Original Research Article

Anthelmintic activity, antioxidant activity, phytochemical profile and microscopic features of *Senna alata* collected in the Democratic Republic of Congo

ABSTRACT

Aim: The objective of this study was to determine the phytochemical profile of *Senna alata* LINN using chemical screening in solution and thin-layer chromatography, and to assess the antioxidant and anthelmintic activities of the plant's aqueous extracts.

Methodology: All the analyses performed in this study were, respectively, done as described by the standard protocols. These were: the microscopic examination of the plant powders performed using a light microscope, the search for secondary metabolites carried out by chemical screening in solution and by thin-layer chromatography, the determination of the secondary metabolites, and the antioxidant activity carried out by UV-visible spectroscopy and the anthelmintic activity performed by dilution in decreasing order of concentration.

Results: Micrographic analysis of the powder of *Senna alata* revealed the histological elements rich in unicellular covering hairs with a punctate surface and in fragments of palisade parenchyma, with elongated cells. The presence of polyphenols (flavonoids, anthocyanins, tannins, leuco anthocyanins, free quinones), steroids, terpenoids, and iridoids was detected by phytochemical screening in solution and confirmed by thin-layer chromatography. The determination of total phenolic compounds, flavonoids, total tannins, and anthocyanins showed that *Senna alata* contains 254.64 mg EQ/g, 12.3%, 9.5%, and 6.5%, respectively, of these metabolites. The aqueous extract of the leaves of *Senna alata* showed a good anthelmintic activity after 41 minutes of exposure to 5.00 mg/mL of the extract and the antioxidant activity was reported, of which the value of IC_{50} (µg/mL) of the extract for the DPPH° tests is 91.42 ± 15.56.

Conclusion: Histological elements rich in unicellular covering hairs with a punctate surface and in fragments of palisade parenchyma, with elongated cells were revealed in the micrographic analysis of *Senna alata*. The plant's leaf methanol extract showed good antioxidant activity, while the anthelmintic activity was demonstrated in its aqueous extract.

Keywords: *Senna alata,* antioxidant activity, anthelmintic activity, microscopic features, RD Congo

1. INTRODUCTION

The vegetable kingdom is a reservoir of the most used popular remedies. Indeed, it is proven that plants contain secondary metabolites that give them therapeutic virtues. This justifies the increasing interest in plants and natural substances in different areas [1].

In fact, the world is increasingly becoming reluctant to consume products containing molecules from chemical synthesis, and a number of industrial sectors (cosmetics, pharmaceuticals, agri-foods)

turned towards the use of medicinal plants [2]. Among these plants is *Senna alata*, belonging to the family of Fabaceae, and with up to 3 m high. This plant is widely used in traditional medicine to treat various conditions, including parasitic conditions caused by gastrointestinal helminths [3-6].

Intestinal parasitoses are diseases caused by various infectious agents whose size varies from micrometer to several meters. They constitute a major tropical public health problem where climatic conditions, absence or insufficient hygiene and sanitation measures, as well as poverty, promote their expansion [7]. The infectious agents of these parasitoses are intestinal worms (helminths) and unicellular parasites (protozoa). The diseases are transmissible by absorbing soiled or contaminated foods and these parasitoses can reach serious shapes and sometimes even cause death. Thus, *Entamoeba histolytica* is the second cause of mortality due to protozoa and the third one is due to parasites, in general [8].

Some of these intestinal parasitoses have an opportunistic character in the event of immune depression, therefore are becoming increasingly important with the advent of HIV AIDS and during certain metabolic diseases such as cancer [9]. It should be indicated that oxidative stress and free radicals are widely involved in immunity and metabolic diseases [10].

Although these intestinal parasitoses raise little interest next to diseases such as AIDS, tuberculosis, malaria, and onchocerciasis, they are tropical, and a public health problem because of favorable climatic conditions, absence or insufficient hygiene, sanitation, and poverty measures. Children are particularly vulnerable to malnutrition, dehydration, and anemia, causing a status-in-weight delay and susceptibility to infections at the root of high infant mortality [11].

