ORIGINAL ARTICLE

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Phytochemical profile, antioxidant and anthelmintic activities of *O. gratissimum leaves* collected in Kinshasa (D.R. Congo)

Etienne M Ngoyi,[1] Dorothée D Tshilanda,[2] Jason T. Kilembe,[3] Emmanuel M Lengbiye,[4] Carlos N Kabengele,[5] Giresse N Kasiama,[6] Damien T S Tshibangu,[7] Daniel O. Opota,[8] Koto-Te-Nyiwa Ngbolua,[9] Pius T Mpiana[10*]

ABSTRACT

Extracts of natural plants contain several chemical groups with various biological activities. This work aimed to evaluate the antioxidant, anthelminthic activities and determining the secondary metabolites, mineral and histological elements of *Ocimum gratissimum* from Democratic Republic of the Congo used for various culinary and therapeutic purposes. Primo Star 200® microscope was used for microscopic analysis further thin layer chromatography and X-ray fluorescence were used for chemical analysis. Antioxidant and anthelminthic activities were evaluated with DPPH° *radical scavenging* method and the rate of Benhamia rosea death, respectively. Spiral vessels, group scleritis, sclerotic fiber, pollen seed, stomata, hairs cells were found by Powder microscopic analysis. Secondary metabolites such as polyphenols (tannins, flavonoids, anthocyanins, leuco anthocyanins), alkaloids, steroids, terpenoids and various mineral elements (K, Ca, Cl, P, S, Mg, Na, Zn, Cu, Mn, Fe, etc.) were found in *O. gratissimum* leaves. Aqueous extract showed a high antioxidant activity with an IC50 of 25.7 ± 1.03 µg/mL. Aqueous extract of *O. gratissimum* leaves is a good antioxidant and it has an interesting anthelmintic activity.

Keywords: antioxidant activity, anthelmintic activity, *O. gratissimum*, microscopic examination, DRC

*Correspondence to: Pius T Mpiana, Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
ptmpiana@gmail.com

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INTRODUCTION

Plants play a very important role in human life, producing a large number of molecules with potential biological activities.[1] Due to the toxicity, cost and hard accessibility of synthetic products, a large part of the world's population uses medicinal plants for their primary healthcare.[2]

According to data from the World Health Organization, more than 80% of African population uses medicinal plants to relieve from their pain.[3] Democratic Republic of the Congo (DRC) owns a large tropical forest and biodiversity that can be used against various pathologies including malaria, diarrhea, sickle cell disease, cough, cancer, helminthiasis, etc.

Helminths are parasitic worms that can cause many diseases in animals as well as humans. Some anthelmintic drugs are ineffective against gastrointestinal nematodes, hence the use of phytotherapy.[4] Several studies have shown the antioxidant and anthelminthic properties of different compounds such as beta-carotene, ascorbic acid, and plant-derived polyphenols.[5,6] Our research team have identified more than 100 plants with interesting therapeutic virtues, including *O. gratissimum* L. This aromatic plant belonging to the Lamiaceae family and found in tropical countries is used for various culinary and therapeutic purposes. It showed strong antiradical, antisickling, anti-inflammatory, antiseptic and antibacterial activities due to some secondary metabolites including polyphenols.[7]

The present work aims to valorize *O. gratissimum* growing in the DRC by evaluating its antioxidant and anthelminthic activities and determining its secondary metabolites, mineral and histological elements.

[1]Etienne M Ngoyi: Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.
[2]Dorothée D Tshilanda: Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.
[3]Jason T. Kilembe: Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.
[4]Emmanuel M Lengbiye : Department of biology, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
[5]Carlos N Kabengele: Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.
[6]Giresse N Kasiama: Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
[7]Damien T S Tshibangu: Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.
[8]Daniel O. Opota, Faculty of pharmaceutical sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.
[9]Koto-Te-Nyiwa Ngbolua: Department of Biology, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.
[10]Pius T Mpiana: Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo

METHODOLOGY

Vegetal material

O. gratissimum leaves were collected in N'sele township in the East of Kinshasa, capital city of DRC and identified at the herbarium of the "Institut National d'Etudes et Recherches Agronomiques (INERA)" in the Faculty of Sciences of the University of Kinshasa. The vegetal material was dried at room temperature and crushed using an "sinbo" electric grinder.

