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Microscopic Studies, Mineral composition and Bioactivity of *Vitex madiensis* Oliv. (Lamiaceae)

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ABSTRACT

Ethanol and organic acid extracts from the leaves of *Vitex madiensis* Oliv. were evaluated for their antihelminthic activity using the animal model of *Benhamia rosea* and *B. itoleisis* (Kinshasa/DRC earthworms), antibacterial activity and antioxidant activity. Different concentrations (5 mg/mL, 2.5 mg/mL, 1.25 mg/mL and 0.625 mg/mL) of standard Albendazole (positive control) and sample solutions (organic acid extracts and ethanol extracts) were used for this study which involved the determination of the paralysis time (vermifuge) and mortality time (vermicidal activity) of the worms. The presence of different secondary metabolites was determined by the thin layer chromatography. The results obtained showed that the ethanolic extract and the organic acid extracts showed significant antihelminthic activity. Microscopic examination of the parts used revealed the presence of various histological elements. Its phytochemical profile remains dominated by various secondary metabolites such as coumarins, anthraquinones, flavonoids, phenolic acids and terpenoids. The mineral composition was determined by inductively coupled argon plasma optical emission spectrometry (ICP-EOS) showed the abundance of various elements, namely calcium, iron, potassium, selenium, sodium, magnesium, manganese, nickel, phosphorus, cobalt, copper, barium and zinc in this plant. The antibacterial activity of the extracts of this plant was weak against the bacterial strains tested. The organic acid and ethanol extracts showed interesting antioxidant activity. The leaves extracts of *Vitex madiensis* Oliv. showed good antihelminthic, antibacterial and antioxidant activities related to their phytochemical compositions. All these results constitute scientific evidence validating the use of this medicinal plant for the management of parasitic infections of small ruminants in the Democratic Republic of the Congo.

Keywords: Gastrointestinal nematodes; medicinal plants; antihelminthic activity; *Vitex madiensis*, Democratic Republic of the Congo

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INTRODUCTION

Gastrointestinal nematodosis is one of the factors limiting the efficient production of small ruminants in Africa, particularly in the Democratic Republic of the Congo.[1] Gastrointestinal nematodosis is usually controlled using synthetic antihelminthics. The emergence of nematode resistance to synthetic anthelminthics has stimulated the search for alternatives, such as the use of medicinal plants.[2] Depending on the circumstances and depending on their efficacy, natural herbal antihelminthics offer an alternative that can overcome some of these problems and is both sustainable and environmentally acceptable. Several species of the *Vitex* genus have been found to have antihelminthic activity.[3]

Vitex madiensis Oliv. is a plant that was described in 1753 by Linnaeus in the *Vitex* genus (Lamiaceae) before Verbenacea. Today, this genus includes between 250 and 300 species of trees and bushes (rarely lianas). These plants are distributed in tropical and subtropical regions[4,5] and sometimes produce a shrub or a small tree, often a suffix, of less than 50 cm. The tips are covered with long fawn hairs and the leaves are digested and composed of five leaflets. These leaflets are obovate, and the lower leaflets clearly smaller, dark green on top, very rough to leathery; paler underneath with softer hairs; rounded to square top, often with a short, abrupt tip. Flowers at axillary heads, white with pale blue to dark purple lip; calyx lobes densely covered with fawn hairs. Fruit ellipsoid-oblong, fleshy, pendant on long stems, black, often white-spotted, cup-shaped in the persistent calyx.[6]

Historically, *Vitex spp*. have been widely used in traditional medicine; and to date, they have a wide range of ethnopharmacological uses such as the treatment of premenstrual and gynaecological

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conditions, bacterial infections, gastrointestinal problems and inflammations as well as insect and venomous animal repellents.[7] Ethanolic extract of *Vitex spp*. has also been reported for its anti-helminthic activity against the Indian earthworm *Pheritima posthuma*.[3] These species contain a variety of potentially bioactive molecules such as iridoids, flavonoids, terpenoids and essential oils.