Population growth, climatic conditions, low socioeconomic level, and precarious hygiene are favorable factors for the extension of parasitism in the population [11]. In Africa, factors such as promiscuity, lack of drinking water, food hygiene, and insufficient health facility have caused the overall prevalence of intestinal parasitoses of 63.3%, including the majority (53 %) which is transmitted by dirty water [12].

In the Democratic Republic of Congo (DRC), intestinal parasitoses are a public health problem because of the economic crisis characterized by the lack of drinking water, food hygiene, and insufficient sanitary facility. According to Kapiteni [13], the prevalence of intestinal parasitoses in the DRC is 94% and the most affected age group is between 18-29 months with a predominance of the female sex.

It is therefore important to verify the antihelminthic and antioxidant activities of *Senna alata* harvested in the DRC and determine its phytochemical composition. This is to contribute to the fight against parasitoses by the local means and thus valuing the traditional Congolese pharmacopeia.

2. MATERIAL AND METHODS

2.1 Material

The leaves of *Senna alata* were harvested in the commune of Kimbaseke, May Engele district, Busulu street, in Kinshasa, in the DRC. The plant has been identified and authenticated at the herbarium of the National Institute of Agricultural Studies (INERA), housed at the Faculty of Sciences of the University of Kinshasa, by the Botanist Technician Nlandu. The animal material used consists of common earthworms of *the Benhamia Rosea* genus*,* collected from the banks of Keni river, in Mont Ngafula township in Kinshasa. This material was identified at the natural resource management laboratory of the Faculty of Agricultural Sciences of the University of Kinshasa.

2.2. Methods

The vegetable material was dried in the open air at room temperature. After drying, it was crushed and sown to obtain a fine powder.

The harvested ground worms have been brought live and placed in the Petri boxes, before putting them in contact with the extracts of the plants, at different concentrations.

The microscopy of the powder was carried out following the procedure described by Tshilanda *et al.* [14]. It serves to characterize the histological elements of the plants and the structures of their cells

[15], [16]. Each plant is characterized by the presence of one or more particular histological elements whose cellular forms are also found in the powder [16].

The thin layer chromatography (CCM) was carried out following the standard protocol described by Wagner, based on the observation of the spots of various colors to identify the different secondary metabolites [17].

The assay of secondary metabolites was carried out following the protocols described by Bahmed [18]. Briefly, the total polyphenol content was determined by the Folin-Ciocalteu method [19]. The dosage of total flavonoids and anthocyanins was carried out according to Le Bretons' method [20]. The condensed and the hydrolyzable tannins of *Senna alata* leaves were dosed, respectively, based on the condensation of the polyphenolic compounds, with vanillin acid and the reaction with iron chloride (III) [21].

The evaluation of the antioxidant activity was carried out using the DPPH test, according to the protocol described by Kabengele [22], and the test at the stones according to Serigne Ibra Mbacke Dieng [23], while anthelmintic activity has been evaluated using the Ongoka *et al.* approach. [24].

3. RESULTS AND DISCUSSION

3.1. Microscopic examination results of powders

Figure 1 below illustrates the different histological elements of *C. alata* leaves

