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Microscopic features and chromatographic fingerprints of selected congolese medicinal plants: *Aframomum alboviolaceum* (Ridley) K. Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook.f.) Skeels

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Abstract

Nearly 80% of people depend on the traditional medicines for their primary health care according to the World Health Organization. In fact, the study of plant chemistry is still a relevant subject despite its antiquity. The main objective of this study was to determine the microscopic features of different Congolese taxa and to determine their chromatographic fingerprints. Three plant species used notably were: *Aframomum alboviolaceum* (Ridley) K. Schum., *Annona senegalensis* Pers. and *Mondia whitei*. The microscopic features of these plants were carried out along with their phytochemical screening by thin layer chromatography and the spectrophotometric determination of various secondary metabolites contained in these plant species. The microscopic study of *A. Alboviolaceum*, *A. senegalensis* and *M. whitei* revealed the presence of paracytic stomata, fibers, fragments of spiral bundles, non-glandular hairs, parenchyma with numerous starch grains, secreting hairs, fragments of punctuated vessels, as well as lignified fibers. Thin layer chromatographic analysis revealed the presence of flavonoids, phenolic acids, coumarins, anthraquinones, anthocyanins, tannins, irridoids, and the absence of alkaloids in all three plants. In the light of these results, it would be desirable to pursue thorough phytochemical studies in order to isolate the bioactive compounds and elucidate their structures.

Keywords microscopic features, phytochemical screening, traditional medicine

Introduction

The World Health Organization (WHO) reported that almost 80% of people rely on the traditional medicines for their primary health care [1]. Significant economic benefits in the development of this medicine are in the use of medicinal plants for the treatment of various diseases [2]. Therefore, the search for active ingredients derived from plants is more relevant than ever. This is mainly due to the fact that the plant kingdom represents an important source of immense variety of bioactive compounds [3], and these molecules have multiple interests and they are used in the food, cosmetics and pharmaceutical industries. Among these compounds, there are coumarins, alkaloids, phenolic acids, tannins, terpenes, flavonoids, etc. [4], which are endowed with interesting pharmacological properties comprising

antioxidant activity. The main objective of this study was to determine the the microscopic features and phytochemical composition of *Aframomum alboviolaceum*, *Annona senegalensis* and *Mondia whitei*. Specific objectives were: (1) to determine the microscopic features of these three plant powders; (2) to evaluate the qualitative chemical composition *of A. alboviolaceum* leaves, *A. senegalensis* and *M. whitei* root barks thru thin layer chromatography (TLC); (3) to extract the organic acids, and; (4) to measure out total polyphenol and flavonoid contents.

Methodology

Plant Material

In the present study, three plant species were used, namely: *A. alboviolaceum* (Ridley) K. Schum., *A. senegalensis* Pers. and *M. whitei*. These plants were selected from previous ethnobotanical surveys [5] and were collected in April 2016 at Mitendi city while *M. whitei* root barks were purchased at Matete market, both in Kinshasa city, Democratic Republic of the Congo (DRC). They were identified at the Laboratory of Systematic Botany and Plant Ecology, Department of Biology, Faculty of Sciences, University of Kinshasa. These samples were dried in ambient air (± 27 °C) at the Laboratory of Molecular Bio-Prospection (Department of Biology) for two weeks and were crushed to obtain the fine powder. Different parts of these plants are described in following figures.