Animal material

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, and identified at the natural resource management laboratory of the Faculty of Agricultural Sciences of the University of Kinshasa.

Microscopic examination

Primo Star 200® microscope was used to determine particular histological elements as previously described.[8] Briefly, two or three drops of the steimetz reagent were deposited on a slide before adding a small amount of the powder and covering with a cover-slide.

Phytochemical study

Chemical screening was carried out according to the standard protocol.[9] Thin layer chromatography (TLC) analysis was performed for the identification of terpenoids, flavonoids, phenolic acids and iridoids according to the protocol described by Wagner.[10]

The determination of the mineral composition in the powder of *O. gratissimum* leaves was carried out by X-ray fluorescence spectrometry method type XEPOS 3 as previously described.[11]

Antioxidant activity

The radical scavenger activity of the extract of the leaves of *O. gratissimum* has been evaluated by the DPPH° test according to the method used by Bongo.[12]

The inhibition concentration values (IC_{50}) of the individual samples are determined using GrapPadPrism 6.0 software.

Anthelmintic activity

Anthelmintic activity was evaluated according to the method used by Yashaswini.[13] Different aqueous extract solutions of *O. gratissimum* were prepared at various concentrations in water in progressive dilutions of second order geometric series (5 mg/mL to 0.625 mg/mL).

Albendazole solution (5 mg/mL) was prepared as the positive control and distilled water was used as the negative control.

The worms, previously washed, were divided into three batches containing three specimens per petri dish. In the first batch, there was a series of plates containing the extract, in descending order of concentration, in which three worms per plate were placed; in the second batch, albendazole used as a positive control was placed. Finally, the last batch consisted of petri dishes containing distilled water as a negative control.

After this contact between the worms and the products, different parameters such as the behaviour of the worms, the time of paralysis and the death time were evaluated.

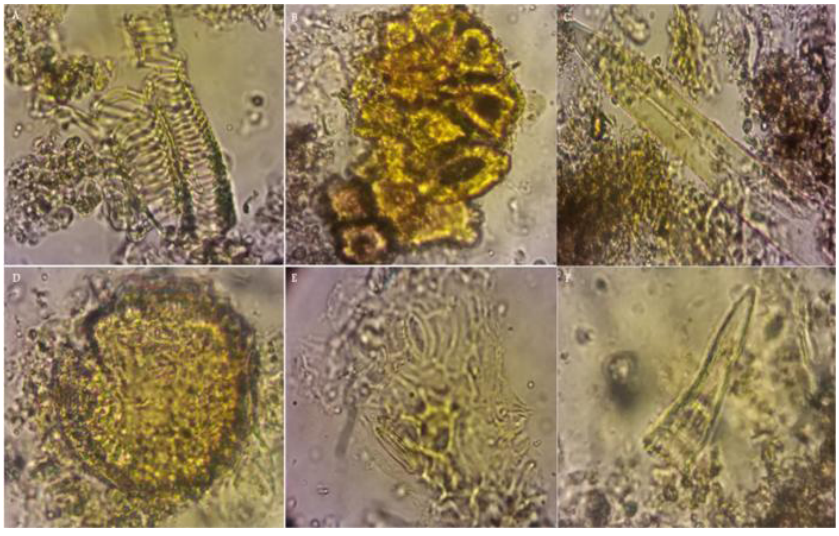

Figure 1 Cells present in *O. gratissimum*: (A) Spiral vessels, (B) Group sclerites, (C) Sclerous fiber, (D) Pollen seed, (E) Stomata, (F) Hairs

RESULT AND DISCUSSION

The figure 1 shows the different cells in *O. gratissimum* leaves.