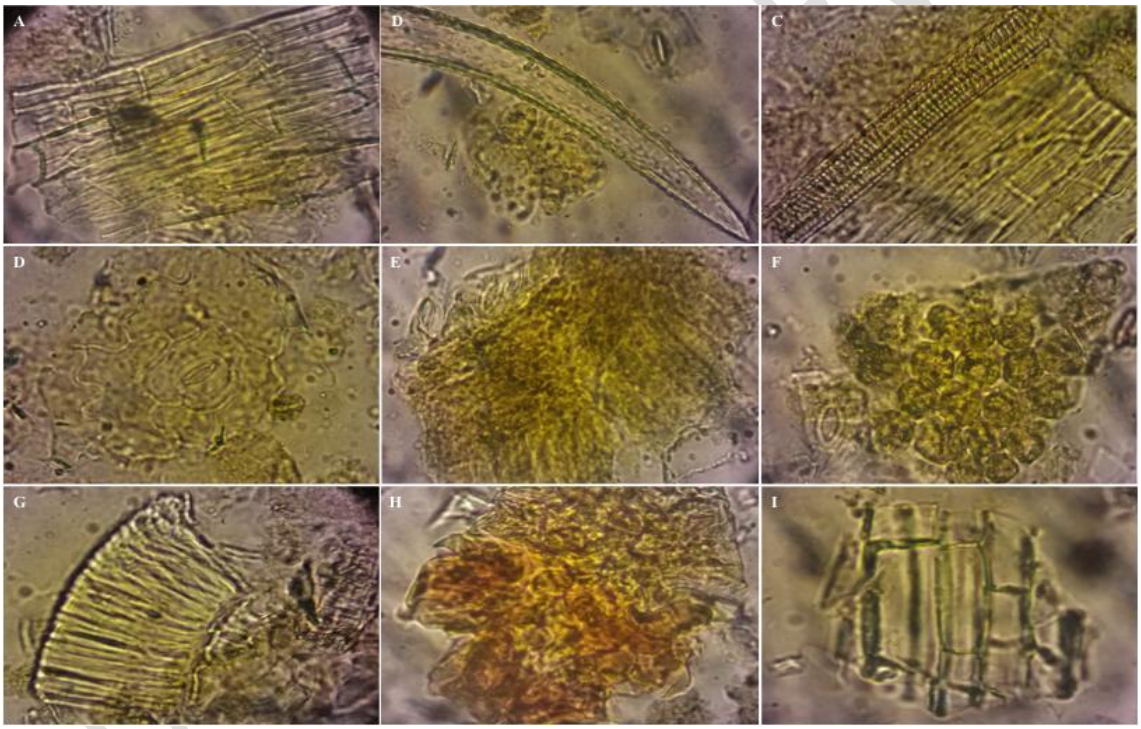

Figure 1: The different cells detected in the powder of the leaves of *Senna alata* L.

Microscopic powder analysis reveals cells such as a fragment of sclerenchymes (a), diacytic stomata (D), fragment of spiral vessels (C), the palm parenchyme fragment with elongated cells (E), hairbripers unicellular with a punctuated surface (b), fiber fragment (I), skin fragment with rounded cells (F) and elements to be characterized (G and H) in *Senna alata* L. sheets, as shown on Figure 1. The presence of the diacytic stomata cells is in accordance with the work performed by Fuzellier *et al.* in the powder of the same plant's leaves harvested in Songkhla (Thailand) [25].

3.2. Phytochemical screening in solution

On one hand, the chemical screening has highlighted the presence of the following chemical groups: polyphenols (flavonoids, anthocyanins, tannins, leuco anthocyanes, free quinones) and steroids; and on the other hand, saponins, alkaloids, bound quinones, and triterpenoid are absent in the excerpt. The presence of polyphenols in *Senna alata* extract thus justifies its use in traditional medicine against dermatoses [26].

The results of this study are similar to those of El-Mahmood & Doughari who have revealed the presence of flavonoids, tannins, polyphenols, saponins, and anthraquinones in the extract from this species, harvested in Benin [27].

Wadre Saidou bearing *Senna obstuntensifolia*, harvested in Burkinafaso, presents the same phytochemical profile, with the exception of alkaloids that are absent in our work. This may be due not only to their environmental conditions but also because they are not the same species [28].

The results of this study are similar to those of Mogode Debete who revealed the presence of flavonoids, tannins, polyphenols and anthraquinones in extracts from the *Senna nigricans* Vahl species harvested in Mali [29].

3.3. Phytochemical screening by TLC

Phytochemical screening by thin-layer chromatography showed the presence of terpenoids, irridoids, and more polar compounds including flavonoids and anthocyanins. The results are shown in Figures 2a-c.

