

# Cellular Antioxidant Activity and Peroxidase Inhibition of Infusions from Different Aerial Parts of *Cassia occidentalis*

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## Abstract

*Cassia occidentalis* L. is widely used in the world in traditional medicine and especially in some African countries for the treatment of various diseases. The aim of this study was to report the microscopic features, the chromatographic fingerprints and the cellular antioxidant activity and the peroxidase inhibition of infusions from different parts of this plant. Microscopically, leaf can be characterized by cells of the spongy mesophyll and parenchyma numerous cluster crystals of calcium oxalate, paracytic stomata, isolated calcium oxalate cluster crystals, covering and glandular trichomes, scalariform vessels, polyedric starch granules, lignified fibers; flowers by abundant covering and glandular trichomes, spirally thickened vessels and associated parenchyma, abundant pollen grains. Seeds were characterized by pluricellular non-glandular trichomes, epidermis of the testa with underlying oil cells, parenchymatous layers of the testa, thicker-walled cells of the endosperm, pollen grain. Phytochemical analysis revealed the presence of phenolic acids, flavonoids, iridoids, tannins and terpenes. TLC fingerprints of different parts were different and characteristic. They showed the presence of glycosylated flavonoids and phenolic acids as main phytochemicals for flowers, leaves and seedpods. ABTS and DPPH assays showed that infusion extracts have the ability to scavenge free radicals connected with their IC<sub>50</sub> values ranging from 21.43 ± 1.25 to 566.24 ± 176.7 mg·mL<sup>-1</sup>. All extracts showed a weaker capacity to scavenge DPPH radical. Aqueous extracts displayed high cellular antioxidant activity at

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the concentrations range of 1 - 20  $\mu\text{g}\cdot\text{mL}^{-1}$  using LO-12 on monocytes HL 60. Flower and leave extracts showed more efficient effects on extracellular ROS production. Phenolic compounds could be major contributors to antioxidant activity of infusions of Cassia parts. In MPO (Myeloperoxidase) direct technique, all infusion extracts exhibited a dose-dependent inhibitory effect on MPO activity in the range concentrations of 1 to 20  $\mu\text{g}\cdot\text{mL}^{-1}$  with the leaves and flowers the most active. Obtained results support the potential therapeutic interest of all aerial parts of Cassia and could justify their use in traditional medicine and local nutraceutical resources.

## Keywords

*Cassia occidentalis*, HRP, Manalaria, Monocytes HL 60, MPO, Nutraceutical

## 1. Introduction

*Cassia occidentalis* L. (Caesalpiniaceae) is widely used in the world in traditional medicine and especially in some African countries for the treatment of various diseases. *Cassia occidentalis* (*Senna occidentalis*) is an Ayurvedic plant with important medicinal values (Figure 1). It is known by various names, e.g. Coffee senna, Fetid cassia and Negro Coffee [1] [2]. In DRC, it is known by its various vernacular names *Pola ekasakasenge*, *Mbandanse*, *Limingolanta*, *Dombati*, *Segbazolo*, *Mbengelimo*, *Bonungolata*, *Bolebe bonse*, *Betshobe awane* and *Lukunda Bajanyi* [3]. This plant is widely consumed by animals and humans in the world. It is widely consumed as a coffee substitute and has many applications in traditional medicine. It is the main ingredient of Liv. 52, a hepatoprotective polyherbal



**Figure 1.** *Cassia occidentalis* (Personal picture: Mont Ngafula City, Kinshasa, DRC, December 2017).

formulation from India. In Africa, *C. occidentalis* is used in a malarial formulation based on a traditional recipe comprising mixture of antimalarial herbs [2]. In Democratic Republic of Congo (DRC), *C. occidentalis* is a component of ameliorated traditional medicine named *Manalaria*<sup>®</sup>.

*C. occidentalis* has been found to possess significant pharmacological properties such as antibacterial, antifungal, antimalarial, anti-inflammatory, laxative, analgesic, choleric and diuretic properties [2].

