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Original Article

Cellular Antioxidant and Peroxidase Inhibition Activities of Decoctions from Different Parts of *Triclisia gilletii* (De Wild) Staner (Menispermaceae)

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ABSTRACT

Introduction: Nutrition is an important aspect of public health and the high intake of vegetables and fruits would greatly reduce the risk of diseases associated with oxidative damage. *Triclisia gilletii*, a traditional vegetable from Kongo Central area (DR. Congo), was studied for establishing the cellular antioxidant activity, the inhibition of a proinflammatory enzyme (MPO) activity of its different parts and their microscopic and Chromatographic features. **Materials and Methods:** The antioxidant activities and the inhibition effect on MPO activity of decoctions from leaves, root bark and stern bark of *Triclisia gilletii* were evaluated using *in vitro* cell-free, cell-based and enzymatic assays. In addition, to better characterize the plant, the microscopic features and chromatographic profiles of different parts of *T. gilletii* were determined. **Results:** Microscopically, leaf can be characterized by anomocytic stomata, spiral vessels, pluricellular non-glandular trichomes, root bark by pitted vessels, abundant starch grains, isodiametric sclereids, cristalline fibers, and stem bark by tracheids with bordered pits, sclereids, ovoids starches grains and pitted vessels. Aqueous extracts of all parts showed the best cellular antioxidant activities on ROS-induced chemiluminescence using L012 on HL 60 monocytes at the concentrations range of 0.1–2 µg/mL with the root bark being the most active plant part. Extracts showed more efficient MPO inhibitory activity measured by the SIEFED technique, with the stem bark being the most active. Antioxidant and anti-inflammatory activities were significantly higher and positively correlated with phytochemicals. **Conclusion:** The obtained bioactivities may justify the use of this plant as a traditional vegetable, potent nutraceutical and medicine.

Keywords: Chromatographic Fingerprint, HL60, Myeloperoxydase, *Triclisia gilletii*, Traditional Food.

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INTRODUCTION

Nutrition is an important aspect of public health and a high intake of vegetables and fruits would greatly reduce the risk of a wide range of diseases, particularly those associated with oxidative damage. Finding alternative ways to reduce oxidative damage might have a beneficial health in the context of people of low-income countries such as Democratic Republic of Congo (DRC). Flora of DRC is abundant in unexploited traditional foods.[1] Among plant foods consumed by people of Kongo Central in DR Congo, we focused our research on *Triclisia gilletii* commonly called in local languages "*Kinuani*" in Kikongo and "*Efiri*" in Lingala.[2]

Triclisia gilletii (De Wild.) Staner (Menispermaceae) is traditionally used in several African countries for the treatment of some pathologies including malaria, edema, anemia, diarrhea, stomach problems, mental health problems, dysentery and respiratory diseases.[3-5] Yombe people in the Kongo Central area use the leaves as a traditional vegetable and they are excellent sources of proteins, minerals and vitamins.[2] To the best of our knowledge, there are few studies on the biological activities of *Triclisia gilletii*. The present work aimed to investigate the antioxidant activities and the inhibition effect on proinflammatory enzyme (MPO) activity of decoctions from different parts of *T. gilletii* using *in vitro* cell-free, cell-based and enzymatic assays. In addition, to better characterize the plant, the microscopic features and chromatographic profiles of different part of *T. gilletii* were determined.

MATERIALS AND METHODS

Plant Materials

The parts of *Triclisia gilletti* were collected from the area of Kimwenza (Kongo Central; DRC). Mr Landu Boniface from the University of Kinshasa confirmed the identity of the plant (Voucher number: Louis 2658). They were dried at room temperature. Leaves, root bark and stem bark were finely ground and stored before extraction.

Chemicals and Reagents

All reagents and solvents used were of analytical and HPLC grade and purchased from Merck VWR (Leuven, Belgium) and Sigma-Aldrich (Bornem, Belgium).

Microscopic Analysis

Powder observations were made using lactic acid reagent (European Pharmacopoeia reagent).[6] Observations and pictures were made with a Zeiss Primo Star microscope coupled to camera (DP 200).

Preparation of extracts

Aqueous extracts were prepared by decoction of 10g of sample powders with 100 mL of water for 10 min. The decoction was cooled before filtration and the evaporation of the solvent was performed under reduced pressure.

