vivo tests in order to formulate anthelmintic plant products in our country.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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