Original Research Article

Anthelmintic activity, antioxidant activity, phytochemical profile and microscopic features of *Cassia alata* collected in the Democratic Republic of Congo *(RD Congo)*

ABSTRACT

Aim: the objective of this study was to determine the phytochemical profile of *Cassia 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 analysis 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 *Cassia 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 *Cassia 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 *Cassia 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 *Cassia alata*. The plant's leaves methanol extract showed a good antioxidant activity, while the anthelmintic activity was demonstrated in its aqueous extract.

Keywords: Cassia alata, antioxidant activity, anthhelmintic 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, a number of industrial sectors (cosmetics, pharmaceuticals, agri-foods) turned

towards the use of medicinal plants [2]. Among these plants is *Cassia alata*, belonging to the family of Fabaceae, with up to 3 m of 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, a public health problem because of favorable climatic conditions, absence or insufficient hygiene, sanitation and poverty measures. Children are particularly vulnerable with malnutrition, dehydration, anemia, causing a status-in-weight delay and susceptibility to infections at the root of high infant mortality [11].

Population growth, climatic conditions, low socio-economic 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 female sex.

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

2. MATERIAL AND METHODS

2.1 Material

The leaves of *Cassia 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 *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 being 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 hydrolysable tannins of *Cassia 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

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

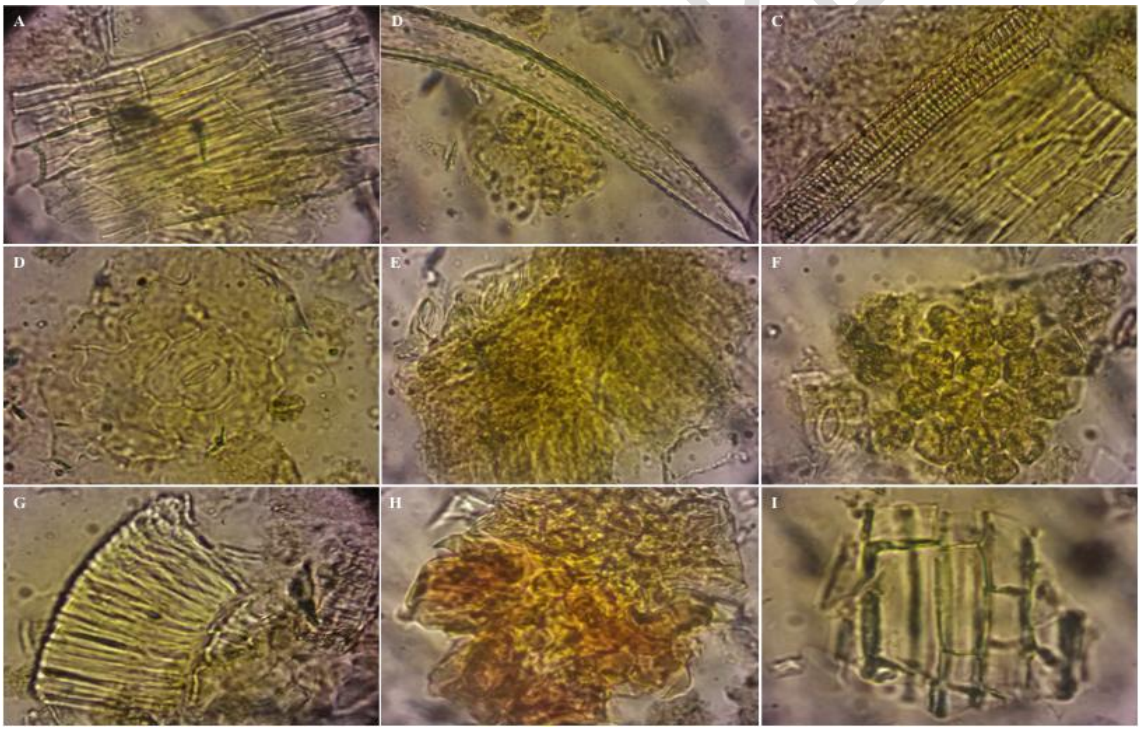

Figure 1: The different cells detected in the powder of the leaves of *Cassia alata* L.