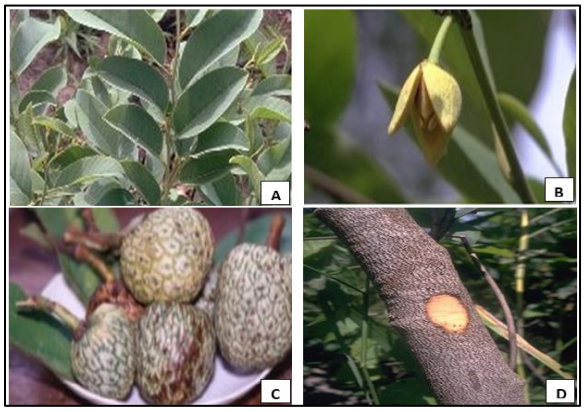


Figure 1. (A) Leaves (B) Inflorescence (C) fruits (D) Trunk of *A. senegalensis* Pers (Source: Orwa et al., [6])

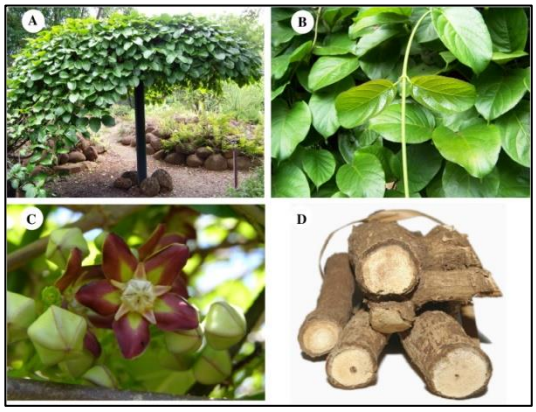


Figure 2. (A) Whole plant (B) Leafy stem (C) Young buds and flowers entirely opened (D) Roots of *M. whitei* (Source :Aremu et al., [7]).

Powder micrography

Microscopic feature is one of the most fundamental methods of controlling plant drug quality. It is very important to carry out well the preparation of the plate to be observed under the microscope in order to distinguish different elements constituting the powder. Two-three drops of selected reagent were placed on a slide and a small amount of powder is added. This slide is covered with a cover-slide in order to homogenize the preparation followed by the microscopic feature examination. It is important to prepare well by thoroughly wiping the outer surface of the slide and light preparations should be made in order to distribute the tissues well and avoid the over-exposure.

Phytochemical Screening

This screening was performed according to the standard protocol described by Bruneton [8] and Tiwari et al. [9].

Search for Flavonoids and Phenolic Acids

One gram of pulverized drug was extracted with 5 mL of methanol by stirring for 10 minutes. Afterwards, 10 mL of filtrate was used for Thin Layer Chromatography (TLC) analysis. Silica gel F_{254} was used as a

stationary phase, and formic acetic acid – glacial acetic acid - water (100:11:11:26) as mobile phase 1 and dichloromethane, formic acid, acetone (80:10:20) as mobile phase 2. As controls, Rutin, hyperoside, isoquercitrin and chlorogenic acid were used. Once developed, the chromatogram was observed under UV at 254 and 366 nm and was then sprayed with DPBAE / PEG reagent and observed under UV at 366 nm. The presence of flavonoids was marked by the presence of fluorescent spots of various colors (yellow-orange-green) varying according to the structure of highlighted compounds.

Search for Iridoids

For iridoids test, Silicagel F_{254} remained stationary phase and ethyl acetate-methanol-water (100: 13.5: 10) were used as mobile phase. The revelation was carried out with 5% sulfuric acid in ethanol by heating for 10 minutes at 100 °C. True iridoids gave colorations, while other terpenes were colored in black.

Search for Anthocyanins

For Anthocyanins test, stationary phase remained same as described above and ethyl acetate-formic acid - water (100: 10: 40) was the mobile phase. The revelation was carried out with phosphoric vanillin on the plate by heating for 10 minutes at 100 °C.

Anthraquinones (anthracene heterosides)

For *Anthraquinones test,* ethyl acetate-methanol-water (100: 13.5: 10) was used as mobile phase. Revelation was performed under UV between 254 and 366 nm and the spraying was performed with ethanolic KOH (10%). The anthraquinones were red colored reflecting red fluorescence at 366 nm, while anthrones were colored in yellow.