Microscopic examination of *O. gratissimum* leaves powder has identified this species at the cellular level; thus this plant contains the following cells: Spiral vessels (A), Group sclerites (B), Sclerous fibre (C), Pollen seed (D), Stomata (E), Hair (F).

Chemical screening showed that *O. gratissimum* leaves contain polyphenols (flavonoids, anthocyanins, tannins, leuco anthocyanins), alkaloids, steroids and triterpenoids; however, they do not contain saponins and bound quinones.

These results for flavonoids, anthocyanins, tannins, leuco anthocyanins, alkaloids, steroids and terpenoids are similar to those found in the leaves of *O. gratissimum* collected in the western part of Kinshasa.[14]

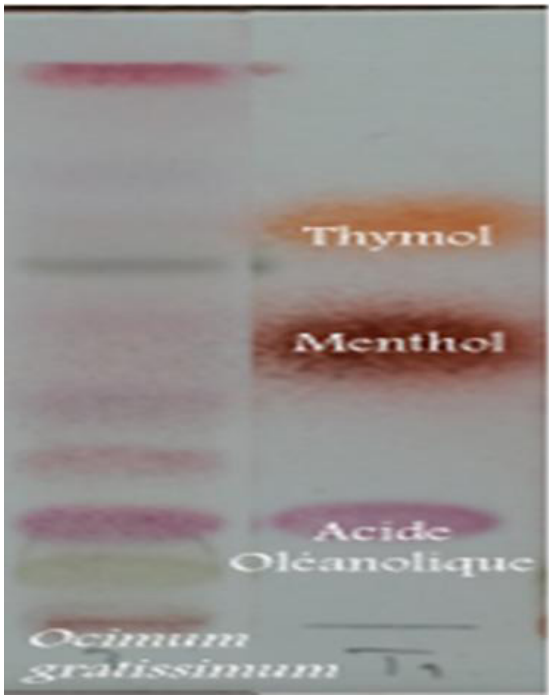

Figure 2 Terpenoids: TLC chromatogram of dichloromethane (DCM) extract of *O. gratissimum*, with oleanolic acid, menthol and thymol as controls. Elution system: toluene ethyl acetate (93:7; v/v) at 366 nm, with vanillin sulfuric acid as developer

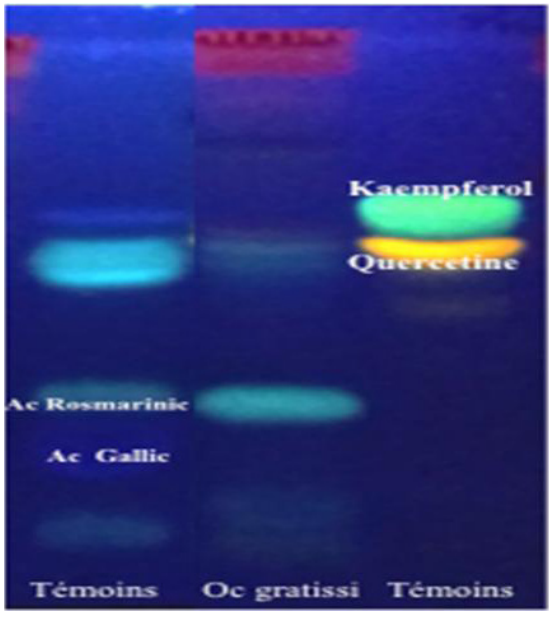

Figure 3 Flavonoids and Phenolic Acids: TLC chromatogram of the methanolic extract of O. *gratissimum* with rosmarinic acid, gallic acid, quercetin and kaempferol as controls. Elution system: ethyl acetate-formic acid-glacial acetic acid-water (100, 11, 11, 26; v/v/v) at 366 nm

Figure 4 Irridoids: TLC chromatogram of methanolic extract of O. *gratissimum*. PM: ethyl acetate-methanol-water (100; 13.5; 10; v/v/v) at 366 nm. H2SO4 as developer

Figures 2, 3 and 4 present the TLC chromatogram of the different secondary metabolites of *O. gratissimum*.