Microscopic powder analysis reveals cells such as: fragment of sclerenchymes (a), diacytic stomata (D), fragment of spiral vessels (C), the palm parenchyme fragment with elongated cells (E), hairbripers unicellular with punctuated surface (b), fiber fragment (I), skin fragment with rounded cells (F) and elements to be characterized (G and H) in *Cassia alata* L. sheets, as shown on the 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

By 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 by the other hand, saponins, alkaloids, bound quinones and triterpenoid are absent in the excerpt. The presence of polyphenols in *Cassia 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 *Cassia 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 *Cassia 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, more polar compounds including flavonoids and anthocyanins. The results are shown in Figures 2a-c.

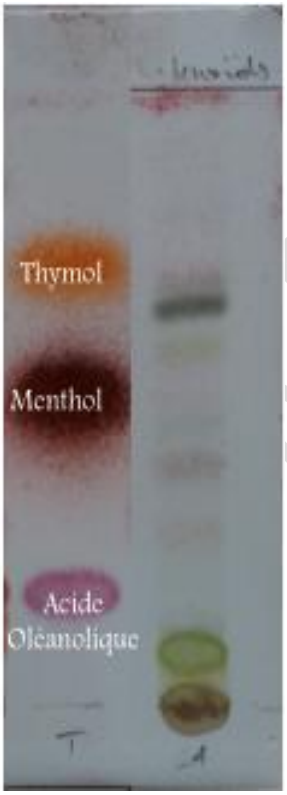

Figure 2a: Terpenoids
SP: Silica gel 60F_{254}
MP: Toluene / Ethyl acetate
Developer: Sulfuric vanillin

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 *Cassia alata* leaves, harvested in 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 irridoids give the fluorescent spots of the various colorations with 5% sulfuric acid in ethanol. These results reveal the presence of irridoids in the leaves of *Cassia 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, the 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 kaempferol-like flavonoid. Caffeic and chlorogenic acids are part of the polyphenols [31].
Our results are similar to those found by Hennebelle team who reported the presence of flavonoids in the leaves of *Cassia alata*, harvested in 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 *Cassia 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 *Cassia 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.

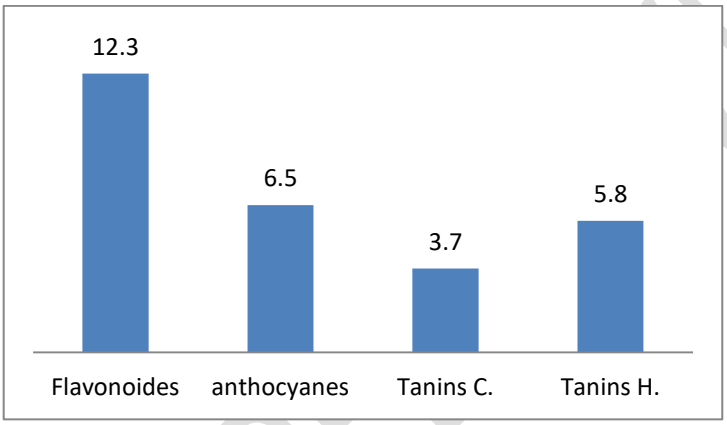

Figure 3: Determination of flavonoids, tannins and anthocyanins

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 %, the content of total tannins being 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 *Cassia alat,* from Bamako [30].

3.5. Evaluation of antioxidant activity

The IC_{50} value (µg/mL) of the *Cassia alata* extract for DPPH° test is 91.42 ± 15.56. *Cassia 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 with 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 *Cassia alata* extract, observed in the present study, would probably be due to polyphenols, in general and to flavonoids, in particular [34-36].

CONCLUSION

In the present study, it was a question to determine the qualitative and quantitative chemical composition of *Cassia alata* LINN leaves' extract and carrying out the powder microscopy of the leaves of this plant by one hand and by the other hand to evaluate the anthelmintic and antioxidant activities from the plant.