Many papers on chemical composition and biological activities on *Cassia occidentalis* exist but few data are reported on the comparison of bioactivities of infusion extracts from their different parts. The aim of this study was to evaluate the cellular antioxidant activity and the inhibition of peroxidase oxidant of infusion extracts from different parts of *Cassia occidentalis*. Additionally, to better characterize the plant investigated, the microscopical characteristics and thin layer chromatography (TLC) profiles of different parts were determined.

## 2. Material and Methods

### 2.1. Materials

The flowers, leaves, the seeds and seedpods of *Cassia occidentalis* were collected during the period of December 2017 to March 2018 from the area of Mont Ngafula (Kinshasa, Democratic Republic of Congo). The identity of the species plant was confirmed by biologists of the University of Kinshasa (Voucher number: Kalanda n°8). They were finely ground in a high-speed mill (Retsch ZM 100 Model) and sieved at 180 µm particle size.

### 2.2. Chemicals and Reagents

All solvents used were of analytical and HPLC grade and purchased from Merck VWR (Leuven, Belgium). 2-Aminoethyldiphenylborat and Phorbol-12-myristate-13-acetate (PMA) were purchased from Sigma (Bornem, Belgium). L0-12 (8-amino-5-chloro-7-phenylpyrido [3,4-d]pyridazine-1,4(2H,3H)dione) was purchased from Wako Chemicals GmbH (Neuss, Germany).

Horseradish peroxidase (HRP) was obtained from Roche (Mannheim, Germany) and human Myeloperoxidase was from Calbiochem, EMD Millipore (Bellirica, MA USA). Caffeic acid, Chlorogenic acid (purity: 95%), Gallic acid (purity: 97%) was purchased from Sigma-Aldrich. Rutin (purity ≥ 99%), isoquercitrin (purity ≥ 99%) and, quercetin (purity ≥ 98.5%) were HPLC grade and purchased from Extrasynthese. Water was treated using a Milli-Q water ultra-purification system before use.

### 2.3. Microscopic Analysis

Powder observations were made using European Pharmacopeia reagent [4]. Observations were made with a Zeiss Primo Star 200<sup>®</sup> microscope and pictures were taken with Smart Phone Samsung E7.

## 2.4. Preparation of Extracts

Aqueous extracts were prepared by infusion of 10 g of sample powders with 150 mL of water for 10 minutes. The infusion was cooled to room temperature before filtration and the evaporation of the solvent was performed by lyophilisation (Apparatus: Christ Alpha 1 - 4 LSC®). The extracts were then weighed and kept in dark hermetic flasks at 4 °C.

## 2.5. Phytochemical Analysis

### 2.5.1. Thin Layer Chromatographic Analysis

Analytical TLC of 10 µL of solution for 10 mg·mL<sup>-1</sup> of methanolic and dichloromethane extracts was carried out on normal phase Silica gel 60 F<sub>254</sub> plates (Merck), using different eluents for the identification of secondary metabolites [5].

### 2.5.2. Phenolics Contents

#### 1) Total phenolics content

Total phenolic content of methanolic extracts (Methanol 80%) was determined according to the Folin-Ciocalteu method as described previously [4]. A calibration curve of gallic acid (0.025 - 0.4 mg·mL<sup>-1</sup>) was prepared, and phenolic contents were determined in triplicate from the linear regression equation of this curve. The results were expressed as milligrams of gallic acid (GA) equivalent per gram of dried matter.

#### 2) Total flavonoids content

The flavonoid content of the extracts was determined by UV-Vis spectrophotometry. Results are expressed in mg equivalent of quercetin per g (mg QE/g) of dry vegetal material [4].

#### 3) Total anthocyanins content

The determination of the anthocyanin content of the extracts was performed by the procedure reported by Bahati *et al.* (2017) [4].

