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Microscopic Studies, Phytochemical and Biological Screenings of *Ocimum canum*

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Abstract: It has been reported that more than 80% of the African population uses traditional medicine to primary healthcare. The aim of the study was to determine the histological elements, the phytochemical composition and evaluate the bioactivities of *Ocimum canum* in order to promote this plant used in Congolese traditional medicine. The earthworms were collected from the ponds of the secondary forest of Monastère Prieure Notre Dame de l'Assomption in Kinshasa city and the blood used in this study was provided by the Centre de Médecine Mixte et d'Anémie SS in Yolo-Sud quarter, Kinshasa, Democratic Republic of the Congo. The histological elements were determined by microscopic examination while different metabolites were determined using thin layer chromatography. Microscopic examination revealed the presence of crystalliferous fibres, fragment of parenchyma, grandular and non-glandular pluricellular trichomes, spheroidal pollen grains, diacytic stomata, spiral vessels. With regard to chromatographic analysis, phytochemicals identified were anthocyanins, coumarins, flavonoids, terpenoids and iridoids. The extracts from *O. canum* showed good antihelminthic, antisickling and antioxidant activities related to their phytochemicalsAll these findings constitute a scientific evidence validating the use of this medicinal plant for the management of various ailments in the Democratic Republic of the Congo.

Keywords: Antihelminthic Activity, Microscopic Features, Oleanolic Acid, *Ocimum canum*, Rosmarinic Acid

1. Introduction

Phytotherapy has undergone a major expansion in recent years throughout the world, because not only that compounds synthetized from plants are infinitely more varied than those found in drugs that have several side effects, but they are also better tolerated by the human body [1]. Thus, medicinal plants are considered as a major and inexhaustible source of substances with therapeutic properties. They have shown through several studies various important biological activities including antisickling activity. The advantages offered by these compounds are constantly attracting the interest of several research projects in order to discover even many other active principles, particularly from endemic plants that have been less studied or not at all [1, 2]. Following the statistics of the World Health Organization (WHO), more than 80% of the African population uses traditional medicine to solve their primary health problem. The use of medicinal plants for various health problems is not only a choice, but is also linked to poverty and the high costs of modern medicines [3-6]. The

Democratic Republic of Congo (DRC), through its cultural diversity, the richness and diversity of its flora, is one of the world's reservoirs of biodiversity [7-8]. This is why we chose *Ocimum canum* Sims, used in Congolese pharmacopoeia in the treatment of several diseases such as cough, hemorrhoid, diarrhea and sickle cell disease [9]. The main aim of this study was to enhance the value of *O. canum* species growing in the DRC. The specific objectives of this work were: (i) to determine the histological elements of the plant, (ii) to evaluate its qualitative phytochemical composition, and (iii) to evaluate the biological activities of this plant.

2. Material and Methods

2.1. Materials

In this study, the aerial part of *Ocimum canum* (stems, leaves and flowers) was used. These organs were harvested in the Kinkole district, N'sele municipality, Kinshasa, DRC. These samples were identified at the herbarium of the Institut National des Etudes et Recherches Agronomiques (INERA) located at the Faculty of Sciences of the University of Kinshasa. They were dried at room temperature (± 27°C) within 15 days, and then ground in order to obtain a fine powder. The worm specimens were collected from the ponds of Monastère Prieure Notre Dame de l'Assomption and identified at the Natural Resources Management Laboratory, Faculty of Agricultural Sciences, University of Kinshasa. The blood used in this study for the antisickling activity was provided by the Centre de Médecine Mixte et d'Anémie SS, located in Yolo-Sud quarter, Kalamu municipality in Kinshasa, DRC.

2.2. Methods

2.2.1. Microscopic Examination of the Powder

Powder micrography is one of the fundamental methods of controlling the quality of plant drugs. 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) were placed on the object slide and a small amount of powder was added. The preparation was covered properly with a cover-slide in order to homogenize the preparation which is followed by the microscope observation of different histological features. It is important to carefully wipe the outer surface of the blade and make very light preparations in order to distribute tissues evenly and avoid overlapping. Observations were made with a Zeiss Primo Star 200® microscope and pictures were taken with Smart Phone Samsung E7 [10-11].

