Antihelminthic Activity, Phytochemical Profile and Microscopic Features of *Ocimum basilicum* Collected in DR Congo

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors CNK, EMN, KNN and PTM designed the study and wrote the protocol. Authors GNK, JTK, AM, CLI, EML and CMM wrote the first draft of the manuscript. Authors DSTT, DDT, JDA, BZG and GNB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

**Aim:** The main aim of the present study is to validate the bioactivity of *O. basilicum*, a medicinal plant traditionally used to treat helminthiasis in DRC.

**Place and Duration of Study:** Department of Chemistry, Science Faculty, University of Kinshasa, between June 2018 and March 2019.

**Methodology:** Different parts (stems, leaves and flowers) of *O. basilicum* and specimens of

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earthworms of *Benhamia rosea* were collected and identified at University of Kinshasa. Micrographic examination was carried out using Biolux NV microscope and X-ray fluorescence method for mineral content determination. Radical scavenging activity was performed using the ABTS radical test.

**Results:** Microscopic analysis of *O. basilicum* powder revealed various histological elements like crystalline fibres, sclerotic fibres, fragments of spiral vessels, pluricellular hairs and glandular hairs. Phytochemical screening in solution and by TLC revealed the presence of polyphenols (flavonoids, anthocyanins, saponins), alkaloids, steroids, coumarins, terpenoids, irridoids and anthraquinones. 20 elements including calcium, sodium, potassium, phosphorus, magnesium, sulphur, chlorine, aluminium, manganese, iron, zinc, copper, strontium, rubidium, bromine, silver, vanadium, neodymium, silicium and lead were determined. The aqueous extract of *O. basilicum* showed good dose-dependent antihelminthic activity and radical scavenging activity with IC₅₀: 27.04 ± 4.58 µg/mL.

**Conclusion:** This study provides an additional information on the mineral composition and antihelminthic activity of *O. basilicum* growing in DRC. The antihelminthic activity of this plant could be associated to the presence of flavonoids. To the current knowledge, no study on the histological elements of *O. basilicum* is available in the literature.

**Keywords:** Ocimum basilicum; DR Congo; mineral content; microscopic examination; antihelminthic activity; antioxidant activity.

### 1. INTRODUCTION

The poor diet and the consumption of unsuitable water in the majority of countries throughout the world expose certain populations to many water-borne diseases such as diarrhea, dysentery, typhoid fever, urinary tract infections and parasitic infections caused by helminths [1]. However, parasitic infections from gastrointestinal helminths are a constant concern and a major challenge for families living in rural and peri-urban areas in Africa who do not have sufficient financial income to ensure good medical coverage [2]. The main reason remains the fact that synthetic anthelmintics are not available everywhere in all places and anthelmintics are encountering resistance from the parasites [3,4].

Medicinal plants have been widely considered as sources of secondary metabolites and nutrients, which is due to their high numbers and varied chemical structures; possess interesting therapeutic properties [1]. Thus, in search of an alternative or complementary solution to control and fight against these animal parasitosis, most people from developing countries are increasingly resorting to the use of potentially antihelminthic medicinal plants since they are accessible, less toxic and possess a diversified bioactivity [4,5].

*Ocimum basilicum* (*O. basilicum*) is one of the cultivated plants often crop in many gardens in the Democratic Republic of the Congo (DRC) [6]. Considered as a potential source of vitamins and minerals, *O. basilicum* is used in DRC as spices and it is also involved in the treatment of several diseases such as: Haemorrhoids, cough, diarrhoea, sickle cell disease and in women's intimate hygiene [7,8,9]. Several studies have also reported its anti-inflammatory [8,10], anti-hyperglycemic [6], antiviral [11], antihelminthic properties [10] and as an important source of antioxidants [12].

The main aim of the present study is to validate the bioactivity of *O. basilicum*, a medicinal plant traditionally used to treat helminthiasis in DRC. The specific objectives are: (i) to assess the effect of this plant on earthworm in vitro, (ii) to assess the antioxidant activity, (iii) to determine the mineral composition and TLC profile of selected secondary metabolites, and (iv) to identify histological elements present in the leaves.

### 2. MATERIALS AND METHODS

#### 2.1 Materials

Different parts (stems, leaves and flowers) of *O. basilicum* were collected from a plantation in Mitendi, a district located in Mont-Ngafula municipality, in the western part of the Kinshasa city (DRC). These samples were identified at the herbaruim of the “Institut National des Etudes et Recherches Agronomiques (INERA)” at the Faculty of Sciences of the University of Kinshasa. These different parts of *O. basilicum*
were dried at room temperature and in the shade for two weeks, then ground to powder using an electric grinder.