Phytochemical Analysis

TLC Analysis

TLC of 10 µL of solution for 100 mg/mL of organic extracts from leaves, root bark and stem bark were carried out on normal phase Silica gel 60 F_{254} plates (Merck, Darmstadt, Germany), using different eluents and detection reagents.[7]

Total phenolic content

Total phenolic contents were determined according to the Folin-Ciocalteu method as described previously.[6]

Total Flavonoid Content

The flavonoid contents were determined by UV-Vis spectrophotometry method described previously.[6,8]

Cell free antioxidant assays

Radical scavenging capacities were performed using ABTS and DPPH assays based on the methods described by Kapepula *et al*. (2017).[9]

Cellular and enzymatic assays

Cellular Antioxidant Activity

The ROS production by activated human promyelocytic leukemia cells (HL60) was measured by L012-enhanced chemiluminescence (CL). To HL 60 suspensions ($25x10^4$ cells/well) distributed in a 96-well microtiter plate (White Combiplate 8, Thermo Labsystems, Finland), 2 uL solutions of the extracts and the standard (quercetin) were added at the final concentrations of 0.1, 0.5, 1 and 2 µg/mL; and 10^{-4}, 10^{-5}, 10^{-6} M respectively. Then, 5 µL of $CaCl_2$ (10^{-1} M), 5 µL of HRP (25 µg/mL), 20 µL of L012 (5 µM) were added and, just before CL measurement, 15 µL of the 16 µM PMA stock solution were added to the cell suspensions. The CL response of the HL 60 was monitored for 30 min at 37°C with a Fluoroskan Ascent FL (Fisher Scientific, Tournai, Belgium) and expressed as the integral value of the total CL emission.[10-11]

Peroxidase inhibition: Inhibition of Myeloperoxidase Activity

The SIEFED, (a licensed method developed by Franck *et al*. (2013) for the specific detection myeloperoxidase (MPO)) was used for the screening of molecules or extracts from natural products that could modulate the activity of MPO.[12]

Statistical Analysis

GraphPad 7.0 (GraphPad Software, San Diego California, USA) was used to perfom the statistical analysis and the IC_{50} values were calculated under application of the function "log (inhibitor) vs. normalized response-variable slope", with the concentrations converted in decimal logarithms. ANOVA, Student's paired *t*-test and Sidak Multiple Comparisons Test were used with $p < 0.05$ such as the level of statistical significance.

RESULTS AND DISCUSSION

Botanical Characters

Powders of the leaves, stem bark and root bark of *Triclisia gilletii* treated with lactic acid reagent, showed the following specific botanical microscopic characters. As shown in the Figure 1, the leaves showed starches grains, upper epiderm with underlying pallisadic cells up to ~35 x 10 µm (LxW) and spongy mesophyll cells up to ~20 µm diameter, helical vessels up to ~30 µm diameter, isolated or grouped elongated sclereids up to ~125 x 25 µm µm (LxW), fibrous sclereids up to ~750 µm long, smooth and uniseriate covering trichomes sometimes widened in their middle part up to ~290 µm long, ponctuated fibers, anomocytic stomata. The root bark showed pitted vessels, abundant spheric starch grains up to 17 µm diameter, oxalate prism to ~40x 30 µm (LxW), isolated sclereids, group of isodiametric sclereids, cork, fibrous sclereids up to ~900 µm long cristalline fibers, non glandular trichome, bordered pitted vessels and bundle of parenchym with elongated cells (Figure 2). For the stem bark, microscopic analysis showed tracheids with bordered pits, sclereids, long non-glandular trichomes up to 320 µm, inner portion of the testa showing thinner walled cells, fibrous sclereids and ovoids starches up to 29 µm diameter grains and scalariform vessels (Figure 3).

Microscopic evaluation constitutes one of the reliable methods used to correctly identify the raw materials from natural products. This technique is useful for their identification and authentification of plant parts, and for revealing adulteration.[13] The characterization of microscopic details of different parts of *T. gilletii* in powder form is important for the appreciation of their quality.