In the Democratic Republic of the Congo, the fruits of *Vitex madiensis* Oliv. are sweet, edible and refreshing. The decoction of the young leaves is used in case of cough, cold, diarrhea and dysentery. The roots cooked in water are used for the treatment of diabetes and anaemia. Elsewhere in Africa, the leaves, fruits, stem and bark of the roots are used in the treatment of conjunctivitis, dysentery, diarrhoea, fatigue, headaches, mental disorders, respiratory problems, back pain in women, leprosy, fever and jaundice.[2]

To our knowledge, few studies have been conducted on the chemical compositions and biological properties of this species. The main objective of this work is to study the antihelminthic, antioxidant and antibacterial activities of ethanol and organic acid extracts. In this study, ethanol and organic acid extracts of *Vitex madiensis* Oliv. leaves were tested on adult earthworms, which were used as an animal model to assess antihelminthic activity.

MATERIAL AND METHODS

Plant Material Collection

The selection of the plant species used in this study was based on previous ethnobotanical surveys.[2] The leaves of *Vitex mandiensis* Oliv. were collected from Mbanza-Ngungu in the Kongo Central Province, located in the Democratic Republic of the Congo (DRC). This species was identified at the Institut National d'Etudes et de Recherche Agronomique, Départment of Biology, Faculty of Science, University of Kinshasa. The leaves were dried at room temperature (±27°C) at the Laboratory of Ethnobiology and Medical Phytochemistry of the Department of Biology for 2 weeks, and then milled to obtain a fine powder for further analysis.

Preparation of extracts

The dried and powdered plant material (50 g) was extracted several times with 400 mL of ethanol (95%) by cold percolation for 48 hours.[8] The resulting filtrate was dry concentrated under reduced pressure using a rotary evaporator. The organic acid fraction (triterpenic acids) was extracted as follows: powdered leaves of *Vitex madiensis* Oliv. (40 g) were macerated with 400 mL of dichloromethane-methanol-NH_4OH (100:1:1; v/v/v) and then percolated with 300 mL of the same solvent mixture at room temperature. The extract was concentrated under reduced pressure to 100 mL (pH 10). The resulting solution was then mixed with 5% citric acid (1:1; v/v) to precipitate the organic/triterpenic acids. The resulting fractions were evaporated to dryness on an evaporation apparatus. All extracts were weighed and stored in sealed vials and kept refrigerated at 4°C.

Phytochemical analysis

Phytochemical screening was carried out on aqueous and organic extracts for the identification of chemical groups by thin layer chromatography according to the standard protocol described by Bruneton.[9] and Tiwari et al.[10]

Determination of mineral content

The determination of mineral content was carried out by inductively coupled argon plasma optical emission spectrometry (ICP-EOS). The water-nitric acid method is based on the principle of dissolving the sample in a mixture of 5 mL distilled water placed in PM60 Teflon bombs (Analytikjena 40Bar) containing 0.3 g of the sample which was heated to 60°C and 10 mL nitric acid (HNO_3 65%) (Merck) were added. The mixture was kept at room temperature for 30 minutes to allow oxidation to take place and then covered first with caps and then with lids previously pickled with HNO_3/H_2O (v/v, 1:1). The bombs were placed in the high-frequency microwave digestor (Analytikjena AG Top wave: 2.5 Ghz, Germany) controlled by a microcomputer by selecting the vegetable leaf mode as the digestion mode at 180°C, 50 bar for one hour. At the end of mixing, the digester stops by letting the bombs rest for 3 hours until they are completely cooled down. The cooled analyte is then carefully transferred by filtration through Whatman filter paper from the bombs to the previously pickled 50 mL volumetric flasks. To reduce the initial volume to 50 mL with distilled water, then place 13 mL of analyte in a previously pickled 15 mL conical flask for reading by inductively coupled argon plasma optical emission spectrometry (ICP-OES) [Optima 8300 Perkin Elmer, USA]. The analysis was performed in triplicate. Calibration of the ICP-EOS is performed using the working standard prepared from commercially available multi-element standard solution 3 at two points (1 mg/L and 2.5 mg/L, Perkin Elmer, USA). The most suitable wavelength, argon gas flow, plasma stabilization and other parameters of the ICP-EOS instrument for minerals are selected and measurements are performed within the linear range of the working standards used for calibration.[1] The working conditions were: Instrument: ICP-EOS [Optima 8300 Perkin Elmer,