2.2.2. Preparation of extracts

Aqueous extract: 10.0 g of powdered leaves were mixed with 100 mL of boiling water for 5 min. The infusion was cooled to room temperature before filtration and the filtrate evaporated to dryness under reduced pressure (40°C) in a rotative evaporator to provide dry extracts which were kept in hermetic and opaque flasks at 4°C.

Ethyl acetate extract: 10.0 g of each powdered leaves were prepared by maceration with ethyl acetate to obtain 200 mL of filtrate. The filtrate was evaporated to dryness under reduced pressure (40°C) in a rotative evaporator to provide dry extracts which were kept in hermetic and opaque flasks at 4°C.

2.2.3. Phytochemical Screening

Phytochemical screening using thin layer chromatography (TLC) was performed according to the standard protocol used by Ngombe *et al.* [10], Inkoto *et al.* [11] and Tiwari *et al.* [12].

A. Search of flavonoids and phenolic acids

One g of powder was macerated and stirred in an ultrasonic bath with 10 mL of methanol for 10 minutes. Then, 10µL of the filtrate were used for TLC analysis. The stationary phase consisted of silicagel F_{254}, while the mobile phase 1 consisted of the system: ethyl acetate - formic acid - glacial acetic acid - water (100:11:11:26). Kaempferol, rosmarinic acid, gallic acid and quercetin: 1mg/ml (methanol) were used as controls. Detection: the chromatogram, once developed, was observed under UV at 254 and 366 nm. The DPBAE/PEG reagent is sprayed followed by observation under UV at 366 nm. The presence of flavonoids is visualized by fluorescent spots of various colors (yellow-orange-green), coloration varying according to the structure of the compounds highlighted. Blue fluorescence are often due to phenolic acids.

B. Search for Irridoids

Ten µL of the extract prepared for the flavonoid test were used. Silicagel F_{254} was used in the stationary phase, while the mobile phase consisted of the ethyl-methanol-water acetate system (100:13.5:10) while sulphuric acid 5% in ethanol was used as a reagent. After heating for 10 minutes at 100°C, true irridoids give various colours and other terpenes are coloured in black.

C. Search of Anthocyanins

Ten µL obtained in the flavonoid test was used for anthocyanin research. Silica gel F_{254} was used in the mobile phase and ethyl acetate formic acid - water system (100:10:40) in the stationary phase. Phosphoric vanillin was used as a reagent. After heating for 10 minutes at 100°C, the pink colouring reveals anthocyanins.

D. Search of Terpenes

One g of powder is macerated in an ultrasonic bath with 10 mL of dichloromethane for 15 minutes and 10 µL of the filtrate were used for the TLC analysis. Silicagel F_{254} plate was used as the stationary phase, while the toluene-ethyl acetate mixture (93:7) was used in the mobile phase. Thymol and menthol: 1 mg/mL (methanol) were used as controls. The plate was revealed by sulfuric vanillin. After heating for 10 minutes at 100°C, terpenes give various colours with this reagent.

E. Search of Alkaloids

To one g of drug powder is macerated with about 1ml of ammonia 10% in an erlenmeyer, 5mL of ethyl acetate (or methanol) was added and the mixture was stirred for 30 minutes. So, 10µL of the filtrate were used for the TLC analysis. The mobile phase consisted of the dichloromethane-methanol-ammonia system 25% (8:2:0.5). Caffeine 5 mg/ml deposit: 10µL was used as a control. Revelation: the chromatogram, once the chromatogram has been developed,

is observed under UV light at 254 and 366 nm, then sprayed with the Draggendorff reagent and observed in visible light. The presence of alkaloids is characterized by the presence of spots ranging from yellow-orange to yellow-brown.

F. Search of tannins

To 2mL of the solution obtained in the general flavonoid test, a few drops of a 1% ferric chloride 1% solution were added: obtaining a dark blue, black or green coloration if tannins. To 3mL of the solution obtained in the general flavonoid test, add 1.5mL of Stiasny reagent (formaldehyde and HCl 30% concentrated 2:1). Heating in a water bath at 90°C causes the catechic tannins to precipitate quantitatively (pink precipitate). After filtration, the sodium acetate saturated filtrate is added with few drops of ferric chloride 1% (presence of gallic tannins if a blue or black blue is obtained).