TLC of a few leaves extracts of *O. gratissimum* allowed the detection of thymol, oleanolic acid, rosmarinic acid and irridoids by comparison their RF to that of standards. Rosmarinic acid was also found in leaves extract of this plant by Tshilanda.[2] Saponins which were not detected in the present sample, were found in leaves of *O. gratissimum* harvested in the western part of Kinshasa in DRC.[15] This difference of phytochemical composition would be due to ecological changes.[14]

The presence of these various secondary metabolites, would justify the therapeutic activities of this plant.[16]

X-ray fluorescence analysis revealed the presence of some minerals in *O. gratissimum* leaves as shown in Tables 1 and 2.

Table 1 Macro-elements content of *O. gratissimum* leaves

Elements	Concentration (g/kg)
K	31.39 ± 0.07
Ca	21.77 ± 0.06
Cl	6.27 ± 0.01
P	4.95 ± 0.01
S	2.86 ± 0.008
Mg	0.79 ± 0.04
Na	0.37 ± 0.37

Table 2 Micro-elements content of *O. gratissimum* leaves

Elements	Concentration (ppm)	Elements	Concentration (ppm)	Elements	Concentration (ppm)
Al	681 ± 14	Cu	716 ± 12	Y	< 0.5
Sc	0.1 ± 0.1	Zn	83.0 ± 3.6	Zr	< 1.0
Ti	46.7 ± 1.6	Ga	3.2 ± 1.6	Nb	< 1.0
V	< 1.0	Ge	< 0.5	Mo	< 1.0
Cr	3.8 ± 0.1	As	< 0.5	Rh	< 5.1
Mn	60.3 ± 1.0	Se	< 0.5	Ag	9.9 ± 1.9
Fe	349 ± 15	Br	7.5 ± 1.0	Cd	3.6 ± 1.4
Co	< 3	Rb	37.3 ± 1.2	In	0.1 ± 0.1
Ni	12.7 ± 3.2	Sr	63.6 ± 1.4	Sn	53.0 ± 2.5
Sb	< 3.0	Te	10.2 ± 1.9	I	7.7 ± 2.6
Cs	< 4.0	Ba	< 2.0	La	< 2.0
Ce	< 2.0	Pr	9.5 ± 3.2	Nd	19.4 ± 3.6
Sm	0.1± 0.0	Eu	0.1± 0.1	Gd	0.1± 0.1
Tb	0.1± 0.1	Dy	0.1± 0.1	Ho	0.1± 0.1
Tm	0.1± 0.1	Hf	< 1.0	Ta	< 1.0
W	< 1.0	Hg	< 1.0	Tl	< 1.0
Pb	< 1.0	Bi	< 1.0	Th	< 1.0
U	3.5 ± 1.2	Si	189 ± 1.0		

Table 3 Paralysis time (minutes) for helminths at different concentrations

	Albendazol		Extrait aqueux de *O. gratissimum*	
Concentrations (mg/mL)	Temps de paralysie (en minutes)	Temps de mortalité (en minutes)	Temps de paralysie (en minutes)	Temps de mortalité (en minutes)
0.625	87	110	128	139
1.25	63	91	99	130
2.5	42	78	90	100
5	30	48	45	60

The results reported in Table 1 show that the leaves of *O. gratissimum* contain seven macro-elements among which K and Ca are predominant. These results have also been found by other researchers but in different concentrations.[17] This would be due to ecological factors.

Several of those mineral elements are essential for the organism. Mg plays a significant role in stress treatment, it participates in numerous intracellular enzymatic reactions and regulate the heart rhythm; Mg deficiency is believed to cause hypertension, diabetes and vasoconstriction.