Owing to their similarity to intestinal helminths, specimens of earthworms of *Benhamia rosea* species have been chosen and collected from a pond at the Priory Notre Dame de l'Assomption, monastery in Kinshasa. Their identification was made at the Natural Resource Management Laboratory within the Faculty of Agronomic Sciences of the University of Kinshasa.

### 2.2 Methods

#### 2.2.1 Micrographic examination

Histological elements were determined using Primo Star 200® microscope as previously described [13,14]. Briefly, two or three drops of the Steimetz reagent were deposited on a slide before adding a small amount of the powder and covered with a cover-slip. The histological elements were analyzed using a computer assisted image analysis program (Motic Images 2000, version 1.3; Motic Chine Group Co LTD). All microscopic analysis were performed in duplicate (X500 magnification).

#### 2.2.2 Thin Layer Chromatography (TLC)

TLC was performed according to the standard protocol described by Wagner based on the observation of spots of various colours to identify different secondary metabolites [15,16,17] as follows:

##### 2.2.2.1 Flavonoids and phenolic acids

One gram of plant powder was extracted with 5 mL of methanol by stirring for 10 minutes. Afterwards, 10 mL of filtrate was used for the TLC analysis using Silica gel F254 as a stationary phase, and formic acetic acid – glacial acetic acid - water (100:11:11:28) as mobile phase 1 and dichloromethane, formic acid, acetone (80:10:20) as mobile phase 2. As controls, rutin, hyperoside, isocoumeritin 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.

##### 2.2.2.2 Iridoids

For irridoids test, Silicagel F254 remained the stationary phase and ethyl acetate-methanol-water (100:13.5:10) were used as the mobile phase. The revelation was carried out with 5% sulfuric acid in ethanol by heating for 10 minutes at 100°C. True irridoids gave colorations, while other terpenes were colored in black.

##### 2.2.2.3 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.

##### 2.2.2.4 Anthraquinones

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 ethanol KOH (10%). The anthraquinones were red colored reflecting red fluorescence at 366 nm, while anthrones were colored in yellow.

##### 2.2.2.5 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 F254 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.

##### 2.2.2.6 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 ethanol KOH (10%).
The blue color was characteristic of coumarins.

2.2.3 Mineral composition

The detection and quantification of mineral elements in O. basilicum powder were carried out using the X-ray fluorescence spectrometry method (XEPOS) as previously described [18,19]. Five grams of the powder of each plant sample was compressed into pellets through the hydraulic press for each plant and the resulting pellets were introduced into the fluorescence spectrophotometer for reading. Briefly, the sample or pellet to be analyzed is placed under a beam of X-rays and under the effect of these rays, the sample resonates and re-emits X-rays, which are its own and these emissions are fluorescent. If we have a look at the energy spectrum of fluorescent X-rays, we can perceive characteristic peaks of different elements present in the sample. Therefore, it helps to know what elements are present and the height of these peaks helps to determine in what quantity are these elements. The Kα1 peak (3.313 Kev) of the K was used for the calculation; Bragg's HOPG Crystal target (17.4KV voltage and 1.99 mA of current) gave surfaces that were normalized compared to the peak from coherent and incoherent diffusion.

2.2.4 Antihelminthic activity

The antihelminthic activity was evaluated according to the method described by Guissou [20]. Different concentrations of aqueous extract from the sample were prepared by diluting the stock solution to concentrations ranging from 5 mg/mL to 0.625 mg/mL. A positive control solution of Albendazole (a drug used against helminths) was prepared under the same conditions and distilled water was used as the negative control. The pre-washed helminths were divided into three batches containing three specimens per batch. The first batch consisted of the plates containing the extract at different concentrations, the second, the dewormer (Albendazole) was dissolved and finally in the last batch containing distilled water. After contacting the helminths with different preparations (extract, Albendazole and distilled water), different parameters were then observed in particular; the behavior of the helminths, the time of paralysis and the mortality rate. This observation was carried out for 48 hours and the experiment was performed in triplicate under the same conditions.

2.2.5 Evaluation of antioxidant activity by the ABTS radical

The procedure described by Bongo was used for the evaluation of the antiradical activity [21]. By reaction with potassium or sodium persulphate (K2S2O8), ABTS⁺ (2,2'-azino-bis-3-ethyl-Benz-Thiazoline-6-Sulphonic Acid) forms the blue to green coloured cationic radical ABTS⁺. The addition of antioxidant reduces this radical and causes discoloration of the mixture. The discoloration of the radical, measured by UV-Visible (Perkin elmer lamba 8) at 734 nm is proportional to the antioxidant concentration.