Figure 1 Leaves of Vitex madiensis Oliv. (Source: www.google image.com)

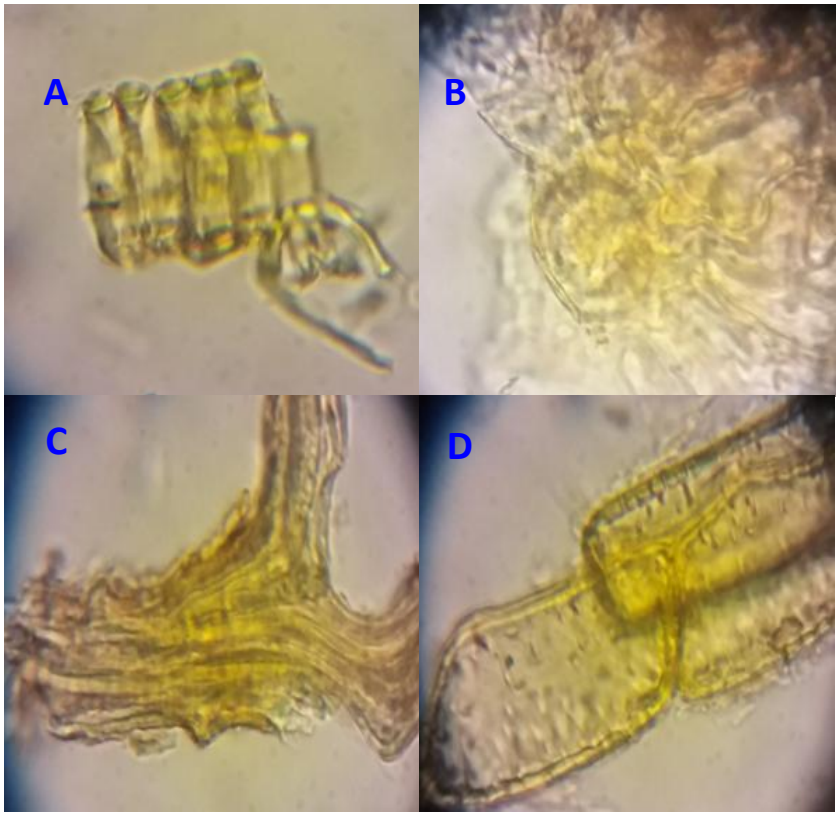

Figure 2 Micrographic characteristics of leaves of Vitex madiensis Oliv. Palisadic parenchyma (A), Hair (B), fragment of fiber (C), sclerites grouped (D)

USA]; Power of Rf: 1500Watt; Plasma gas flow (Ar): 8L/min; Nebulizer: 0.70L/min; Auxiliary gas flow (Ar): 0.2L/min; Viewing size: 5-22 mm; Copy and playback time: 1-5s (maximum 45s); Flow time: 1s (maximum 10s); View: Radial.

Evaluation of Radical Scavenging Activity

The evaluation of the antioxidant activity was performed using the ABTS and DPPH assays in accordance with Kapepula et al. protocol.[11]

(i) ABTS radical scavenging capacity

Its reaction with potassium or sodium persulfate ($K_2S_2O_8$), ABTS (2,2'-azino-bis-3-ethylbenz-Thiazo line-6-sulfonic acid) forms the blue to green ABTS cationic radical. The addition of an antioxidant reduces this radical and causes discoloration of the mixture. The discoloration of the radical measured by spectrophotometry at 734 nm is proportional to the antioxidant concentration. Dissolve in 500 µL of distilled water a quantity of ABTS reagent corresponding to 20 millimoles: solution A. Then dissolve in 500 µL of distilled water a quantity of potassium persulfate ($K_2S_2O_8$) corresponding to 10 millimoles: solution B. Mix the two solutions A and B at an equal volume and keep the mixture away from light between 12 and 16 hours: this solution is the mother solution of the ABTS radical. Then dilute x times the radical stock solution with methanol to obtain an analytical solution with an absorbance between 0.800 and 1.000.