Phytochemicals

Phytochemical analysis of the different parts of *T. gilletii* showed the presence of alkaloids, phenolic compounds, terpenes in all parts and iridoids in leaves only. Leaves contained phenolic acids as major phenolic

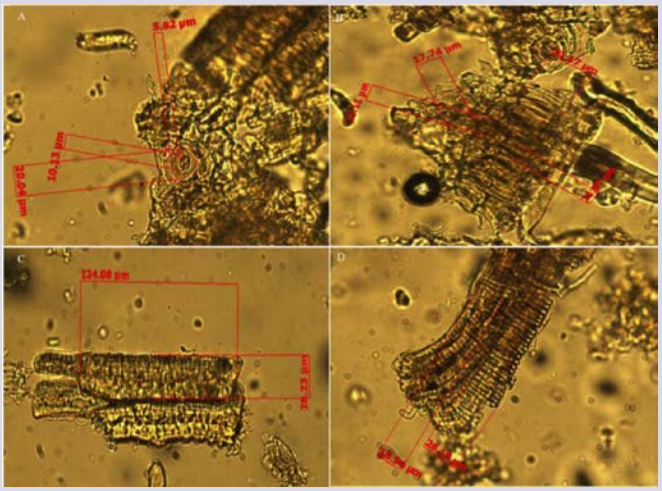

Figure 1: Diacytic stomata (A), fragment of underlying palisade cells and spongy mesophil cells (B), group of sclereids (C) and scalariform vessels (D) from leaves of *Triclisia gilletti* at 40X.

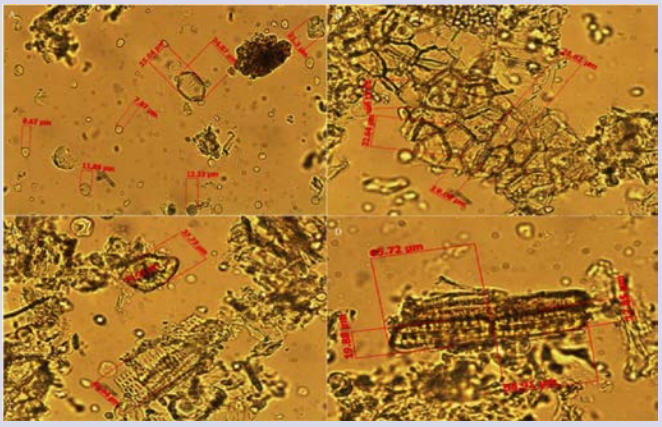

Figure 2: Starches grains, oxalate prism (A), fragment of cork (B), Isolated sclereid and fragment of pitted vessel (C) and grouped of sclereids (D) from root bark of *Triclisia gilletti* at 40X.

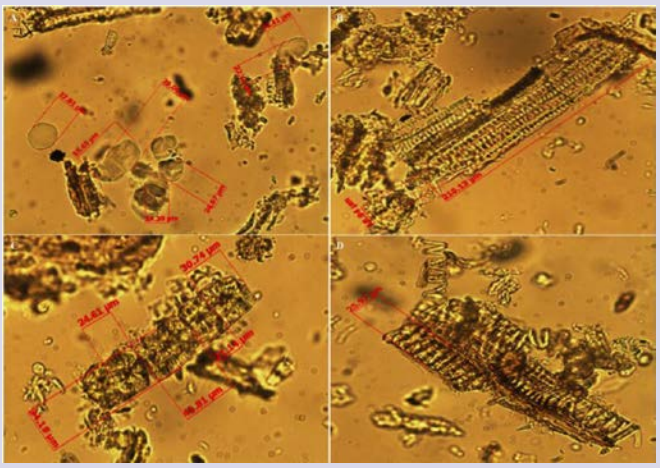

Figure 3: Starches grains (A), fragment of fibrous sclereids (B), group of sclereids (C) and scalariform vessels (D) from stem bark of *Triclisia gilletti* at 40X.