After the reaction of the ABTS⁺ radical with the extract, the percentage inhibition of the radical is determined using the following formula:

% = [1 - \frac{Ax}{Ac}] × 100

Ax: The absorbance of the ABTS⁺⁺ radical in the presence of the extract.
Ac: The absorbance of the ABTS⁺⁺ radical (control solution).
The IC50 value of the aqueous extract of O. basilicum was determined using GrapPad Prism 6.0 software.

3. RESULTS AND DISCUSSION

3.1 Micrographic Test

Microscopic images of the histological elements of O. basilicum are given in Fig. 1.

The microscopic analysis of the powder of O. basilicum revealed a variety of histological elements including crystalline fibres (A), sclerotic fibres (B), fragments of spiral vessels (C), multicellular hairs (D) and glandular hairs (E, F). The determination of the histological elements of O. basilicum is very important for the characterization of its specific properties relating to its identification [12].

3.2 Phytochemical Analysis

3.2.1 Screening

Phytochemical screening of O. basilicum revealed the presence of polyphenols (flavonoids, anthocyanins, saponins), alkaloids, steroids and the absence of triterpenes, bound quinones and leuco-anthocyanins.

Figs. 2, 3, 4 and 5 show the TLC chromatogram of some secondary metabolites of O. basilicum.
Fig. 1. Histological elements of *O. basilicum* on microscope

Fig. 2. Methanol extract of *O. basilicum* for anthocyanins. Elution system: Ethyl acetate / Methanol / Water (100: 10: 40: v / v / v), development with phosphoric vanillin

Fig. 3. Methanol extract of *O. basilicum* for coumarins. Elution system: Toluene / Ether (1: 1; v / v), development with sulfuric anisaldehyde. Alcoholic KOH as developer at 366 nm

Fig. 4. Dichloromethane extract of *O. basilicum* for terpenes. References: thymol, menthol, oleanic acid. Elution system: Toluene / Ethyl acetate (9: 1; v / v), development with sulfuric anisaldehyde

Fig. 5. Ethyl acetate extract of *O. basilicum* for anthraquinones. Elution system: Ethyl acetate / Methanol / Ether (100: 13.5: 10; v / v / v). Alcoholic KOH as developer at 366 nm
The TLC showed the presence of phenolic compounds including phenolic acids, anthocyanins, coumarins and flavonoids; terpenoids and irridoids as well as anthraquinones. The anthocyanins were identified by the pink coloring spots (Fig. 2). Coumarins were detected by the fluorescent blue spot (Fig. 3). In figure 4, terpenes were detected by spots of various colors. Thymol has been identified with an RF of 0.75; Menthol at 0.5 and Oleanolic acid at 0.25. In figure 5, anthraquinones were detected by the red coloring spot.

3.2.2 Mineral content

Table 1 shows the concentrations of different macro and micro elements found in O. basilicum.

From this table, it can be observed that O. basilicum contains seven macro-elements (K, P, Ca, Na, Mg, S and Cl,) with K and Ca as the most abundant elements. Compared to the results found by Łośykowska [22] on O. basilicum collected in Poland, the concentration values of K, P and Ca in % are very close to those reported in the present study, with a large deviation being observed with regard to the concentration of Mg, Ca and Mg can act as co-factors in enzymatic reactions, participating in the biological functions of different tissues in the body. K and Na play an important role in the maintenance of physiological fluid and in the transmission of nerve impulses [23]. Zn and Cu play an essential role in cellular metabolism, enzymatic reactions, hemoglobin production and have antioxidant activity. Fe, which is one of the constituents of hemoglobin, is also involved in the transport of oxygen in the cell. The presence of these elements in sufficient quantities would justify the use of O. basilicum in the management of certain diseases such as diabetes and Sickle cell anemia [22,24,25,26]. The Comparison of the findings of this study with those carried out by Ozcan [27], on the same plant reveals huge differences in the concentration values of certain trace microelements. In addition, the presence of selenium was reported by Ozcan, which is not the case in this study. Furthermore, these differences are often due to the composition of the soil where the samples were collected [7,24]. Elements such as Mn, Zn, Cu and Mg are known to play an essential role in strengthening the immune system and preventing certain viral diseases such as COVID-19 [23,25].

3.3 Antioxidant Activity

The value of (IC\(_{50}\)) after reaction of the extract with the ABTS\(^{+}\) radical is 27.04 ± 4.58 (µg/mL). The lower the IC\(_{50}\) value is, the higher is the antioxidant activity of the extract. This value of IC\(_{50}\) far below 100 µg/mL shows that O. basilicum has an interesting antiradical power, which justifies its use in the fight against sickle cell disease [21].