## 2.6. Cell-Free, Cellular and Enzymatic Assays

The extracts and standard molecules were solubilized in water, thus their effect was compared to a control test performed with water alone.

### 2.6.1. Radical Scavenging Activity

ABTS and DPPH assay were performed according to the method described by Kapepula *et al.* (2017) [6].

### 2.6.2. Cellular Antioxidant Activity

#### 1) Cell culture and treatment

Human promyelocytic leukemia cells (HL-60) were obtained from the American Type Culture Collection (ATCC, USA) and cultured in appropriate medium (IMDM) obtained from Biowest (France) [6].

#### 2) Measurement of Cellular Antioxidant Activity (CAA)

The cellular antioxidant activity consists of evaluating the capacity of the ex-

tracts to modulate the production of ROS in cell models often used to study inflammatory response. The L012 probe was used to monitor the extracellular ROS production in HL-60 cells. The ROS produced by PMA-activated monocytes HL60 were measured by L012-enhanced chemiluminescence (CL). This experiment was based on this method: Two microliters of extract solutions at final concentrations of 0.5, 1, 5 and 10  $\mu\text{g mL}^{-1}$ , 5  $\mu\text{L}$  of HRP (30  $\mu\text{g mL}^{-1}$ ), 5  $\mu\text{L}$  of  $\text{CaCl}_2$  (11  $\mu\text{g mL}^{-1}$ ), 20  $\mu\text{L}$  of L012 (0.25  $\mu\text{g mL}^{-1}$ ) and as last 10  $\mu\text{L}$  PMA (0.486  $\mu\text{M}$ ) were added to a volume of 158  $\mu\text{L}$  cell suspension (250,000 cells). In each assay, three wells were loaded without plant extract and were taken as control for the ROS production (100% ROS induced chemiluminescence). To measure the basic ROS production of the cells at the absence of activation, three other wells without plant extract received no PMA (NA, not activated). The CL produced was measured for 30 min at 37°C on a Fluoreskan (Fluoreskan Ascent FL, Fischer Scientific, Tournai, Belgium).

### 2.6.3. Inhibition of Myeloperoxidase Oxidant Activity

PBS solutions of the plant extracts to the final concentrations of 1, 10 and 20  $\mu\text{g mL}^{-1}$  were incubated for 10 min with human MPO at a final concentration of 25  $\text{ng}\cdot\text{mL}^{-1}$ . After incubation, 100  $\mu\text{L}$  of the mixtures were loaded into the 96-well microtitration plates and incubated for 10 minutes at 37°C in darkness. The MPO peroxidase activity (enzymatic activity) was detected by adding 100  $\mu\text{L}$  of Amplex<sup>®</sup> Red solution (80  $\mu\text{M}$ ) solution dissolved in phosphate buffer (50 mM, at pH 7.5),  $\text{H}_2\text{O}_2$  (10  $\mu\text{M}$ ) and 10  $\mu\text{L}$  sodium nitrite (10 mM). The oxidation of Amplex<sup>®</sup> Red into the fluorescent adduct resorufin ( $\lambda$  excitation = 544 nm;  $\lambda$  emission = 590 nm) was monitored for 30 min at 37°C with a fluorescent plate reader (Fluoroskan Ascent, Fisher Scientific). A control assay set as 100% MPO activity was performed with MPO in the presence of PBS instead of the plant extracts [7].