Terpenes

One gram of pulverized drug was extracted with 10 mL of dichloromethane by stirring for 15 minutes. The filtrate was evaporated to dryness and the residue was dissolved in 0.5 mL of toluene. Silicagel F_{254} was used as stationary phase and ethyl toluene-acetate (93:7) was used as mobile phase. Thymol, menthol, oleanic acid and 1 mg/mL (methanol) were used as controls. The revelation was carried out with sulfuric vanillin by heating for 10 minutes at 100 °C. Terpenes gave various colors using this reagent.

Coumarins

The solution prepared for terpenes was used with a deposit of 10 µL. The mobile phase used was toluene-ether (1:1, saturated with 10% acetic acid). This mobile phase was prepared from the mixture of 10 mL of toluene, 10 mL of ether and 10 mL of 10% acetic acid in a separatory funnel, where lower phase was removed and the upper phase was used as mobile phase. The revelation was carried out under UV between 254 and 366 nm and the spraying is performed with ethanolic KOH (10%). The blue color was charasterisitic of coumarins.

Alkaloids

In an acidic medium, 0.3 g of drug powder was introduced into an Erlenmeyer flask and 3 mL of 5% diluted hydrochloric acid were added. The mixture was sealed and stirred for 30 minutes and the filtrate was collected. In a test tube, five drops of Mayer's reagent was added to one mL of the filtrate. The presence of alkaloids was observed by the appearance of a white precipitate or turbidity. In case, where this general test was positive, a thin layer chromatography (TLC) was required. One g of the drug powder was macerated into one mL of 10% ammonia in an Erlenmeyer flask; 5 mL of ethyl acetate (or methanol to extract the quaternaries) was added and stirred for 30 minutes. Further, 20 µL and 50 µL of filtrate were used for TLC analysis. Dichloromethane-methanol ammonia 25% (8:2:0.5) served as a mobile phase and 5 mg/mL caffeine was used as control with a 10 µL deposition. The chromatogram was observed under UV between 254 and 366 nm and was then sprayed using. Draggendorff reagent and observed under visible light. The presence of alkaloids was marked by the presence of spots ranging from yellow-orange to yellow-brown.

Preparation of ethanol extracts

Fifty grams of *A. alboviolaceum* (leaves), *A. senegalensis* (bark) and *M. whitei* (barks) were macerated in 500 mL of ethanol (polar solvent) for 48 hours. After filtration, filtrates were concentrated on a rotary evaporator and then, evaporated to dryness in an oven at 40 °C for 48 hours.

Dosage of secondary metabolites

Dosage of total polyphenols

Total polyphenol content of extracts was determined using Folin-Ciocalteu method [10]. Ten mg/mL of each extract was diluted in methanol 80% in order to obtain a 1 mg/mL solution for each extract. Afterwards, for each extract, a reaction mixture composed of 0.5 mL of extract 5.0 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent was prepared. Three minutes later, 1.0 mL of saturated 20% Na_2CO_3 solution was added to the mixture. Different mixtures prepared were stirred and incubated at laboratory temperature under shade for one hour. Absorbances were read at 725 nm and the analysis was performed in triplicate. The amount of total polyphenols was expressed in mg equivalents of gallic acid (GAE) / g of dry extract using the following equation from the calibration line: $y = 1.7097 \ln(x) + 5.2062$ and $R^2 = 0.965$, where x is the absorbance and y is the equivalent of gallic acid (mg/g).

Dosage of total flavonoids

The reaction mixture contained one mL of methanolic solution (80%) of each of extracts having a concentration of ten mg/mL and one mL of 2% $AlCl_3$ (dissolved in methanol) and the whole mixture was well stirred. After one hour of incubation at laboratory temperature and under shade, different absorbances were measured at 415 nm using a spectrophotometer (GENESYS 10S). For each analysis, mixtures were prepared in duplicate. For preparing the blank, the procedure was the same as described above but in lieu of the extract, one mL of methanol was added. The flavonoid content of the extracts was expressed in mg equivalent quercetin (QE)/g of corresponding dry extract using the equation from the calibration line: $y = 0.5001 \ln(x) + 3.442$, $R^2 = 0.944$ where x is the absorbance and y is the equivalent of quercetin (mg / g) [11].