In a test tube, place 20 µL of methanol with 1980 µL of ABTS solution: control solution. In another test tube, place 20 µL of the sample solution for each concentration level, and add to this solution 1980 µL of ABTS radical working solution and incubate for 30 minutes in the absence of light. Successive readings of the solutions for each concentration level are taken at 734 nm with the spectrophotometer: blank (methanol), control solution and samples.

The percentage inhibition of the ABTS radical is determined as follows:

$$\% \text{ inhibition} = [1 - (Ax/Ac)] \times 100 \quad (1)$$

Ax: absorbance of the ABTS radical in the presence of the extract Ac: absorbance of the ABTS (control solution) The ABTS trapping activity of the extracts was expressed in IC_{50}. Different IC_{50} values were determined using Graph Pad Prism version 6.0 software. Each sample was measured in triplicate.

(ii) Radical scavenging capacity of DPPH

The method is based on the degradation of the DPPH radical (2,2 DiPhenyl-1-PicrylHydrazyl). The DPPH radical is a purple colored radical, the addition of antioxidant reduces this radical and causes discoloration of the mixture. This radical discoloration measured by spectrophotometer at 517 nm is proportional to the antioxidant concentration. Dissolve 3.2 mg DPPH in 100 mL methanol (80%) and keep this solution in the dark for at least one hour. The absorbance of this solution should be adjusted to 0.7-0.05 using methanol (80%). Therefore, in a test tube, mix 20 µL of methanol with 1980 µL of DPPH radical solution: control solution. In another test tube, place 20 µL of sample for each

concentration by adding to 1980 µL of DPPH radical working solution and incubate for 30 minutes in the absence of light. Successive readings of the solutions for each concentration are taken with a spectrophotometer at 517 nm: blank (methanol), control solution and various sample solutions. The percentage inhibition of DPPH radicals was determined using the following formula:

$$\% = [1 - (Ax/Ac)] \times 100 \quad (2)$$

Ax: absorbance of the DPPH radical in the presence of the extract Ac: absorbance of DPPH (control solution)

The DPPH trapping activity of extracts was expressed in IC_{50}. Different IC_{50} values were determined using Graph PadPrism version 6.0 software. Each sample was measured in triplicate.

In Vitro Antihelminthic Activity

The *in vitro* antihelminthic activity of *Vitex madiensis* Oliv. leaves extracts was evaluated according to the method reported by Yashaswini.[12] Ethanolic and organic acid (dichloromethane) leaves extracts were tested for their antihelminthic activity and two earthworm species collected in Kinshasa city (*Benhamia rosea* and *B. itoleisis*) were used as an animal model. *Benhamia rosea* and *B. itoleisis* were used in this study because of their anatomical and physiological resemblance to gastrointestinal nematodes. These worms were divided into 4 groups or batches of 3 worms each. Group I worms were placed in a Petri dish containing a distilled water solution which served as a negative control. Group II worms were placed in a dish containing Albendazole at various concentrations (5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.625 mg/mL) which served as the standard (positive control). Group III and IV worms were placed in separate boxes containing solutions of the organic acid fraction and ethanolic extract at different concentrations in the same manner as the standard respectively. All test samples were prepared in distilled water. During the study, observations were made on the paralysis time and mortality rate of individual worms. The time of paralysis was noted when no movement of any kind was observed. Death was concluded when the worms completely lost their motility and did not respond, even after contact with the needle followed by discolouration of their body. The earthworms in the control group (I group) were alive for up to 48 hours after the experiment. The mean values for the time of paralysis and time of death of the earthworms *Benhamia rosea* and *Benhamia itoleisis* were calculated separately.

Antibacterial Activity

The antibacterial activity of leaves extracts of *Vitex madiensis* Oliv. was evaluated using the micro-dilution method on two strains of bacteria, *Escherichia coli and Staphylococcus aureus*, and the minimal inhibitory concentration (MIC) was obtained.[8]

Microscopic examination of the powder

Powder micrography is one of the fundamental methods of quality control of herbal medicines. It is very important to prepare the plate to be observed under the microscope in order to distinguish the different components of the powder. Two to three drops of the selected reagent (Steimetz) are placed on the slide and a small amount of powder is added. The preparation has been properly covered with an object slide to homogenize the preparation, which is followed by microscopic observation of various histological features.[13,14]

Data Analysis

Each concentration was tested in triplicate for each test performed. All results were expressed as mean values ± standard deviation (SD). Statistical analysis was performed using GraphPad 7.0 (GraphPad software, San Diego, California, USA). A one-way analysis (ANOVA) was used and the level of statistical significance was set at $p < 0.05$. The IC_{50} values were calculated with GraphPad Prism 7.0 by applying the function "log (inhibitor) vs. normalized slope of the response variable" after converting the concentrations to their decimal logarithm.