2.3. Evaluation of Biological Activities

2.3.1. Evaluation of Antioxidant Activity

DPPH assay is the *in vitro* biochemical method based on the degradation of the DPPH- (2,2 DiPhenyl-1-PicrylHydrazyl) radical. The DPPH° radical is a violet coloured radical, the addition of antioxidant reduces this radical and causes the discoloration of the mixture; this discoloration of the radical was measured by spectrophotometry at 517nm, which is proportional to the antioxidant concentration [11, 14]. Dissolve 3.2 mg of DPPH in 100 mL of methanol 80% and keep the solution away from light for at least one hour. The absorbance of this solution must be adjusted to 0.7 ± 0.05 using methanol 80%. Then, in a test tube, place 20µL of methanol with 1980µL of the solution of DPPH° radical: control solution. In another tubes, place 20µL of the sample solution for each concentration level in the range of concentrations from 50 to 250 µg/ mL , then add to 1980µL of the DPPH° radical analysis solution and incubate away from light for 30 minutes. Read the solutions successively for each concentration (3 replicates) using a spectrophotometer at 517 nm: the white (methanol), the control solution and the sample solutions. The percentage inhibition of the DPPH radical by the sample is determined as follows:

$$\%\ inhibition = [1 - \frac{A_x}{A_c}]\ x\ 100 \qquad (1)$$

Where: Ax: the absorbance of the DPPH radical read in the presence of the extract

Ac: the absorbance of the DPPH radical read for the control solution

The IC_{50} values of different samples were determined using GrapPad Prism 6.0 software.

2.3.2. Evaluation of Antihelminthic Activity

The *in vitro* antihelminthic activity was evaluated using the method described by Dash *et al.* [14] and Guissou *et al.* [15]. The extracts to be tested were previously dissolved in saline solution (0.9%) (5mg/mL) in order to obtain the stock solution. The stock solution is diluted in series in order to optimize concentrations from 10 mg/mL to 0.6125 mg/mL. A standard solution (Albendazol) which was used as the positive control was prepared under the same conditions as the extract and the saline solution was considered as the negative control. The worms, previously washed, were divided into three batches containing three specimens per petri dish. The experiments were carried out in three batches of which the first batch contained the earthworms and the extract while the second batch contained the standard (worming agent) (positive control) and the last batch contained only the saline solution (negative control). For each batch, three earthworms were provided. The three petri dish were kept at room temperature for 48 hours for a good observation. The paralysis rate of each specimen was evaluated as a function of time of the worm by being in contact with different solutions. While the mortality rate (expressed in percentage) was determined as follows:

$$Mortality\ rate\ (\%) = \frac{Number\ of\ dead\ worms}{Total\ number\ of\ worms}\ x\ 100 \qquad (2)$$

2.3.3. Evaluation of the Antisickling Activity

All antisickling experiments should be carried out on freshly collected blood. In order to confirm their SS nature, blood samples taken from sickle cell patients should be first characterized for hemoglobin electrophoresis on cellulose acetate gel. Once the SS nature is determined, these blood samples will be stored in the refrigerator at a temperature of ± 4°C. The stock solutions of plant extracts were prepared by simple dilution of the lyophilisate in physiological saline (NaCl 0.9%) at 1 mg / mL. Two successive dilutions were carried out in order to obtain different solutions at 0.5 mg/mL and 0.25 mg/mL. The sickle cell blood (0.5 mL) was previously diluted five times with 2 mL of NaCl (0.9%) - $Na_2S_2O_5$ mixture (v/v) [13].

Microscopic preparations were carried out by placing on the slide a diluted blood drop mixed with a drop of the drug (extract). The solution was covered with a cover slide and the edges of the slides were covered with supercooled paraffin in order to create hypoxia conditions. These various preparations were observed under the light microscope (Olympus Model CH10BIMF brand) at 600X magnification, after 24 hours.

3. Results and Discussion

3.1. Microscopic Characteristics

Microscopic analysis of the powder of *O. canum* leaves revealed various histological elements. Leaves were characterized by: crystalliferous fibre fragment (A), granular hairs (B), spheroidal pollen grains (C) and pluricellular non-glandular trichomes with a stiff pear (D). Other microscopic features were diacytic stomata, spiral vessel, suber fragments , characteristic to *O. canum*. To our knowledge, few informations on the micrographic study of this plant has been reported before. Nevertheless, Hubert [16] in a cross-sectional study of *O. canum* leaves reported the presence of spiral vessels, trichomes and sclerites. Previous studies reported that spheroidal pollen grains were characteristic to certain species of the family Lamiaceae such as *Ocimum* species [17].