Ca remains a major element in bone mineralization and in human blood; it plays a significant role in muscle contraction and as a second messenger in cellular exchanges. P is also one of the major elements in bones, participating in the formation of hydroxyapatite, and it is present in one of the main human energy source, ATP. K plays an important role in skeletal muscle contraction and nerve impulse. Both Na and K play a significant role in the maintaining of blood pressure.[3,18]

As reported in Table 2 *O. gratissimum* leaves contain 53 micro-elements, and Cu, Al, Fe, Si and Zn concentrations are higher than those of Sm, Tb, Tm, Eu, Dy, In, Ho and Gd.

Zn acts as an antioxidant in human body, produces insulin and participates in several enzymatic reactions. It contributes to the management of diabetics, hypertensives and sickle cell disease and strengthens the immune system. Fe is one of the major constituents of hemoglobin and myoglobin; its deficiency causes anemia, and weakness of the immune system. Cu participates in several enzymatic reactions: cytochrome oxidase, collagen synthesis, elastin, myelin, catecholamines and neuropeptides, in combination with zinc, they exhibit an antioxidant activity, and strengthens cellular immunity in combination with gold(Au) and silver(Hg).[11,19]

Antioxidant activity of *O. gratissimum* aqueous extract was evaluated by DPPH° concentration reduction. The IC_{50} value was 25.7 ± 1.03 µg/mL. This radical scavenging activity would be due to the presence of polyphenols, which are known as free radical scavengers.[20] The IC_{50} value of aqueous extract of *O. gratissimum* leaves harvested in Kinshasa is in the same order of magnitude (< 30 µg/mL) as that of the methanolic extract of the same species harvested in Bandundu and west of Kinshasa (DRC).[14,21] The sample used in this study showed better antioxidant activity than the methanolic extract of *O. gratissimum* leaves harvested in Akure State in southwestern Nigeria.[22] The aqueous extract of *O. gratissimum* leaves has a lower IC_{50} value than the aqueous extract of *O. canum*.[23]

Table 3 gives helminths paralysis and death times in the presence of aqueous extract of *O. gratissimum* leaves.

It can be seen from this table that aqueous extract of *O. gratissimum* shows anthelmintic activity, which is dose dependent. Although this extract shows the paralysis and death times of helminths slightly higher than albendazole used as positive control, it is more lower than those found by others researchers for others plants species.[24,25] This activity is probably due to the presence of polyphenols such as tannins and flavonoids, which have been reported to have the ability to bind proteins and modify the physical and biochemical properties of worms.[3] Comparing to those of Tshilanda for *O. canum*, the results of this work indicate that *O. gratissimum* would also be one of the potential candidates for gastrointestinal parasitosis control.[23]

CONCLUSION

The aim of this work was to valorize *O. gratissimum* used in DRC for its therapeutic uses by determining its chemical composition and evaluating both its antioxidant and anthelmintic activities. This plant contains many secondary metabolites (polyphenols, tannins, leuco anthocyanins, steroids, alkaloids, terpenoids), as well as mineral elements such as K, Ca, P, Mg, Na, Mn, Zn, Fe, Cu, etc. known for their therapeutic uses.

The IC_{50} value of 25.7 ± 1.03 µg/mL found using the DPPH° radical scavenging method showed that the aqueous extract of *O. gratissimum* leaves harvested at the eastern of Kinshasa (DRC) has a high antioxidant activity, which is justified by the presence of polyphenols such as rosmarinic acid. Furthermore, the death time observed during 60 minutes at 5mg/mL indicates that *O. gratissimum* also has an interesting anthelminthic activity thanks to its secondary metabolites such as flavonoids and tannins. These results could justify the use of *O. gratissimum* in the management of several pathologies in traditional medicine.

Other biological activities and structures determination of bioactive molecules on this plant are on going.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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