RESULTS AND DISCUSSION

Microscopic Studies

Phytochemical Analysis

The phytochemical analysis performed by the TLC revealed the presence of phenolic compounds as major compounds. These phenolic compounds are responsible for the antioxidant activity of this plant, since this potential is strongly attributed to them.[8] In comparison with the standards used, phenolic acids such as caffeic acid and chlorogenic acid are identified in this plant species.[15,16] These results are similar to those described previously, which indicate the presence of flavonoids in species of the *Vitex* genus,[1] particularly coumarins (fluorescent blue spots) in almost all the extracts tested. Concerning anthraquinones, we detected the presence of anthrones (red spots).[17,18] Analysis of the figures shows the coloration observed in the chromatographic profiles of each sample (violet, orange) and indicates the presence of terpenes, including iridoids, in all extracts. These results are similar to those described previously by Prakash et al. and

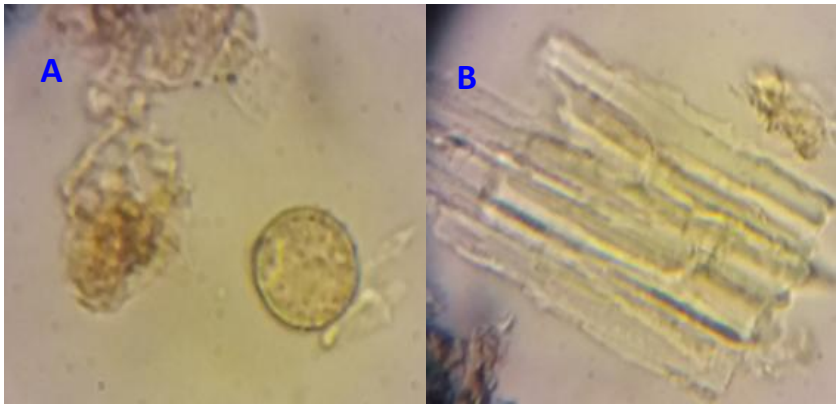

Figure 3 Micrographic characteristics of leaves of Vitex madiensis Oliv. spheroidal pollen grain (A), fragment of fiber (B)