Moreover, given its importance in traditional pharmacopoeia, the determination of its histological elements is essential for the characterization of its properties and the

detection of falsifications [18].

3.2. Secondary Metabolites

Phytochemical analysis revealed the presence of the compounds presented in table 1 below.

Table 1. *Phytochemicals from O. canum.*

Researched substance	test
Flavonoïds	+
Anthocyanins	+
Coumarins	+
Terpenoids	+
Irridoids	+
Anthraquinones	-

Legend: +: presence of the searched substance, -: absence of the searched substance

TLC fingerprints of different phytochemicals were characteristic. For polyphenols, they showed the presence of glycosylated flavonoids and phenolic acids as main compounds (Figure 2). By comparison with used standards, they showed that *O. canum* contain quercetin, rosmarinic acid. Rosmarinic acid is the phenolic common acid to *Ocimum* species [19]. The chromatograms in Figure 3 correspond to the search for terpenoids. By comparison with used standards, they showed that *O. canum* contain oleanolic acid, and terpenic acid. The presence of these phenolic compounds in the plant would justify its antisickling and anti-scavenging properties [2,20]. These findings are consistent with previous studies by Bassole *et al.* [21] and Bondet *et al.* [22].

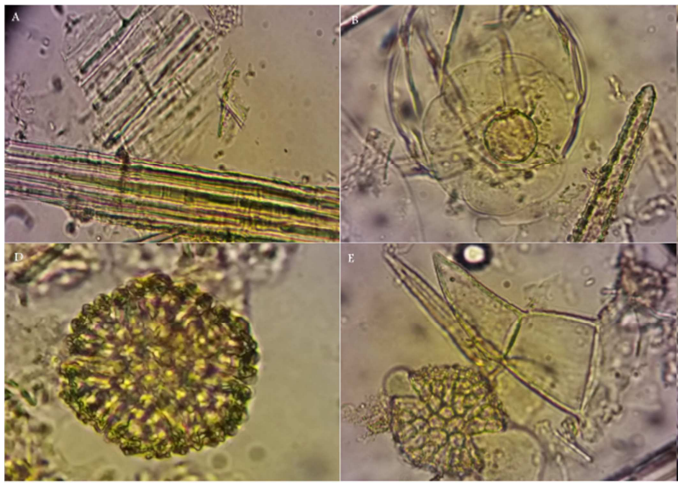


Figure 1. *Micrographic characteristics of O. canum: crystalliferous fibre fragment (A), granular hairs (B), pollen seeds (C) and pluricellular non-glandular trichomes with a stiff pear (D).*

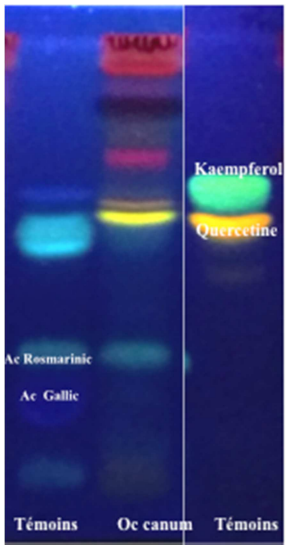
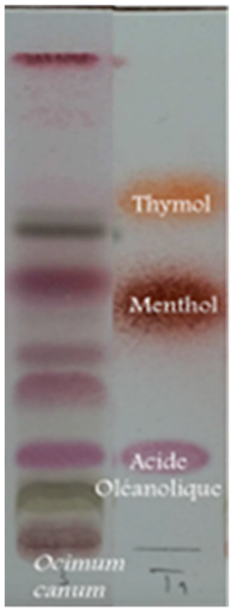


Figure 2. *TLC chromatogram of methanolic extract from Ocimum canum with caffeic acid, gallic acid, rosmarinic acid, ferulic acid, kaempferol quercetine as standards; developed with dichloromethane/formic acid/ acetone(80:10:20; v/v/v) and visualized at 365 nm with Natural Products-PEG reagent. Flavonoids are detected as yellow-orange fluorescent spots and phenolic acids as blue fluorescent spots.*



Figures 3. *TLC chromatogram of dichloromethane extract from Ocimum canum with oleanolic acid, menthol and thymol as standards; developed with toluene/ ethylacetate (97:3; v/v) and visualized at 365 nm with sulfuric anisaldehyde reagent. Terpenes are detected as violet spots.*

3.2. Antioxidant Activity

The antioxidant activity of the aqueous extract of *O. canum* in DPPH assay gives the IC_{50} value equal to 199.99 ± 41.5 µg/ mL. Since the IC_{50} is greater than 100 µg/ mL, this plant has a low radical scavenging activity compared previous studies [23-24].