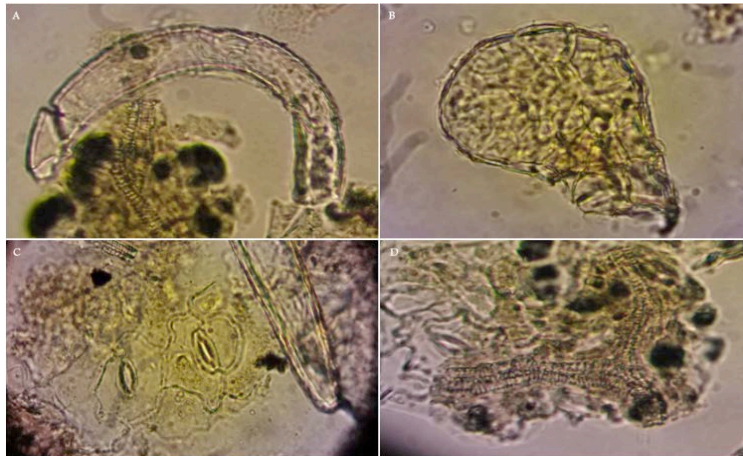
## 2.7. Statistical Analysis

Each concentration was tested in triplicate in each assay, and at least 3 different assays were performed. All results were expressed as mean values  $\pm$  standard deviation (SD). The statistical analysis was performed with GraphPad 7.0 (GraphPad Software, San Diego California, USA). Two-way analysis (ANOVA) was used; multiple comparisons of all data were performed using the “Tukey” Multiple Comparisons Test and the level of statistical significance was set at  $p < 0.05$ . The  $\text{IC}_{50}$  values were calculated with GraphPad Prism 7.0 under application of the function “log (inhibitor) vs. normalized response-variable slope” after converting the concentrations into their decimal logarithm.

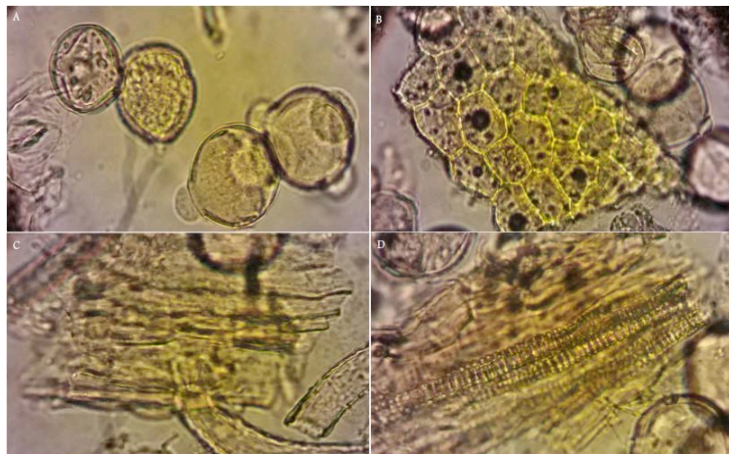
## 3. Results and Discussion

### 3.1. Botanical Microscopic Features

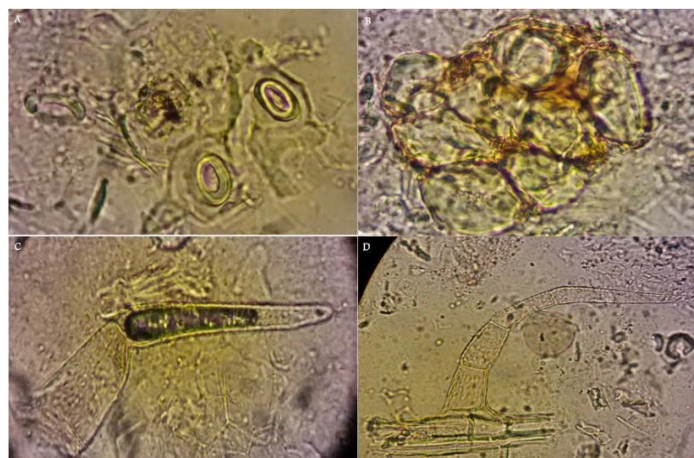
Microscopical analysis of powders of leaves, flowers and seeds of *C. occidentalis* revealed the features below (Figures 2-4). Leaves were characterized by upper



**Figure 2.** Unicellular non glandular trichome (A), Glandular trichome (B), Paracytic stomata (C) and Scalariform vessels (D) from leaves of *C. occidentalis* at 40 $\times$ .