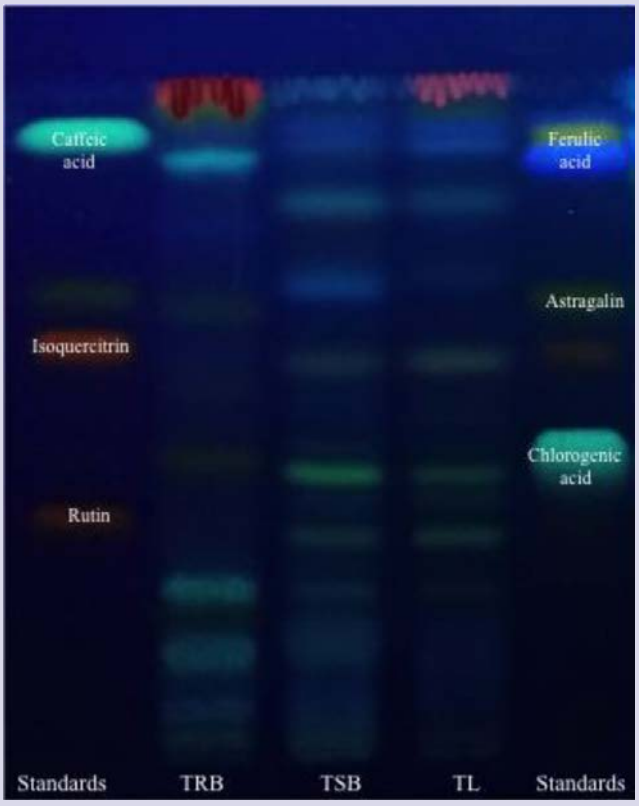

Figure 4: TLC chromatogram of aqueous extracts from different parts of *Triclisia gilletti* (TRB: Root bark; TSB: Stem bark, TL: Leaves) sprayed with Natural Product/PEG (366 nm).

compounds and root bark, stem bark contained flavonoids including kaempferol and phenolic acids (Figure 4).

Kikueta *et al*. (2013) already showed that the leaves contained flavonoids and anthraquinones, the stem-bark contained saponins. The root bark and stem bark contained tannins and proanthocyanidins and terpenes, steroids and alkaloids were identified in all plant parts.[5] Flavonoids were reported in leaves of *T. gilletii*, where Tiam *et al*.[4] identified a new flavone, named triclisinone and others compounds such as stigmasterol. For alkaloids, previous data reported that *Triclisia gilletii* contains bisbenzylisoquinoline (BBIQ) alkaloids, morphinan alkaloids and other amide alkaloids. The quantitative determination of polyphenols showed that the phenolic contents varied significantly (p <0.05) between different parts of *T. gilletii* (Table 1). Leaves had the highest amount of total phenolic compounds and only root bark contained flavonoids such as indicated by TLC analysis. The nature and quantity of phytochemicals differs from one organ to another within the same plant and this could influence their bioactivities.

Cellular Antioxidant Activity

The antioxidant activity determined on cell-free assays by two *in vitro* biochemical methods (ABTS and DPPH assays) showed that the aqueous extracts of all parts of *T. gilletii* have significant free radical scavenging effects with increasing concentrations. Antiradical activities were connected to the ability of extracts to scavenge free radicals based on their IC_{50} (Table 1). The obtained results showed that the antioxidant response of the aqueous extracts appeared to be correlated with the two methods. Extracts tested with a low IC_{50} in ABTS assay also have a lower IC_{50} in the DPPH assay, with the ABTS radical scavenging significantly higher than DPPH values. This phenomena can be explained by different mechanisms evaluated in the analytical methods.[14] In cellular models for inflammation, the addition of aqueous extracts in the range concentrations of 0.1-2 µg/mL and quercetin (10^{-6}-10^{-4}M) resulted in a dose-dependent decrease of the HL60 ROS production (Figure 5). L012-dependent chemiluminescence (CL) was used in the present study as a measure of the extracellular ROS production, resulting mainly from NADPH oxidase activity by stimulated monocytes HL-60.

Our results showed that the cellular antioxidant activity of the extracts is significantly higher (p < 0.001) in the following order: leaves> root barks > Stem bark. To our knowledge, few investigations have been performed on the antioxidant capacities of *T. gilletii*. However, Cimanga et al (2018) showed that a decoction of the stem bark of *T. gilletii* from Kinshasa had a high radical scavenging activity.[15] In addition to conventional cell-free antioxidant assays, the assessment of the antioxidant and anticatalytic potential in cell models is relevant. Several techniques are used for this purpose. In the present work, the lucigenin dependent chemiluminescence (CL) test was used to evaluate the ROS extracellular production by stimulated neutrophils. Lucigenin is considered to be a more specific probe for the detection of superoxide anions directly produced by NADPH oxidase activity.[16] Our results suggest that phytochemicals from *T. gilletti* are efficient as superoxide anion scavengers and could have noteworthy ability to inhibit other radical species or ROS produced by cells. Altogether, the cellular antioxidant activities were in good accordance with radical-scavenging capacities. Decoction extracts of *Triclisia* showed significant antioxidant activity and this activity would depend to synergic effect of polyphenolic compounds. According to obtained cellular antioxidant activity, leaves (which are consumed as traditional vegetable), and the root bark showed stronger activity than the stem bark.