Results and Discussion

Botanical microscopic characters

The results of the microscopic features of plant powders are presented in Figures 3, 4 and 5. As shown in Figure 3, leaves of *A. alboviolaceum* displayed different histological elements including paracytic stomata, fibers, fragments of spiral wraps and secretory hairs. The observed histological elements are the characteristics of the experimental species and the results would provide a database for theses pecies. Microscopic analysis of *A. senegalensis* revealed the presence of fibers, parenchyma with numerous starch grains, secretory and secreting hairs as well as fragments of punctuated vessels (Figure 4). The characteristic histological elements for *M. whitei* were numerous starch grains, parenchyma, punctuated beam fragments and lignified fibers (Figure 5).

To the best of our knowledge, no information has been reported on the micrographic study of the studied species. In view of their use in traditional medicine, it is important to promote these plants while carrying out the standardization, for which the determination of histological elements of drugs for the elaboration of monographs, prove to be paramount for the detection of falsifications.

Secondary metabolites: chromatographic profiles

Flavonoids and Phenolic Acids

The results of chromatographic analysis thru thin layer chromatography (TLC) revealed the richness of phenolic compounds and terpenoids in studied plant extracts. The chromatograms of Figures 6a and 6b correspond to the of the search for flavonoids and phenolic acids, showed the presence of orange yellow, green spots corresponding to flavonoids, and blue spots corresponding to phenolic acids. Figure 6a corresponded to more polar phenolic compounds while Figure 6b will be less polar compounds. The blue

fluorescent spots in *M. whitei* extract will be to chlorogenic and caffeic acids. *A. senegalensis* contained the quercetin that was already reported for this species [12].

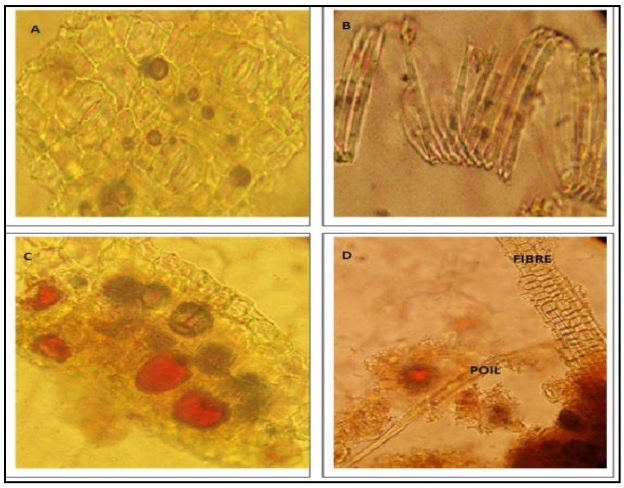


Figure 3. Microscopic features of *A. alboviolaceum* : (A) Paracytic stomata, (B) fragments of spiral vessels, (C) fragments of suber et (D) fibers and secretory hairs

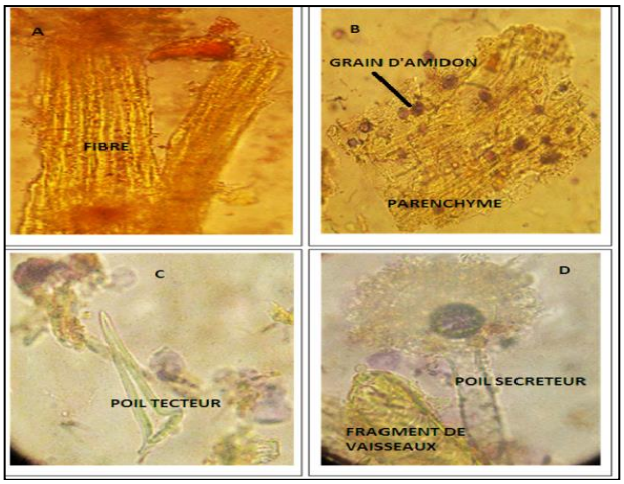


Figure 4. Microscopic features of *A. sengalensis*: (A) fibers (B) Parenchyma with numerous starch grains (C) Secretory and secreting hairs (D) Punctuated vessel fragments