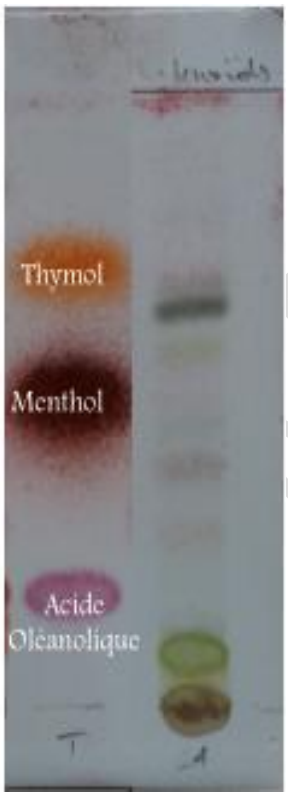

Figure 2a: Terpenoids
SP: Silica gel 60F$_{254}$
MP: Toluene / Ethyl acetate
Developer: Sulfuric vanillin

Figure 2b: Irridoids
SP: Silica gel 60F$_{254}$
MP: Acetated ethyl / Formic acid / Water
Developer: Sulfuric acid

Legend: SP: Stationary Phase; MP: Mobile Phase

The result presented in Fig. 2a reveals the presence of terpenoids which were detected by spots of the various colorations after development with sulfuric vanillin. It should be noted that menthol, oleanolic acid, and thymol are absent in the extract.

The chromatograms of the apolar extracts show several spots corresponding to the different apolar molecules, which could probably be the sterols, terpenoids, and lipids.

The terpenoids in Fig 2a show the fluorescent spots of the various colors with the sulfuric vanillin reagent. By comparing these spots with those of the control, the fluorescent spots would correspond to oleanolic acid, menthol, and thynol which are part of the terpene family.

Our results are similar to those found by Fuzellier *et al*. [30] who reported the presence of terpenoids in *Senna alata* leaves, harvested at Nancy University, in France, whereas traditional chemical screening did not reveal the presence of these compounds. It can be assumed that the content of these compounds in our powder is low.

In figure 2b, the true iridoids give the fluorescent spots of the various colorations with 5% sulfuric acid in ethanol. These results reveal the presence of iridoids in the leaves of *Senna alata*, in traces.

Figure 2c: Flavonoids
SP: Sillicagel 60F$_{254}$
MP: Ethyl acetate / Methanol / Water / Formic acid
Developer: Neu reagent

Figure 2d: Anthocyanins
SP: Sillicagel 60F$_{254}$
MP: Ethyl acetate / Methanol / Water / Formic acid
Developer: Phosphoric vanillin

Legend: SP: Stationary Phase; MP: Mobile Phase

Concerning the flavonoids, figure 2c shows the spots which testify the presence of the following compounds in the plant: Flavonoids with yellow fluorescent spots using Neu's reagent.
By comparing these spots with those of the control, the fluorescent spots would correspond to caffeic and chlorogenic acids and the green spot should correspond to the kaempferol-like flavonoid. Caffeic and chlorogenic acids are part of the polyphenols [31].
Our results are similar to those found by the Hennebelle team who reported the presence of flavonoids in the leaves of *Senna alata*, harvested at the University of Reading [32].
Anthocyanins are also present in the plant as it can be seen in figure 2d. Anthocyanins give pink spots with phosphoric vanillin reagent. Another study done by Bellassoued *et al*. [33], on *Senna Angustifolia* leaves showed that the content of the total polyphenols was 4.38 ± 0.08 mg EAG / g of extract ($p \leq 0.001$).

3.4. Determination of total phenolic compounds, flavonoids, tannins, and anthocyanins

The dosage of total phenolic compounds shows that *Senna alata* contains 254.64 mg EQ/g (milligram equivalent of gallic acid per gram of dry powder of the plant). Figure 3 gives the flavonoids, anthocyanins, and tannins content.