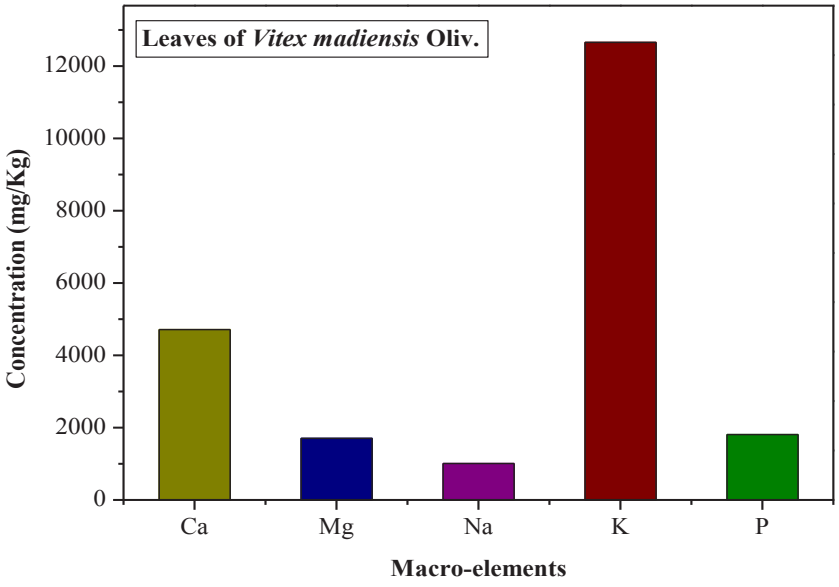

Figure 4 *Concentration of macro-element*

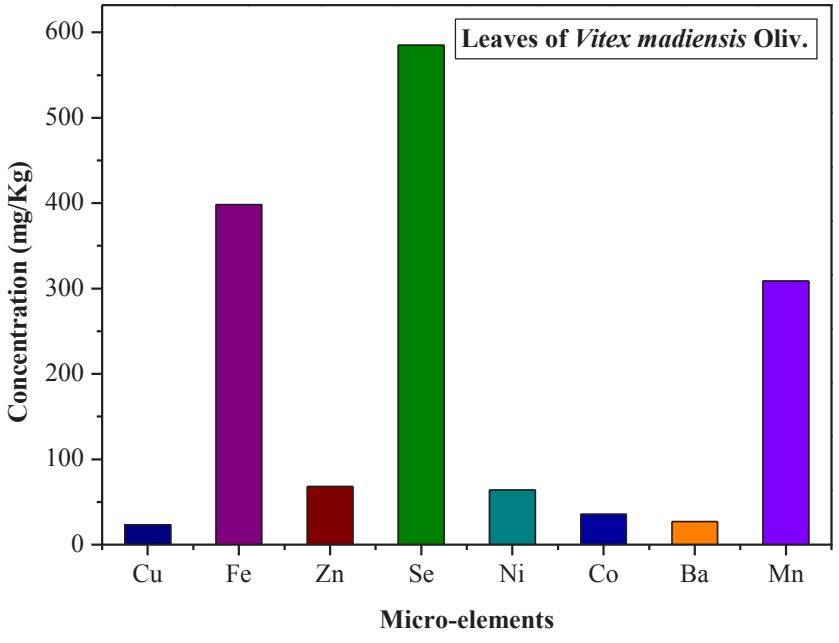

Figure 5 *Concentration of micro-elements*

Sivagnanasundaram and Karunanayake.[19] in *Vitex madiensis* Oliv.[2] which indicated the presence of these chemical compounds in *Vitex madiensis* Oliv. The presence of these compounds in a plant species could confer certain biological properties, such as antioxidant activity, antibacterial activity and anti-helmintic activity.[1]

Mineral Compounds

The mineral composition of the leaves extract of *Vitex madiensis* Oliv. is given in the table and the figures.

The above table shows us that thirteen mineral elements were detected in the powders of the organs of the plants studied.

It emerges from the two figures above that potassium is the macroelement that has a remarkable predominance in terms of concentration in the leaves of *Vitex madiensis* Oliv. followed by calcium in relation to the other macroelements detected (magnesium, sodium and phosphorus) (Figure 3). While selenium is the microelement with the highest concentration, followed by iron, manganese, zinc and nickel. While other microelements such as cobalt, barium and copper have a low concentration (figure 4). Soetan et *al.*[20] reported in the literature that minerals are inorganic nutrients, generally required in small amounts of less than 1-2500 mg per day. As with vitamins and other essential dietary nutrients, mineral requirements vary between animal species. For example, humans and other vertebrates (such as ruminants) require large amounts of calcium for building and maintaining bones and the normal functioning of nerves and muscles. Phosphorus is an important component of adenosine triphosphate (ATP) and nucleic acid and is also essential for acid-base balance, tooth and bone formation.

Ngbolua et *al.*[1] have shown that selenium, magnesium, copper, zinc, iron, nickel and manganese are important cofactors found in the structure of certain enzymes and are essential in many biochemical pathways. Sodium and potassium are important in maintaining osmotic balance between cells and interstitial fluid. The cobalt is required as a constituent of vitamin B12 and its metabolism. In addition to its role in vitamin B12, cobalt is also a cofactor of enzymes involved in DNA biosynthesis and amino acid metabolism.[21] Cobalt deficiency in ruminants causes anorexia, skeletal muscle atrophy, hepatic steatosis, hemosiderosis of the spleen and anemia.[20] It should be noted that selenium is an essential mineral element, a sulphur analogue with high biochemical activity, a component of certain selenoproteins and enzymes: glutathione peroxidase and other peroxidases, blood and tissue proteins. In terms of biological mechanisms of

action, selenium is a much more powerful antioxidant than vitamins C, A and E, beta-carotene and an active immunomodulator. Interactions between nutrition and disease, nutrition and drug metabolism have been reported. Excessive consumption of certain minerals can lead to a disturbance of homeostatic balance and undesirable side effects.