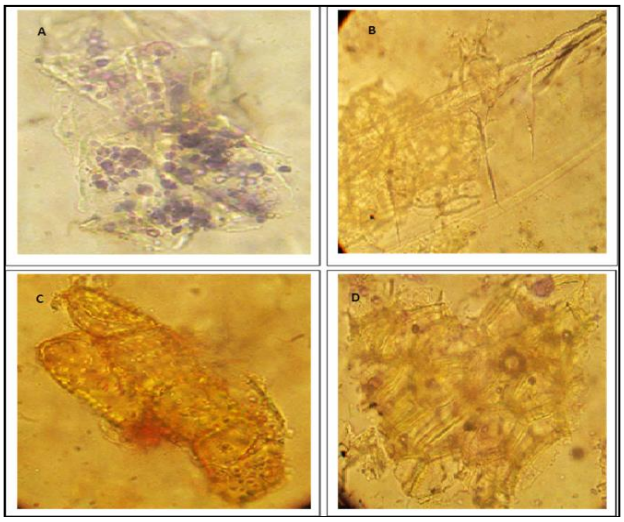


Figure 5. Microscopic features of *M. whitei*, (A) starch grains (B) fiber fragments (C) beam fragments (D) parenchyma

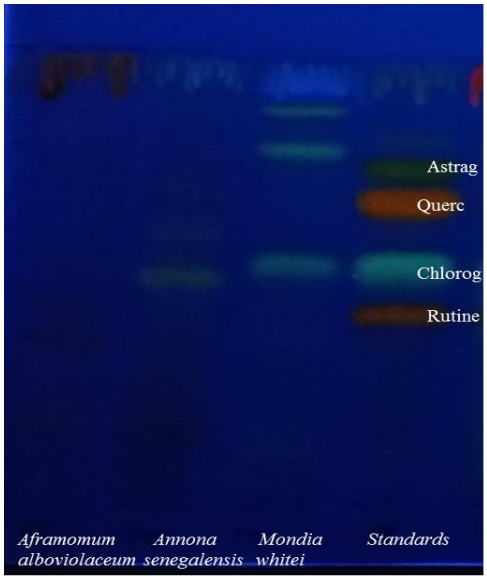


Figure 6a. TLC Chromatogram of methanolic extracts of studied plants using controls at 366 nm

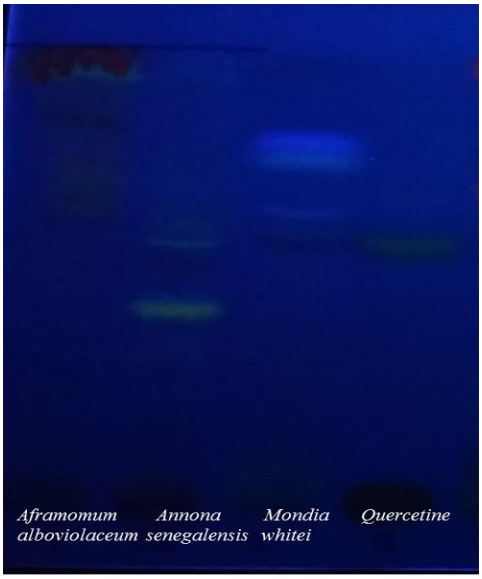


Figure 6b. TLC Chromatogram of methanolic extracts of studied plants using controls at 366 nm