Peroxidase Inhibition

MPO, a pro-oxidant enzyme involved in secondary cell damage is considered as a biological marker of inflammation.[12] In SIEFED technique, all *Triclisia* extracts exhibited a dose-dependent inhibitory effect on MPO activity in the range concentrations of 1 to 20 µg/mL (Figure 6). The obtained inhibitory effect was significantly higher (p < 0.001) in the following order: Stem bark > root bark > leaves and showed a better interaction of molecules of *Triclisia* extracts with the active site of

Table 1: Total flavonoid, phenolic contents and IC_{50} values (µg/mL) of aqueous extracts from different parts of *Tricliisa gilletti* on ABTS and DPPH assays (Mean ± SD, *n* = 6).

	Total phenolic Content (mg GAE/g DW)	Flavonoid content (mg QE/g DW)	ABTS IC_{50} (µg/mL)	DPPH IC_{50} (µg/mL)
Root Barks	20.42 ± 1.5	2.1 ± 0.019	1.01 ± 0.08	65.01 ± 4.33
Stem Barks	4.18 ± 2.8	-	52.36 ± 7.75	242.1 ± 45.64
Leaves	29.20 ± 3.55	-	2.56 ± 0.4	21.63 ± 1.28
Gallic acid	-	-	0.71 ± 0.08	1.07 ± 0.10

Legends: Total polyphenol content were calculated as gallic acid equivalents in milligrams per gram of dry weight (mg GAE/g DW) while flavonoid contents were determined as quercetin equivalents in milligrams per gram of dry weight (QE/g DW)

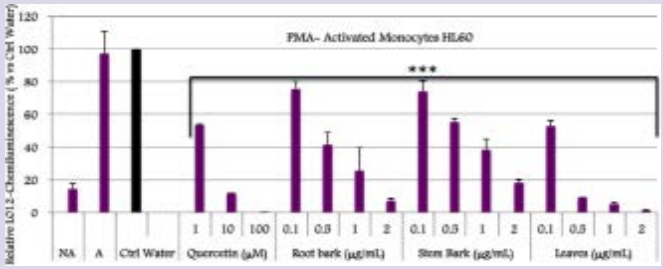

Figure 5: Modulatory effects of quercetin and extracts from *Triclisia giletti* on chemiluminescence response (CL) by PMA stimulated HL60 monocytes (Means ± SD, *n* = 6). *P*- values (*** $p < 0.001$) calculated by two-way ANOVA followed by Sidak Multiple Comparisons Test indicated a significant effect of the extracts vs. water control.

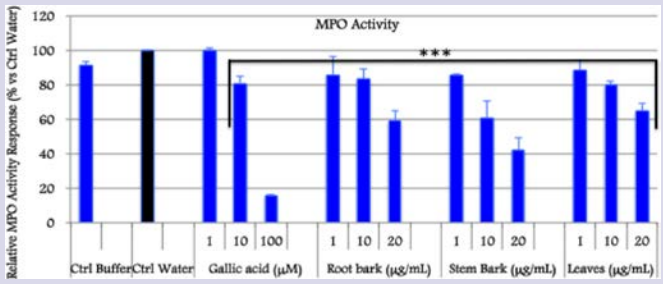

Figure 6: Effects of gallic acid and aqueous extract of *Triclisia gilletti* on MPO activity measured by SIEFED.. P-values (*** < *p* 0.001) calculated by two-way ANOVA indicated a significant effect vs water control set as 100% response. ns = not significant vs. Water control.