**Figure 3.** Pollen grains (A), Inner epidermis (B), Fragment of fiber with unicellular non-glandular trichome (C), and Scalariform vessels (D) from flower of *C. occidentalis* at 40 $\times$ .



**Figure 4.** Epicarp in surface view showing stomata (A), Endosperm layer in surface view (B), Pluricellular non glandular trichomes (C), Parenchymatous layers of the testa in surface view and non glandular trichomes (D) from seeds of *C. occidentalis* at 40 $\times$ .

epidermis in surface view with part of the underlying palisade, cells of the spongy mesophyll containing cluster crystals of calcium oxalate, lower epidermis of the leaf in surface view showing paracytic stomata, parenchyma containing numerous calcium oxalate crystals, isolated calcium oxalate cluster crystals, covering and glandular trichomes, scalariform vessels, polyedric starch granules, lignified fibers. The diagnostic characters of flowers were: abundant covering and glandular trichomes, inner and outer epidermis of the corolla, spirally thickened vessels and associated parenchyma, abundant pollen grains. The diagnostic characters of the seeds were: pluricellular non glandular trichomes, cork, epicarp in surface view showing stomata, epidermis of the testa with underlying oil cells, part of the pericarp showing the innermost layer of the mesocarp, the endocarp, parenchymatous layers of the testa in surface view, epiderms and palisade of the cotyledons in sectional view, thicker-walled cells of the endosperm, pollen grain.

Microscopic analysis is one of the cheapest methods to correctly identify the raw materials from herbal medicines and is useful for the identification and authentication of botanicals and for detecting the adulterated and poor quality [8]. The knowledge of botanical microscopic details of different parts of *C. occidentalis*, is important for the evaluation of their quality such as raw materials for traditional medicines. Microscopy analysis allows the identification of herbal drugs and the detection of individual components of a mixture.

### 3.2. Phytochemicals

Phytochemical analysis revealed the presence of phenolic acids, flavonoids, iridoids, tannins and terpenes. TLC fingerprints of different parts were different and characteristic. They showed the presence of glycosylated flavonoids and phenolic acids as main compounds for flowers, leaves and seed pods. By comparison with used standards, they showed that flowers and leaves contain quercetin. Menthol is detected equally in all extracts from different parts (Figure 5).

Previous phytochemical screening revealed the presence of alkaloids, saponins, tannins, reducing sugar, phenols, anthraquinones, cardiotoxic glycosides, resins, sterols in different parts of this plant. Leaves contained phlobatannins, chrysophanol, emodin, physcion, tetrahydroanthracene derivatives, germichryson and occidentalins A and B [1] [9] [10]. Flowers contained anthraquinones, emodin, physcion and physcion-1-O- $\beta$ -D-glucoside. The study of phytochemicals of *C. occidentalis* reveals that the nature and amount of phytochemicals vary according to climatic conditions. Stems, leaves and the root bark of the plant from Ivory Coast, Africa contained small amount of saponins, no alkaloids, sterols, triterpenes, quinines, tannins and flavonoids. However, a large amount of alkaloids were found in the stem, leaves and fruits of the species from Ethiopia [2].

### 3.3. Antioxidant Activities

ABTS and DPPH assays showed that infusion extracts of the *Cassia* parts have the ability to scavenge free radicals connected with their IC<sub>50</sub> values (Table 1).

**Table 1.** IC<sub>50</sub> values ( $\mu\text{g}\cdot\text{mL}^{-1}$ ) of infusion extracts of parts of *C. occidentalis* on ABTS and DPPH assays (Mean  $\pm$  SD, n = 6) and their phenolic contents.