3.3. Antihelminthic Activity

The antihelminthic activity of *O. canum* leaf extracts is presented in the tables below.

Table 2. *Paralysis time of earthworms in the presence of plant extracts.*

Concentrations (mg/ml)	Paralysis time (minutes)	
	Albendazol	*O. canum*
0.625	7.5	98
1.25	63	84
2.5	34	64
5	28	51

Table 3. *Mortality rate of earthworms in the presence of plant extracts.*

Concentrations (mg/mL)	Paralysis time (minutes)	
	Albendazol	*O. canum*
0.625	100	100
1.25	75	100
2.5	75	100
5	91.5	100

As observed in the above tables, the extract of the plant used possesses antihelminthic properties due to the paralysis observed in treated groups where different concentrations were used (Table 2). However, at the lowest concentration (0.625 mg/mL) of *O. canum*, the worm paralysis was observed just after 98 minutes of contact. By comparing the plant extract with the positive control (Albendazol) at low doses (0.625 mg/mL), it was observed that the extract has a lower activity than albendazol. These findings are consistent with previous studies that evaluated the antihelminthic activity of medicinal plants [25-27]. With regard to the mortality rate (Table 3), the plant extract has a high antihelminthic activity. By comparing this plant with the positive control (mebendazol), we notice that at low concentration (0.625mg/mL), aqueous extract from *O. canum* shows significant activity (100% of the mortality rate) than Albendazole (50% of the mortality rate), which is a modern product used in the treatment of gastrointestinal worms. This result is justified by the fact that this species contains secondary metabolites such as flavonoids, which confer plant potential antihelminthic properties to plants [28-30]. Since parasitic infections by gastrointestinal helminths remain among the main causes of school absenteeism in children, including sickle cell disease, the antihelminthic activities of this plant studied constitute an alternative management method and would reduce the risk of parasitic infections.

3.4. Evaluation of Antisickling Activity

The microscopy of the antisickling test is presented in the figures below.

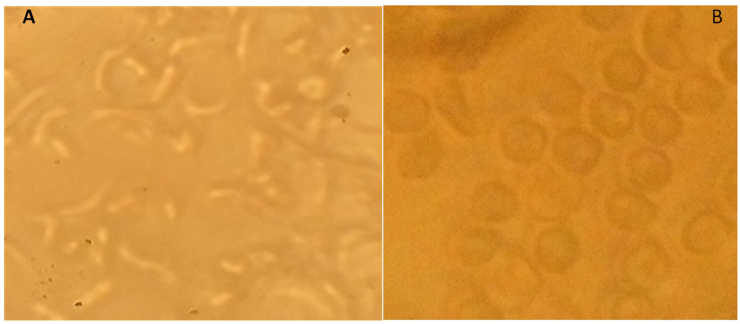


Figure 4. *Microscopy of the anti-sickling test: untreated erythrocytes (A); erythrocytes treated with ethyl acetate extract (250mg/ml) of O. canum (B).*

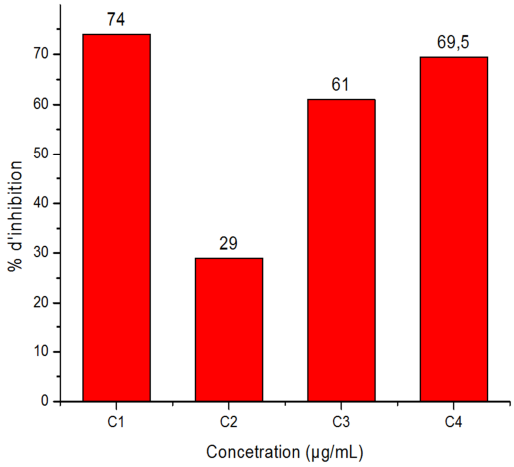


Figure 5. *Percentage of inhibition of erythrocyte hemolysis in the presence of ethyl acetate extracts).*