3.4 Antihelminthic Activity

The antihelminthic activity of the aqueous extract of O. basilicum and the positive control are shown in Table 2.

From these results, it can be noted that the positive control has lower paralysis and mortality times than the extract. This is justified by the fact that the control is essentially made up of the active principle, whereas in the O. basilicum sample there are still many molecules, not all of which play the role of antihelminthic. The comparison of these results with those of previous studies like Akoto et al. [28] revealed huge differences in the time of paralysis,

| Elements     | Concentration in ppm | Elements     | Concentration in ppm |
|--------------|----------------------|--------------|----------------------|
| Potassium (K)| 3390.0 ± 8.0         | Iron (Fe)    | 59.8 ± 1.9           |
| Phosphorus (P)| 468.4 ± 1.3         | Zinc (Zinc)| 135.4 ± 4.8          |
| Calcium (Ca)| 3034.0 ± 7.0        | Copper (Cu) | 17.3 ± 3.2           |
| Sodium (Na)| 370.0 ± 1.3         | Strontium (Sr)| 98.8 ± 1.6        |
| Magnesium (Mg)| 122.0 ± 5.0        | Rubidium (Rb)| 39.3 ± 1.2          |
| Sulfure (S)| 370.5 ± 0.9         | Bromine (Br)| 7.3 ± 1.0            |
| Chlorine (Cl)| 576.9 ± 0.9         | Silver (Ag)| 5.6 ± 2.0            |
| Manganese (Mn)| 80.3 ± 1.2         | Vanadium (V)| 7.9 ± 1.1            |
| Aluminium (Al)| 122.8 ± 1.8        | Silicium (Si)| 305.4 ± 1.7        |
| Neodymium (Nd)| 21.4 ± 3.5         | Lead (Pb)| 7.9 ± 1.1            |

Table 1. Elements content of O. basilicum
Table 2. Worms death and paralysis time in the presence of the extract and the positive control

| Concentrations (mg/mL) | Worm paralysis time in minutes | Worm death time in minutes |
|------------------------|--------------------------------|----------------------------|
|                        | Albendazole                   | Aqueous extract            | Albendazole                   | Aqueous extract            |
| 0.3125                 | 86.0 ± 9.4                    | 112.5 ± 3.4                | 122.0 ± 4.6                   | 161.0 ± 8.2                |
| 0.6250                 | 67.5 ± 8.7                    | 98.0 ± 2.6                 | 108.0 ± 3.2                   | 140.5 ± 6.7                |
| 1.2500                 | 63.0 ± 4.6                    | 84.0 ± 3.9                 | 100.5 ± 5.7                   | 128.0 ± 4.2                |
| 2.5000                 | 34.5 ± 3.2                    | 54.5 ± 4.3                 | 83.0 ± 2.1                    | 103.5 ± 3.3                |
| 5.0000                 | 28.0 ± 3.2                    | 41.0 ± 1.8                 | 50.5 ± 1.8                    | 67.0 ± 2.4                 |

The aqueous extract of *O. basilicum* showed antihelminthic activity with 8 ± 0.72 minutes as the mortality time at a maximum concentration of 40 mg/mL [29].

At 5 mg/mL the mortality and paralysis times induced by the extract decrease, thus *O. basilicum* has an interesting activity against helminths, thus justifying its presence in the list of antihelminthic or vermifuge plants in studies conducted in Benin and Congo Brazzaville [30,31]. In addition, this property is believed to be due to the presence of polyphenols. Flavonoids have been reported to play a major role in antihelminthic activity [32,33].

4. CONCLUSION

This work involved a phytochemical study, microscopic analysis and evaluation of the antiradical and antihelminthic activities of *O. basilicum*. It appears from this study that *O. basilicum* is rich in secondary metabolites such as polyphenols (flavonoids, bound quinons, anthocyanins, saponins); alkaloids, anthraquinones, iridoids, coumarins. The mineralogical analysis of the powder revealed the presence of 19 mineral elements, of which potassium and calcium are the most abundant. Microscopic examination of the *O. basilicum* powder showed the presence of histological elements (crystaliferous fibres, sclerotic fibres, fragments of spiral vessels, multi-cellular sensory hairs, glandular hairs) which are characteristic of this species. *O. basilicum* showed antioxidant activity with an IC₅₀ value far below 100 µg/mL, considered interesting with ABTS⁺ and good antihelminthic activity towards helminths compared to a synthetic dewormer (Albendazole). Bioguided fractionning is undergoing in order to determine main molecules responsible of antihelminthic activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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