Samples	IC <sub>50</sub> ( $\mu\text{g}\cdot\text{mL}^{-1}$ )		Phenolic Contents		
	ABTS	DPPH	Total phenolic (mgGAE/100g DW)	Flavonoids (mgQE/100g DW)	Anthocyanins (mgCE/100g DW)
Flowers	23.44 $\pm$ 2.35	232.27 $\pm$ 6.51	151.25 $\pm$ 6.78	1.73 $\pm$ 0.4	-
Leaves	25.06 $\pm$ 2.15	216.77 $\pm$ 9.69	83.91 $\pm$ 12.28	4.98 $\pm$ 0.01	1.25 $\pm$ 0.12
Seed Pods	21.43 $\pm$ 1.25	535.8 $\pm$ 99.53	75.69 $\pm$ 3.73	0.43 $\pm$ 0.07	-
Seeds	45.08 $\pm$ 6.62	566.24 $\pm$ 176.7	48.81 $\pm$ 4.31	1.07 $\pm$ 0.19	1.46 $\pm$ 0.15
Gallic acid	0.71 $\pm$ 0.08	1.07 $\pm$ 0.10	-	-	-

**Figure 5:** TLC chromatogram of dichloromethane extracts from *C. occidentalis* with oleanolic acid, menthol, thymol as standards; developed with toluene/ethyl acetate (93:7; v/v) and visualized at visible with sulfuric anisaldehyde reagent. Terpenes are detected as violet spots.

All extracts showed a weaker capacity to scavenge DPPH radical. DPPH assay only detect hydrophilic antioxidants in the contrary of ABTS assay which detect hydrophilic and lipophilic antioxidants. Phenolic compounds could be major contributors to antioxidant activity of infusions of Cassia parts. The total phenolic contents varied significantly ( $p < 0.05$ ) between the parts of Cassia. Results showed that flowers was the part with the highest amount of total phenolic content. Leaves had the highest amount of flavonoids (**Table 1**). Previous studies reported the antioxidant activity of different parts of *C. occidentalis*. Singh *et al.* (2017) showed that methanolic extract from seeds of this plant had moderate DPPH radical scavenging activity [11]. Methanol extract from leaves was proved



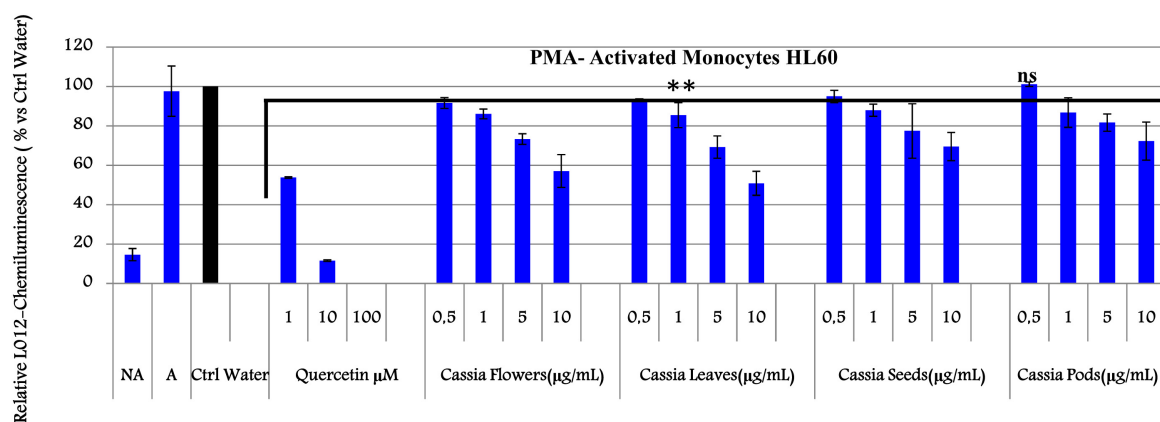
to be the most effective among the organic extracts for scavenging hydrogen peroxide radical [9].