The obtained results show that *Cassia alata* LINN leaves contain, by one hand polyphenols (flavonoids, anthocyanins, tannins, leuco anthocyanins, free quinones) and steroids and by the other hand, saponins, alkaloids, bound quinones and triterpenoids are absent in the extract. Quantitative analysis of *Cassia alata* LINN leaves' extract shows a high content of secondary metabolites, including total polyphenols 254.64 mg EAG/g, 12.3% flavonoids, 6.5% anthocyanins, condensed tannins at 3.7% and hydrolysable tannins at 5.8%.

The antioxidant test made it possible to conclude that the aqueous extract has a strong anti-radical activity linked to the content of polyphenols.

Regarding the antihelminthic activity, the leaves of *Cassia alata* LINN seem to have an activity against helminths linked to the presence of polyphenols.

To our knowledge, this is the first time that the anti-free radical activity of *Cassia alata* LINN leaves' extract and the antihelminthic activity of these leaves have been evaluated.

All of these results show that the formulation of a phytomedicine from the leaves of *Cassia alata* LINN, would make it possible to fight against both parasitic worms of the gastrointestinal tract and the free radicals produced in the animal organism. The determination of the specific chemical groups responsible for these activities is in progress.

REFERENCES

[1]. Beloud A. Medicinal plants of Algeria. OPU. Algiers, 1998.

[2]. Matou Mélissa, Composition and biological properties of extracts of Phyllanthus amarus Schumacher α thonning (1827) used in traditional medicine in the West Indies, 2019, P-16.

[3]. Kaboré A., Antihelminthic activity of two tropical plants tested in vitro and in vivo on the gastrointestinal strongyles of sheep of the Mossi breed from burkina faso. Doctoral thesis at the Polytechnic University of Bobo-dioulasso Unique doctorate in rural development, 2009, (167): 46-47.

[4]. Githiori JB, Hoglund J, Waller PJ, Baker RL. Anthelminthic activity of preparations derived from Myrsine africana and Rapanea melanophloeos against the nematode parasite, Haemonchus contortus of sheep. J. Ethnopharmacol, 2002, 45(18):312-324.

[5]. Perry, B., Randolph T. H., Mcdermont, J. J., Sones K. R. And Thornton P. K., Investing in Animal Health Research to alleviate Poverty. International Livestock Research Institute (ILLRI), Nairobi, Kenya, 2002, 43(20): 104-112.

[6]. Pamo E.T., Awah-Ndukum J., Boukila B., Kana J.R., Tendonkeng F., Essie F.M.N., A study of anthelmintic property of fresh cassava (Manihot esculenta) leaves incorporated in the diet of West African dwarf goats. Food Research International, 2006, 44 (2011) 1800–1811.
[7]. Alpha Seydou Yaro, Fadima Camara, Moussa Sacko. Prevalence of Human Intestinal Parasites in Patients of the Parasitology Department of INRSP Bamako from 2010 to 2015. July 2019 Ed. 2010 2015;15(21):377.
[8]. Nanfah Woda Murielle Patricia. Study of intestinal polyparasitism at the INRSP in the district of Bamako - Mali. Thesis in Pharmacy; University of Bamako, Faculty of Medicine, Pharmacy and Odontostomatology; 2007.
[9]. Ousmane Konate. Analysis of the prescription and dispensing of intestinal antiparasitics in six pharmacies in the district of Bamako: pilot study. Pharmacy doctoral theses. University of Sciences, Techniques and Technologies of Bamako, Mali, 2020.