Antihelminthic Activity

The *in vitro* antihelminthic activity is described in table 1, which shows that the organic acid extract has better antihelminthic activity than the ethanolic extract from the leaves of *Vitex madiensis* Oliv. This indicates that the compounds with strong antihelminthic activity are those that pass easily through the less polar solvent (dichloromethane). It should be noted that both extracts show dose-dependent antihelminthic activity, with the shorter paralysis time being observed in the dichloromethane extract. The positive control has a similar paralysis time and mortality rate (i.e. 54.50 ± 3.53 minutes per 5 mg/mL) compared to our extracts, particularly for the organic acid extract 32.50 ± 2.88 minutes and the ethanolic extract 97.50 ± 3.53 minutes per 5 mg/mL. In reality, the positive control is composed of a single well identified molecule whose family is well known;[22] whereas our extracts are composed of a mixture of bioactive compounds.

Phytochemical analysis has shown that the leaves of *Vitex madiensis* Oliv. contain phenolic compounds, terpenoids and organic acids.[2] Other authors have reported in the literature that tannins and phenolic compounds are known to interfere with energy production in parasitic helminths by decoupling oxidative phosphorylation or that they bind to the free protein of the gastrointestinal tract of worms and cause death, demonstrating that the presence of flavonoids and polyphenolic compounds in these leaves could justify the antihelminthic activity.[23]

It should also be noted that the presence of these compounds in this plant species could attribute certain biological properties, such as antioxidant activity, antibacterial activity and antihelminthic activity.[24]

Indeed, it has been reported in the literature that antihelminthic drugs with an imidazothiazole nucleus such as Albendazole have the property of binding to the nicotinic acetylcholine receptors of parasitic worms, thereby mimicking the action of acetylcholine. This binding induces a change in acetylcholine permeability of the post-synaptic membrane, leading to muscle contraction, followed by spastic paralysis and eventual death of the worms.[25] On the other hand, polyphenols, such as tannins, have the ability to bind to proteins, and thus modify the physical and biochemical properties of parasitic worms. Indeed, they interact with the worms by binding to macromolecules in the cuticle or sheath of L3s larvae (containing proteins rich in proline and hydroxyproline) or bind to the enzymes secreted by the worms and thus block their activity.[26] It has also been envisaged that interactions between proteins in the exoskeleton (cuticle) and tannins, the digestive tract or even the genital structures of the worms could affect certain essential functions such as reproduction or nutrition.[27]

The antibacterial activity of *Vitex madiensis* Oliv. leaves extracts presented in table 3 indicates that *E. coli* and *S. aureus* are not sensitive to these extracts (CMI ≥ 1000 µg/mL). However, this activity is low[28] and is thought to be due to the presence of phenolic and triterpenic compounds in the extracts tested. Indeed, the bioactivity would be due to the nature of their wall,[29] *S. aureus* is a Gram-positive bacterium. Its wall is thick (several layers) and would be the pharmacological target of the active compounds present in the leaves of *Vitex madiensis* Oliv. These results corroborate those found in our previous studies on the antibacterial property of secondary plant metabolites.[1]

To our knowledge, this is the first time that the antibacterial activity of the organic/triterpenic acids in the leaves of *Vitex madiensis* Oliv. is reported in the literature.

Antioxidant activity

The antioxidant activity of the extracts tested as determined by ABTS and DPPH is presented in the table above and is expressed as IC_{50} values. The IC_{50} is the amount of antioxidant required to inhibit the initial radical concentration by 50%. Antioxidant activity is higher when its IC_{50} value is lower. Oleanolic acid, organic acid extract and ethanolic

Table 1 Mineral composition of *Vitex madiensis* Oliv. Leaves

Minerals elements	Concentration (mg/Kg)
Cu	23.166±0.86
Fe	398.40±5.44
Zn	68.00±9.67
Mn	308.83±2.00
Ca	4715.00±39.15
Mg	1703.33±16.50
Na	1008.73±9.17
K	12660.00±657.95
Se	585.10±16.02
Ni	63.89±41.31
Co	35.52±25.55
Ba	26.93±5.80
P	1810.00±196.77