Beside conventional cell-free antioxidant assays, it can be pertinent to evaluate the antioxidant and anticatalytic potentials of plant extracts in cellular models such as those involved in ROS production and inflammatory responses. The addition of increasing concentrations of the infusion extracts from different parts of *C. occidentalis* (0.5, 1, 5 and 10  $\mu\text{g}\cdot\text{mL}^{-1}$ ), and quercetin ( $10^{-6}$  to  $10^{-4}$  M) resulted in a dose-dependent decrease of HL-60 ROS production in comparison to the control test performed with water (Figure 6). With this cellular model involved in inflammation, our results showed that the activities of aqueous extracts are significantly higher ( $p < 0.05$ ) in the following order: Leaves > Flowers > Seeds > Seed Pods.

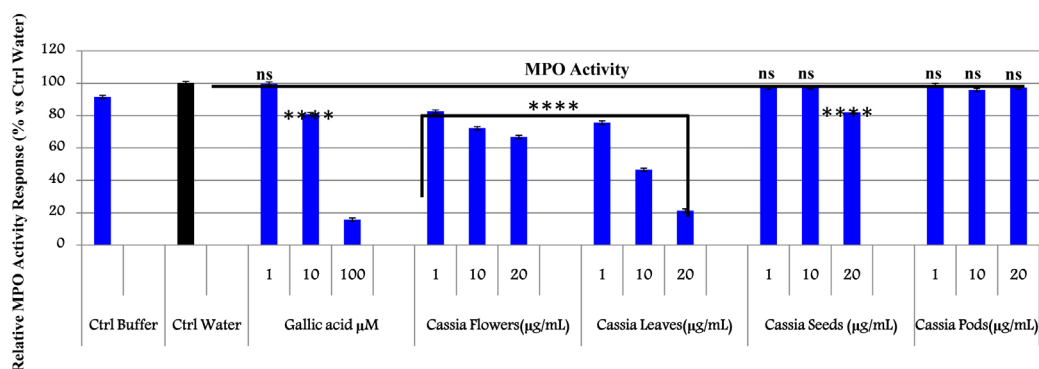
These results suggest that the compounds of infusion extracts are good extra-cellular ROS scavengers. Antioxidants especially phenolic acids and flavonoids have ability to scavenge biological free radicals. The aqueous extracts used in local medicine preserved an antioxidant capacity. Interestingly, leaves and flowers exhibited a higher cellular antioxidant activity.

### 3.4. Inhibition of Peroxidasic Activity

Peroxidases generally use  $\text{H}_2\text{O}_2$  as one of the substrates and participate in oxidizing drug and xenobiotic detoxification, innate immunity, hormone biosynthesis and the pathogenesis of inflammatory diseases [7] [12]. In MPO direct technique, all infusion extracts of parts of *Cassia* exhibited a dose-dependent inhibitory effect on MPO activity in the range concentrations of 1 to 20  $\mu\text{g}\cdot\text{mL}^{-1}$  (Figure 7). The leave and flowers extracts showed higher inhibition effect as compared to other plant parts. Seeds showed weak activity at tested concentrations and seedpods were practically not active. This inhibitory effect was significantly higher



**Figure 6.** Modulatory effects of quercetin and extracts from parts of *Cassia occidentalis* on chemiluminescence response (CL) by PMA stimulated HL60 monocytes (Means  $\pm$  SD,  $n = 6$ ). The CL intensity results from the reaction between L012 and the ROS produced by the non-activated (NA) and activated HL60 (A). The CL response of stimulated HL60 in the presence of water used to solubilize the extracts was defined as 100%. P-values (\*\* $p < 0.01$ ) calculated by two-way ANOVA followed by Sidak Multiple Comparisons Test indicated a significant effect of the extracts vs. water control.