Figure 4 (A) shows the red sickle-shaped blood cells, which effectively indicates that it is SS blood. Under our experimental conditions, this falciformation was maintained by the hypoxia created by surrounding the microscope slide preparations with paraffin. On the other hand, Figure 4 (B) shows the behaviour of erythrocytes that return to their normal biconcave form in the presence of plant extracts, when they are placed under the same hypoxic conditions as the control in the presence of the saline solution. As observed in Figure 4 (B), in the presence of the plant extract (250 µg/mL), erythrocytes return to their normal form, which is biconcave, while they are placed in the same condition as the control. These results are justified by the presence of terpenic acids such as oleanolic acid, ursolic acid… contained in the plant with antisickling properties. These results are similar to those obtained by Tshilanda [31] who evaluated the

antisickling properties of *Ocimum* species growing in DRC. The membrane fragility test is based on the ability of plant extracts to prevent hemolysis of sickle cells, induced by hypotonia and heat, and thus prevent the release of hemoglobin. As observed, the extracts of *O. canum* have shown inhibitory properties for erythrocyte hemolysis (Figure 5).

4. Conclusion

In this current study, it was necessary to determine the histological elements of the powder, the phytochemical composition and to evaluate the antisickling, antioxidant and anti-helminthic activities of *O. canum* extracts, plant used in Congolese traditional medicine. The results obtained revealed that *O. canum* has various histological elements, its phytochemical profile remains dominated by anthocyanins, flavonoids, alkaloids, terpenes and coumarins. Moreover, this plant also possesses antioxidant, antisickling and antihelminthic properties. This study opens new perspectives for the discovery of new antihelminthic and anti-sickling drugs based on the ethno-medical knowledge. It would therefore be desirable for further studies to be carried out using gastrointestinal helminths as an animal model to confirm or refute the results obtained in this work. The structural determination of the bioactive compounds contained in the extracts from *O. canum*, would facilitate a better pharmaceutical formulation for the management of sickle cell disease and helminthiases.

References

[1] Nassima C. (2011). Activité biologique *in vitro* des extraits de *Pistacia lentiscus* contre les radicaux ABTS•+, O_2•$^-$ et •NO et caractérisation des fractions actives. Mémoire de Magister, Biochimie Appliquée aux Substances Végétales Bioactives, Département de Biologie Physico-Chimique, Faculté des Sciences de la Nature et de la Vie, Université Abderahmane Mira de Bejaia, Alger, Algérie.

[2] Ipona EN, Inkoto CL, Bongo GN, Mulenga CM, Ilinga BL, Shetonde OM, Mbala BM., Tshilanda DD, Mvingu BK, Kayembe JS, Mpiana PT, Ngbolua KN (2018). Ethno-Botanical Survey and Ecological Study of Medicinal Plants Traditionally Used Against Erectile Dysfunction in Democratic Republic of the Congo. *Bioscience and Bioengineering,* 4 (4): 85-91.

[3] WHO. Organisation Mondiale de la santé (OMS) Rapport sur la médecine traditionnelle: Besoins et potential, 2002. N° 4. 6 p.

[4] Ngbolua, KN, Mihigo, SO, Inkoto CL, Masengo C, Tshibangu, DST, Zoawe, BG, Robijaona B, Fatiany RP and Mpiana P. T. Ethno-botanical survey of plant species used in traditional medicine in Kinshasa city (Democratic Republic of the Congo). *Tropical Plant Research*, 2016, 3 (8): 413–427.

[5] Masunda AT, Inkoto CI, Bongo GN, Oleko Wa Oloko JD, Ngbolua KN, Tshibangu DST, Tshilanda DD, Pius Tshimankinda Mpiana. Ethnobotanical and Ecological Studies of Plants Used in the Treatment of Diabetes in Kwango, Kongo Central and Kinshasa in the Democratic Republic of the Congo. *International Journal of Diabetes and Endocrinology*, 4 (1): 18-25.

[6] Ngbolua KN, Inkoto CL, Mongo LN, Masengo AC, Masens Da Musa YB, Mpiana PT. (2019). Etudes ethnobotanique et floristique de quelques plantes médicinales commercialisées à Kinshasa, République Démocratique du Congo. Rev. Mar. Sci. Agr. Vet. 9 (1): (Sous presse).