[10]. Defraigne J.O., A central physiopathological mechanism behind the complications of diabetes, Rev Med Liege, 2005, 60, 472-478.
[11]. Africad. Y., Epidemiology of intestinal parasitosis in the population of the city of Agadir. Doctoral thesis in Medicine. Cadi Ayyad University, Marrakech, Morocco, 2018.
[12]. Ohouya. D.G., Prevalence of intestinal parasitosis in children aged 0 to 5 in the community of Anonkoi 3. Pharmacy thesis, Felix Houphouët Boigny University, Abidjian Ivory Coast, 2015. p 149.
[13].Woolf Kapiteni, John Kivukuto Mutendila, Celestin Mamba. Impact of the seasons on intestinal parasitosis in young children under 5 years old at the Afia-Sake Reference Health Center in the province of North Kivu in DR Congo, 2019, pp.145-151
[14]. Tshilanda DD, Inkoto CL, Mpongu K, Mata S, Mutwale PK, Tshibangu DST, Bongo GN, Ngbolua KN, Mpiana PT. Microscopic studies, phytochemicals and biological screenings of Ocimum canum. International Journal of Pharmacy and Chemistry. 2019;5(5):61-67. DOI: 10.11648/j.ijpc.20190505.13
[15]. Inkoto CL, Bongo GN, Kapepula PM, Masengo CA, Gbolo BZ, Tshiama C, Ngombe NK, Iteku JB, Fundu TM, Mpiana PT, Ngbolua KN. Microscopic features and chromatographic fingerprints of selected Congolese medicinal plants: Aframomum alboviolaceum (Ridley) K. Schum, Annona senegalensis Pers. and Mondia whitei (Hook.f.) Skeels. Emerging Life Science Research. 2018;4(1):1-10. Available: http://dx.doi.org/10.7324/ELSR.2 018.410110
[16]. Gurav SS, Gurav NS. Indian herbal drug microscopy. Springer New York Heidelberg Dordrecht London; 2013. DOI: 10.1007/978-1-4614-9515-4
[17]. Wagner H., Drogenanalyse, Dünschichtchromatographische Analyse von Arzneidrogen. Springer Verlag BerlinHeidelberg New York, 1983, 522 pp.
[18]. Bahmed Amira-Imane, Composition of phenolic compounds and evaluation of their potential antioxidants: Case of Citrullus lanatus and Cucumis melo. Master memory. Abdelhamid Ibn Badis Mostaganem University. .Mostaganem, Algeria, 2018.
[19]. Goli A H., Barzeger M and Sahari M A. Antioxidant activity and total phenolic compound of pistachio (pistachia vera) hull extracts. Food chemistry, 2005, 92:521-525.
[20]. Lebreton P., Jay M., Voirin B. On the qualitative and quantitative analysis of flavonoids. Chem. Anal. (Paris), 1967, 49(7), 375-383.
[21]. Dohou N., Yamni K., Tahrouch S., Hassani L M., Badoc A., Gmiran. Phytochemical screening of an Ibero-Moroccan endemic, thymelaea lythroides. Bull .Soc. Pharma. Bordeaux, 2003.
[22]. Carlos N. Kabengele1, Etienne M. Ngoyi1, Giresse N. Kasiama1, Jason T. Kilembe1, Aristote Matondo1, Clement L. Inkoto2, Emmanuel M. Lengbiye2, Clement M. Mbadiko2, Jean Jacques D. Amogu2, Gedeon N. Bongo2,3 , Benjamin Z. Gbolo2,3, Damien ST Tshibangu1, Koto-te-Nyiwa Ngbolua2,3, Dorothée D. Tshilanda1 and Pius T. Mpiana1. Antihelminthic Activity, Phytochemical Profile and Microscopic Features of Ocimum basilicum Collected in DR Congo. Asian Journal of Biology. 10(3): 42-50, 2020; Article no.AJOB.62090 ISSN: 2456-7124

[23]. Serigne Ibra Mbacke Dieng, Alioune Dior Fall, Kady Diatta-Badji, Abdou Sarr, Madieye Sene, Moussa Sene, Amadou Mbaye, William Diatta et Emmanuel Bassene. Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de Piliostigma thonningii Schumach, 2017, Int. J. Biol. Chem. Sci. 11(2): 768-776.