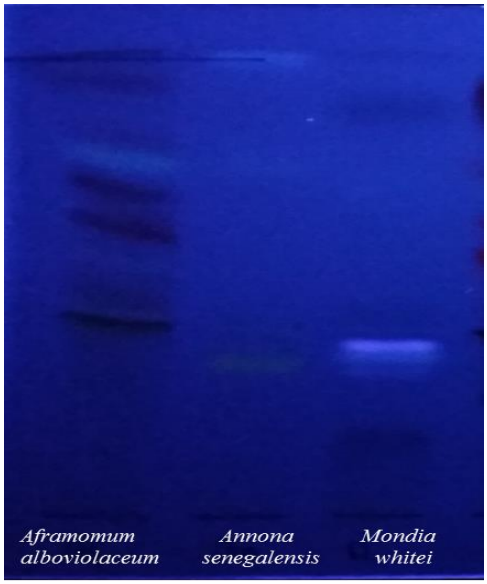


Figure7. TLC Chromatogram of methanolic extracts of studied plants without controls at 366 nm



Figure 8. TLC Chromatogram of methanolic extracts of studied plants without controls at 366 nm

In general, the presence of these phenolic compounds is responsible for their anti-radical activity, since this potential is strongly attributed to them [13-15]. Chromatograms of Figures 7 and 8 showed the presence of coumarins and anthraquinones. Figure 7 shows the presence of coumarins (blue fluorescent spots) in almost all the extracts of the three tested plant species. Fatoumata [12] reported the presence of these compounds in *A. senegalensis*. Concerning anthraquinones, Figure 8 shows the presence of anthrones (yellow spots) in all three plants with a low content in *A. alboviolaceum* and *M. whitei*, but distinct in *A. senegalensis*.

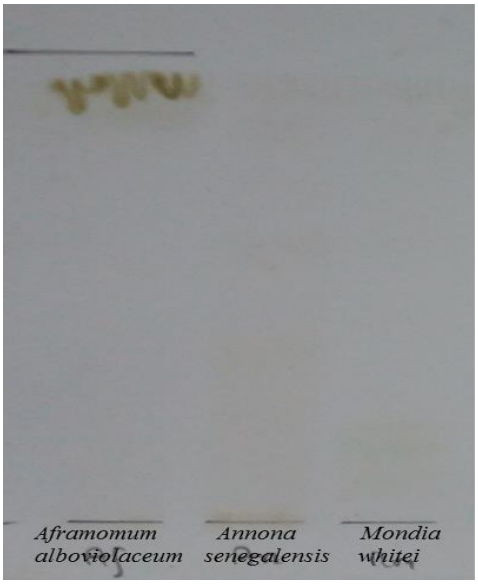


Figure 9. TLC of Chromatogram of methanolic extracts of studied plants without controls in the visible

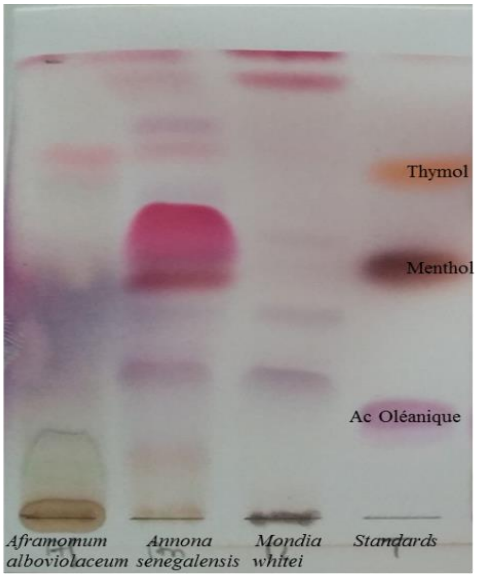


Figure 10. TLC Chromatogram of dichloromethane extract of studied plants with controls in the visible