[7] Kambale JLK, Asimonyio JA, Shutsha RE, Katembo EW, Tsongo JM, Kavira PK, Yokana EI, Bukasa KK, Nshimba HS, Mpiana PT, Ngbolua KN (2016). Etudes floristique et structurale des forets dans le domaine de chasse de Rubi-Tele (Province de Bas-Uele, Republique Democratique du Congo). International Journal of Innovation and Scientific Research, 24 (2): 309-321.

[8] Asimonyio JA, J. C. Ngabu JC, C. B. Lomba CB, C. M. Falanga CM, P. T. Mpiana PT, K. N. Ngbolua KN 2015). Structure et diversité d'un peuplement forestier hétérogène dans le bloc sud de la réserve forestière de Yoko (Ubundu, République Démocratique du Congo). International Journal of Innovation and Scientific Research, 18 (2): 241-251.

[9] Ngbolua KN., Mandjo BL., Munsebi J. M., Masengo CA., Lengbiye EM., Asamboa L. S., Konda RK., Dianzuangani DL, Ilumbe M, Nzudjom AB, Kadimanche M, Mpiana P. T. (2016e). Études ethnobotanique et écologique des plantes utilisées en médecine traditionnelle dans le District de la Lukunga à Kinshasa (RD du Congo). International Journal of Innovation and Scientific Research, 26: 612-633.

[10] Ngombe NK, Ngolo CN, Kialengila DM, Wamba AL, Mungisthi PM, Tshibangu PT, Dibungi PKT, Kantola PT, Kapepula PM. (2019). Cellular Antioxidant Activity and Peroxidase Inhibition of Infusions from Different Aerial Parts of Cassia occidentalis. Journal of Biosciences and Medecine. 7, 83–94.

[11] Inkoto LC, Bongo NG, Kapepula MP, Masengo AC, Gbolo ZB, Tshiama C, Ngombe KB, Iteku BJ, Mbemba FT, Mpiana PT, Ngbolua KN (2018). Microscopic features and chromatographic fingerprints of selected congolese medicinal plants: *Aframomum alboviolaceum* (Ridley) K. Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook. f.) Skeels. *Emergent Life Sciences Research*, 4 (1): 1-10.

[12] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. (2011). Phytochemical Screening and Extraction: A review. *International Pharmaceutical Sciences*., 1 (1): 98-106.

[13] Bongo G, Inkoto C, Masengo C, Tshiama C, Lengbiye E, Djolu R. and Kapepula M et *al*. (2017). Antisickling, Antioxidant and Antibacterial Activities of *Afromomum alboviolaceum* (Ridley) K. Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook. f.) Skeels. *American Journal of Laboratory Medicine*, 2: 52-59.

[14] Dash GK, Suresh P, Sahu SK, Kar DM, Ganapaty S, and Panda SB (2002). Evaluation of *Evolutus alsinoides* Linn. for antihelminthic and antimicrobial activities. *Journal of Natural Remedies* (2): 182-185.

[15] Guissou LP, Ouedraogo S, Sanfo A, Some N, Lompo M (1988). Mise au point d'un modèle biologique de test antiparasitaire appliqué aux plantes médicinales. *Pharmacopée Médicale Traditionnelle Africaine*, 10: 105-133.

[16] Hubert R. (2007). Les plantes aromatiques et huiles essentielles à grasse, 4éme partie: Les lamiacées, Les basilics «*Ocimum basilicum*», Botanique-Culture-Chimie Production et marché, Ed: L'Harmattan, France (Paris), 414: 254-256.

[17] Doaigey AR, El-Zaidy M, Alfarhan A, Milagy AES, Jacob T. Pollen morphology of certain species of the family Lamiaceae in Saudi Arabia. (2018). Saudi Journal of Biological Sciences. 25 (2): 354–60. https://doi.org/10.1016/j.sjbs.2017.03.001

[18] Gurav SS, Gurav NS. (2013). Indian Herbal Drug Microscopy. Springer New York.