Table 2 **The *in vitro* antihelminthic activity of ethanol and organic acid extracts of leaves of *Vitex madiensis* Oliv. against *Benhamia rosea* and *B. itoleisis***

Groupes	Drug treatment	Concentration (mg/mL)	Death rate (%)	Time taken for paralysis (min)
GP-I	Distilled water	-	-	-
GP-II	Standard (Albendazol)	5	54,50 ± 3,53	33,30
		2,5	74,50 ± 3,53	55,60
		1,25	84,50 ±3,53	66,70
		0,625	99,50 ±10,60	66,70
GP-III	OAE	5	32,50 ± 2,88	100
		2,5	45,00± 5,77	100
		1,25	57,50 ±2,88	66,66
		0,625	67,50 ± 2,88	66,66
GP-IV	EtOH	5	97,50 ± 3,53	100
		2,5	102,50 ± 3,53	100
		1,25	112,50 ± 3,53	100
		0,625	127,50 ±3,53	66,66

Legend: OAE: organic acid extract; EtOH: ethanolic extract; GP: group

Table 3 **Antibacterial effect of plant extracts**

	Samples Concentrations (µg/ mL)	
	OAE	EtOH
Microorganisms (Gram)	**1000**	**1000**
Escherichia coli ATCC 27195 (-)	MIC	MIC
Staphylococcus aureus ATCC 25923 (+)	MIC	MIC

Legend : OAE: organic acid extract; EtOH: ethanol extract; +: gram positive; -: gram negative, ATCC : American Type Cell Collection.

Table 4 **IC_{50} values (µg/mL) of organic acid fraction of *Vitex madiensis* Oliv. on ABTS and DPPH assays**

	IC_{50} (µg/mL)	
Drugs	**ABTS**	**DPPH**
OAC	40,74 ± 6,76	277140,50 ± 277137,60
EtOH	35,66 ± 4,80	59,70 ± 28,52
Oléanolic Acid	42,17 ± 1,66	-

acid from these leaves showed lower IC_{50} values, i.e. less than 100 µg/mL in the ABTS test. In the DPPH test, oleanolic acid and organic acid extract appeared inactive at the concentrations tested. Whereas the ethanolic extract showed antioxidant activity with the DPPH test. The capacities of the different extracts used to inhibit radicals vary considerably depending on the type of test. This difference may be due to the quality and/or quantity of secondary metabolites in the extracts. In addition, it should be noted that the IC_{50} values obtained with the ABTS test are of interest compared to the DPPH test. This difference in activity is attributed to the reaction mechanisms. Indeed, the ABTS radical reacts with hydrophilic and lipophilic compounds at the same time whereas the DPPH° radical reacts only with hydrophilic compounds.[11] A lower IC_{50} value of *Vitex madiensis* Oliv. leaves extracts indicates a higher antioxidant activity. To our knowledge, this is the first time that the antioxidant activity of the organic acid extracts from the leaves of *Vitex madiensis* Oliv. has been reported in the literature.

CONCLUSION

In this study, the histological elements of the powder were determined, the chemical composition, the mineral compounds were determined, and the antihelminthic, antioxidant and antibacterial activities of *Vitex madiensis* Oliv. leaves extracts were evaluated. The results obtained showed that the leaves of *Vitex madiensis* Oliv. possess various histological

elements, its phytochemical profile remains dominated by various secondary metabolites such as coumarins, anthraquinones, flavonoids, phenolic acids and terpenoids. The leaves of *Vitex madiensis* Oliv. also contain various minerals such as potassium, sodium, magnesium, manganese, cobalt, barium, calcium, iron, zinc, copper, selenium, nickel and phosphorus. Ethanol and organic acid extracts have significant antihelminthic activity. The antibacterial activity of the extracts of this plant is weak with respect to the bacterial strains tested. The extracts revealed an interesting antioxidant activity. These extracts can be further studied for other pharmacological properties, which could be useful in the design of new antihelminthic drugs.

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CONFLICT OF INTEREST

Authors have not conflict of interest.

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