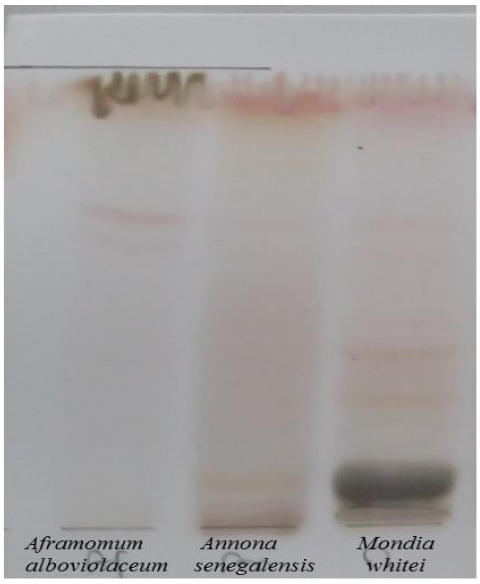


Figure 11. TLC Chromatogramof methanolic extracts of studied plants without controls

The presence of anthocyanins although in the form of traces in experimental extracts is described in the chromatogram below (Figures 9 and 10).

The analysis of Figures 9 and 10 shows various colorations observed in the chromatographic profiles of each sample (violet, orange) and indicate the presence of terpenes, including irridoids in all the three plants. These results were similar to those previously described by Djeussi et al. [16] in *A. alboviolaceum*. Igweh and Onabango [17] indicated the presence of these chemical compounds in *A. senegalensis* and Quasie et al. [18] reported their presence in *M. whitei*.

True irridoids were obtained in all the three plants with spots of different colorations and the black spots correspond to terpenes (Figure 11). The presence of these compounds in a plant species could confer some biological properties, such as anti-rheumatic activity, hypotensive, sedative to central nervous system (CNS) and antioxidant activity [19]. It should be noted that, with the exception of the compounds for which chromatograms are shown above, other compounds have also been detected. These include tannins that were reported in *A. senegalensis* by Fatoumata [12].

The alkaloid test gave a negative response using Draggendorff reagent. By comparing these results with previous work, we noted that the absence of these compounds in *M. whitei* was also reported by Quasie et al [16]. In addition, the absence of alkaloids was noted in *A. senegalensis* [12], while Igweh and Onabango [15] reported that the leaves of *A. senegalensis* contained alkaloids. The work of Djeussi et al [16] showed the presence of alkaloids in the fruits of *A. alboviolaceum*.

Quantitative analysis of secondary metabolites

The results of secondary metabolite assays are presented in Table 1 below. From table 1, it is noted that *A. senegalensis* was the richest in phenolic compounds followed by *A. alboviolaceum* and *M. whitei*, respectively.

Table 1. Results of total polyphenol and flavonoid assays

Extracts	Total Polyphenol (mgGAE/g)	Flavonoids (mgQE/g) (%R)
Aframomum alboviolaceum	51.97 ± 0.30	1.44 ± 0.04 (2.77)
Annona senegalensis	55.13 ± 0.28	1.51 ± 0.01 (2.74)
Mondia whitei	51.48 ± 0.48	1.27± 0.03 (2.47)

Legend : GAE/g Equivalent of gallic acid (GAE) per g of dry extract ;
QE/g Equivalent of quercetin (QE) per g of dry extract. %R= [(flavonoid ratio/ total polyphenols)] x 100.

However, there was no difference between the three plants in terms of flavonoid content. Several factors can have influence on the content of phenolic compounds. 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 [20-22].

Conclusion

In the present work, the main aim was to carry out the microscopy of three plant powders for the determination of their qualitative and quantitative chemical composition. Another objectives was to extract organic acids from various extracts of these three taxa in order to contribute to the promotion of the local plants used in Congolese traditional medicines for the management of sickle cell anemia. Each plant species studied had characteristic histological elements, and all the three plants tested contain various secondary metabolites such as flavonoids, phenolic acids, coumarins, anthrones, tannins, terpenoids and irridoids. All these results constitute scientific evidence validating the use of these medicinal plants for the management of sickle-cell anemia in the Democratic Republic of Congo. It would also be interesting to pursue more in-depth phytochemical studies in order to isolate the involved bioactive compounds and to determine their structure.

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