[19] Tshilanda DD, Mutwale PK, Onyamboko DVN, Frederich M, Ngombe NK, Ngbolua K., & Mpiana PT. (2016). Chemical Fingerprint and Anti-Sickling Activity of Rosmarinic Acid and Methanolic Extracts from Three Species of Ocimum from DR Congo. Journal of Biosciences and Medicines, 4: 59–68.

[20] Yayi-Ladekan E., Kpoviessi DSS., Gbaguidi F., KpadonouKpoviessi BGH., Gbenou J., Jolivalt C., Moudachirou M., Accrombessi GC., Quetin-Leclercq J. (2011). Variation diurne de la composition chimique et influence sur les propriétés antimicrobiennes de l'huile essentielle de Ocimum canum Sims cultivé au Bénin. *International Journal of Biological and Chemical Sciences*, 5 (4): 1462-1475.

[21] Bassole IHN, Ouattara AS, Nebie R, Outtara CAT, Kaboré ZI and Traoré SA (2001). Composition chimique et activites antibactériennes des huiles essentielles des feuilles et des fleurs de *Cymbopogon proximus* (STAPF) et *Ocimum canum* (SIMS). *Pharmacopée Médicale Traditionnelle Africaine,* 11: 37-51.

[22] Bondet V., Brand-Williams W., & Berset C. (1997). Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method. Lebensmitte-Wissenschaft und-Technologie, 30 (6): 609–615.

[23] Ngbolua KN, Bishola TT, Mpiana PT, Mudogo V, Tshibangu DST, Ngombe KN, Tshilanda DD, Baholy R (2014a). *In vitro* antisickling and free radical scavenging activities of *Pentaclethra macrophylla* Benth (Fabaceae). *Journal of Advancement in Medical and Life Sciences*, DOI: 10.15297/JALS.V1I2.03.

[24] Ngbolua K, Mubindukila REN, Mpiana PT, Tshibangu, D. S. T., & Ashande, M. C. (2014b). Phytochemical Screening, Antibacterial and Antioxidant Activities of *Anthocleista liebrechtsiana* Wild & T. Durand (Gentianaceae) Originated from Democratic Republic of the Congo. *Journal of Advancement in Medical and Life Sciences*, 1: 1–6. https://doi.org/10.15297/JALS.

[25] Wone B. (1982). Les plantes fébrifuges et antimalariques de Kisangani. Bulletin de la société royale de Botanique, République du Zaïre, ISTR.

[26] Mensah GA., Sobakin LJ., Koudande D., Pomalegni CB., Kpera GN. (2006). Inventaire préliminaire des plantes médicinales utilisées pour traiter les aulacodes d'élevages malades et pour la prophylaxie sanitaire dans les aulacodicultures installés au Sud-Bénin. Bulletin de la Recherche Agronomique du Bénin, No. 54.

[27] Ongoka PR, Diatewa M, Ampa R, Ekouya A, Ouamba JM, Gbeassor M, Abena AA (2012). Evaluation *in vitro* de l'activité anthelminthique des plantes utilisées au Congo Brazzaville dans le traitement des maladies parasitaires. *Annales de l'Université Marien Ngouabi*, 13 (4): 101-107.

[28] Vidyadhar S, Saidulu M, Gopal TK, Chamundeeswari D, Umamaheswara R. & Banji D. (2010). *In vitro* anthelmintic activity of the whole plant of Enicostemma littorale by using various extracts. *International Journal of Applied Biology and Pharmaceutical Technology*; 1 (3): 1119-1125.

[29] Deore SL, Khadabadi SS, Kamdi KS, Ingle VP, Kawalkar NG, Sawarkar PS, Patil UA and Vyas AJ (2009). *In vitro* anthelmintic activity of *Cassia tora*. *International Journal of ChemTech Research*, 1 (2): 177-179.

[30] Vedha HBN, Saravana KP & Ramya DD (2011). Comparative *in vitro* anthelminthic activity of the latex of *Ficus Religinosa, Ficus elastica* and *Ficus bengalensis*. *Journal of Phytol and Phytopharmacy,* 3 (3): 26-30.

[31] Tshilanda DD (2016). Etude phytochimique et actvité anti-drepanocytaire de trois espèces du genre *Ocimum*: *Ocimum basilicum* L., *O. canum* SIMS, *O. gratissimum* L. (Lamiaceae). Thèse de doctorat, Département de Chimie, Faculté des Sciences, Université de Kinshasa, 272 pp, inédit.