[24]. Ongoka P.R., Diatewa M., Ampa R., Ekouya A., Ouamba J.M., Gbeassor M., Abena A.A.. In vitro evaluation of the anthelmintic activity of plants used in Congo Brazzaville in the treatment of parasitic diseases. Annals of Marien Ngouabi University. 2011-2012; 12-13 (4): 101-107

[25]. Fuzellier M.C., Mortier F. and Lectard P. Antifungal activity of Cassia alata L. Ann. Pharma. 1982, 40,4.357-363.
[26]. Agban A., Karou D.S., Tchacondo T., Atchou K., Batawila K. Evaluation of the antifungal activity of Cassia alata L. and Piliostigma thonningii (Schum) Milne Redhead extracts. Rev. CAMES-Series A, 2012, 13(1).
[27]. El-Mahmood A.M. and Doughari J.H. Phytochemical screening and antibacterial evaluation of the leaf and root extracts of Cassia alata Linn. African Journal of Pharmacy and Pharmacology, 2008; 2(7): 124-129.
[28]. Fuzellier M.C., Les folioles de Cassia alata L. Chemical and pharmacological study of anthracene derivatives Doctorate thesis in Pharmaceutical Sciences, University of Nancy 1, 1983.
[29]. Defraigne, J. O. and Pincemail, J. Oxidative stress and antioxidants: myths and realities. Rev Med Liege, 2008; 63(14): 10-19.
[30]. Wadre Saïdou. Anthelmintic activity of Cassia obtusifolia L. in Djallonké sheep artificially infested with Hœmonchus contortus in Burkina Faso. End of cycle memory. Polytechnic University of Bobo Dioulasso, 2016.
[31]. Hennebelle, T., Weniger, B. et al. Senna alata. Fitoterapia, 2009, 80, pp. 385–393.
[32]. Mogode Debete. Phytochemical and pharmacological study of Cassia Nigricans Vahl (Caesalpiniaceae) used in the treatment of dermatoses in Chad. Doctoral Thesis, University of Bamako. 2005
[33]. Bellassoued, K., Hamed, H., Ghrab, F., Kallel, R., Van Pelt, J., Makni Ayadi, F. & Elfeki, A. (2019). Antioxidant and hepatopreventive effects of *Cassia angustifolia* extract against carbon tetrachloride-induced hepatotoxicity in rats, Archives of Physiology and Biochemistry, DOI:10.1080/13813455.2019.1650778.
[34]. Aïda Diallo, in vivo study of the antiplasmodial activity of aqueous extracts of baye compound tea (cassia alata linn; cochlospermum planchonii book; phyllantus amarus sehum and thann) in nmri mice infested with plasmodium berghei, 2004.
[35]. Serigne Omar Sarr, Alioune Dior Fall, Rokhaya Gueye, Amadou Diop, Khady Diatta, Ndeye Diop, Bara Ndiaye and Yérim Mbagnick Diop. Study of the antioxidant activity of Vitex doniana (Verbenacea) leaf extracts, 2015, Int. J. Biol. Chem. Science. 9(3): 1263-1269.
[36]. Quintin A. and Frank, J. Veterinary anthelmintics: old and new. Trends in Parasitology, 2004. 23(20): 17-25.
[37]. Vidyadhar S., Saidulu, M. Gopal, T K; Chamundeeswari, D; Rao, Umamaheswara; Banji, David. In vitro anthelmintic activity of the whole plant of enicostemma littorale by using various extracts, 2011. 161184
[38]. Deore SL., Khadabadi SS., Kamdi KS., Ingle VP., Kawalkar NG., Sawarkar PS., Patil U.A. and Vyas A.J. In vitro anthelmintic activity of Cassia tora. Int J Chem Tech Res, 2009; 1(2): 177-179.
[39]. Vedha HBN., Saravana KP. & Ramya DD., Comparative in vitro anthelmintic activity of the latex of Ficus Religinosa, Ficus elastica and Ficus bengalensis. J Phytol Phytopharm, 2011, 3(3): 26-30.