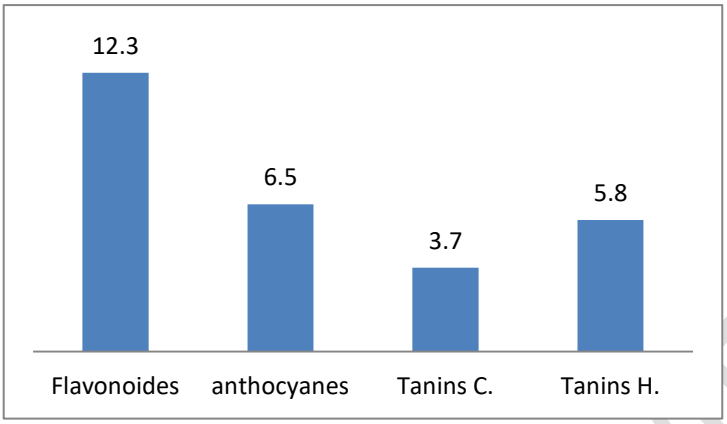

Figure 3: Determination of flavonoids, tannins, and anthocyanins

It emerges from this figure that the content of flavonoids is the highest (12.3%), while the anthocyanins have the lowest content of 6.5 %, and the content of total tannins is 9.5%. Hydrolysable tannins are in higher content (5.8%) compared to condensed tannins (3.7%). Our results are similar to those found by Diallo who reported the concentration of flavonoids and anthocyanins in the leaves of *Senna alata,* from Bamako [30].

3.5. Evaluation of antioxidant activity

The IC_{50} value (µg/mL) of the *Senna alata* extract for DPPH° test is 91.42 ± 15.56. *Senna alata* leaves extract shows good antioxidant activity with the DPPH radical, probably due to the presence of phenolic compounds [31]. Indeed, phenolic compounds are known for their anti-radical properties [31]. It should be noted that the ABTS radical did not react with our extracts, so we could not find its IC_{50}.

3.6. Evaluation of anthelmintic activity

The table 1 below shows the results of the antihelminthic activity.

Table 1: Paralysis time of helminths in different concentrations of the extract

Concentration (mg/mL)	**Time (min)**		
	Positive control (Albendazole)	Extract	Negative control
5.00	34	41	-
2.50	53	80	-
1.25	87	98	-
0.63	116	126	-

It appears from this table that the aqueous extract of *C. alata* shows good antihelminthic activity at high concentration. This indicates that compounds with strong deworming activity pass easily into the polar solvent (water) [32].

By one hand, at a concentration of 0.63 mg/mL, we note that the positive control batch (Albendazole) did not cause helminth mortality throughout the experiment; by the other hand, at the concentration of 5.00 mg/mL, the table 1 shows that the efficacy of Albendazole (positive control) appears after 34

minutes and that of the extract appears after 41 minutes of exposure. At this time, the antihelminthic efficacy observed between these two concentrations was statistically different, hence Albendazole exhibits a short paralysis time compared to the extract. This may be due to the composition of the extract. In reality, the positive control is composed of a single well-identified molecule whose family is well known [33], while our extract consists of a mixture of bioactive compounds. Thus, the vermicidal activity of *Senna alata* extract, observed in the present study, would probably be due to polyphenols, in general, and flavonoids, in particular [34-36].

CONCLUSION

The aim of the study was to determine the chemical composition, and histological elements and to evaluate the anthelmintic and antioxidant activities of *Senna alata* LINN leaves' extract.
The results revealed that *Senna alata* LINN leaves contain various secondary metabolites like polyphenols (flavonoids, anthocyanins, tannins, leuco anthocyanins, free quinones), steroids, while saponins, alkaloids, linked quinones and triterpenoids are absent in the extract. Quantitative analysis of *Senna alata* LINN leaves' extract shows a high content of total polyphenols (254.64 mg EAG/g) of which 12.3% of flavonoids, 6.5% of anthocyanins, 3.7% of condensed tannins and 5.8% of hydrolysable tannins. The aqueous extract displayed also
a strong anti-radical and anthelmintic activities.
To the best of our knowledge, this is the first time that the anti-free radical and antihelmintic activities of *Senna alata* LINN leaves' extract are reported.
The phytochemical analyses on the active extract are in progress.

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