MPO. The SIEFED method that was used here to measure MPO activity allowed the detection of compounds that have a direct interaction with the hydrophobic pocket at the entrance of the active site of MPO.[12]

The results showed that the extracts showed the highest antioxidant activities and the highest inhibition on MPO activity. Root bark and leaves showed better antiradical and antioxidant activities than the stem bark, which exhibited a high inhibitory effect on MPO activity. The molecules or the extracts with good antiradical, antioxidant activities are not necessarily good inhibitors of MPO activity. Gallic acid is less efficient MPO inhibitor in comparison to quercetin, which is less antiradical.[15-16] Previous studies reported for phenolic acids that pyrogallol and the elongation of the carboxylic group yielding a propenoic group seem to be essential for the inhibition of MPO activity, probably by facilitating the interaction with the MPO active site.[12] Flavonoids were reported to be excellent inhibitors of MPO and the pyrogallol group in the B ring, the 3, 4', and 5-OHs, and of the C2-C3 double bond are essential for both binding and reactivity with peroxidase such as MPO.[17-18] Polyphenols compounds have been found to be the major compounds of *Triclisia* extracts and could be responsible for the obtained biological activities. The decoction extracts used in food consumption and in local medicine preserved antioxidant and anti-inflammatory capacities. The effect of thermal exposure on phytochemicals lead to the enhancement of quantity of certain metabolites such as phenolic compounds associated with thermal destruction of cell walls and subcellular compartments.[19-20] The antioxidant and anti-inflammatory properties of differents parts of *Triclisia gilletti* could have beneficial effects on the health of Congolese people.

CONCLUSION

This study reported the botanical microscopic features, chromatographic fingerprints, antioxidant activities and the inhibition peroxidasic activity of leaves, root bark and stem bark of *Triclisia gilletii*. Microscopic analysis showed the presence of characteristic botanical features for each part of this plant. Phytochemical screening by TLC revealed the presence of alkaloids, phenolic compounds, terpenes as major phytochemicals. Aqueous extracts from all parts exhibited the highest antioxidant activities and the highest inhibition on MPO activity. Root bark and leaves showed greater antiradical and antioxidant activities than stem bark, which exhibited a high inhibitory effect on MPO activity. However, phytochemical studies to characterize chemicals components are needed and the *in vitro* antioxidant and anti-inflammatory activities should be complemented in the future. This could lead to the valorization of *T. gilletii,* a traditional vegetable which could be promoted as a functional food, nutraceutical, or phytomedicine resource with high antioxidant capacity.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

ABTS: 2,2'-Azino- bis (3-ethylbenzothiazoline-6-sulphonic acid; **CL:** Chemiluminescence; **DPPH:** 1, 1-Diphenyl-2-picrylhydrazyl; **HRP:** Horsh Radish Peroxidase; **IC_{50}:** Half maximal Inhibitory Concentration; **MPO:** Myeloperoxydase; **PMA:** Phorbol Myristate Acetate; **SIEFED** (Specific Immunological Extraction Followed by Enzymatic Detection **TLC:** Thin Layer Chromatography.

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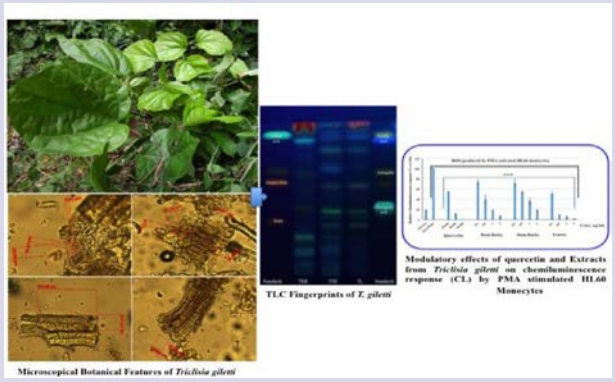
PICTORIAL ABSTRACT



SUMMARY

- Biological evaluation on several parts of *Triclisia gilletii* used in folk medicine.
- Noteworthy cellular antioxidant activity and inhibition of a proinflammatory enzyme
- Microscopic features and chromatographic fingerprints monitored and set for aqueous extracts

About Authors

Paulin Mutwale Kapepula is pharmacist and associate professor of Faculty of Pharmaceutical Sciences of University of Kinshasa. He is expert in natural products, pharmacognosy, quality control of herbal medecines and traditional medecine. He performs his research activities mainly in the field of bioactivities of traditional Foods (herbal teas, vegetables, mushrooms, edible insects) and medicinal plants as well as quality control of herbal medecines, improved traditional medicines and dietary supplements. He works in the projet on research and intervention on a tropical negleted disease named konzo. Prof Mutwale has professional memberships with the one health Congolese plateform.

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