**Figure 7.** Effects of gallic acid and infusion extracts of parts of *Cassia* on MPO activity. The percentage of inhibition was calculated for each sample concentration versus the corresponding control (MPO + Water), taken as 100% (mean  $\pm$  SD,  $n = 6$ ). Samples vs. Control Water: ns: no significance. P-values (\*\*\* $p < 0.0001$ ) calculated by two-way ANOVA indicated a significant effect vs. water control set as 100% response. ns = not significant vs. Water control. There are statistical differences between extracts of Flowers and Leaves and control.

( $p < 0.001$ ) and showed a better interaction of molecules of *Cassia* extracts with the enzyme.

Phenolic compounds such as flavonoids found in different parts were reported to be excellent ROS scavengers and inhibitors of MPO [6] [13] [14]. MPO and its metabolites are biomarkers for infectious diseases and a wide array of non-infectious and neurodegenerative disorders [15]. The inhibitors of MPO activity are promising therapeutic agents [16]. Altogether the results showed that the extracts tested showed the highest acellular and cellular antioxidant activity, also the highest inhibition on MPO activity related to their phytochemicals. *Cassia occidentalis* is known such as a plant with many applications in traditional medicine. Pharmacological properties such as anti-inflammatory, anticarcinogenic, antimutagenic, antiplasmodial, antirheumatic and hepatoprotective were reported on this species [2]. Oxidative damage and inflammatory process play a crucial role in the diseases treated by herbal formulations from *Cassia* [17] [18]. Obtained results support the potential therapeutic interest of all aerial parts of *Cassia* and could justify their use in traditional medicine and local nutraceutical resources.

#### 4. Conclusion

The microscopic analysis of powders from flowers, leaves and seeds of *C. occidentalis* allowed the identification of specific microscopical features. TLC analysis indicated that phenolic compounds and terpenes are major secondary metabolites in this plant. Aqueous extracts from all parts exhibited good antioxidant activity with flowers and leaves as the most active, which is consistent with the higher phenolic content. The leaves and flowers extracts showed higher inhibition effect on MPO as compared to other plant parts. However, the *in vitro* activities should be complemented in the future with *in vivo* evaluation which could be promoted as medicine and as nutraceutical resource with high antioxi-

dant capacity.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding this publication.

## References

- [1] Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., Bruyne, T., Apers, S., Vlietinck, A.J., *et al.* (2004) *In Vitro* Antiplasmodial Activity of Extracts and Fractions from Seven Medicinal Plants Used in the Democratic Republic of Congo. *Journal of Ethnopharmacology*, **93**, 27-32. <https://doi.org/10.1016/j.jep.2004.02.022>
- [2] Yadav, J.P., Arya, V., Yadav, S., Panghal, M., Kumar, S. and Dhankhar, S. (2010) *Cassia occidentalis* L.: A Review on Its Ethnobotany, Phytochemical and Pharmacological Profile. *Fitoterapia*, **81**, 223-230. <https://doi.org/10.1016/j.fitote.2009.09.008>
- [3] Mbuta, K. (2014) *Quelques Plantes Utiles du Province de Bas-Congo, République Démocratique du Congo* Paul Latham. Mystole Publications, Canterbury.
- [4] Bahati, L.M., Kapepula, P.M., Kabamba, N.N., Moni, B., Kafuti, G.M., Mungitshi, M., Fundu, T.M., *et al.* (2017) Microscopic Features, Chromatographic Fingerprints and Antioxidant Property of Some Unconventional Green Leafy Vegetables Consumed in Bandundu, DR Congo. *Pharmacognosy Communications*, **7**, 158-163. <https://doi.org/10.5530/pc.2017.4.23>
- [5] Wagner, H., Bauer, R., Melchart, D., Xia, P.-G. and Staudinger, A. (2013) *Chromatographic Fingerprint Analysis of Herbal Medicinal: Thin-Layer High Performance Liquid Chromatography of Chinese Drugs*. Vol. 3, Springer International Publishing, Berlin.
- [6] Kapepula, P.M., Kabamba Ngombe, N., Tshisekedi Tshibangu, P., Tsumbu, C., Franck, T., Mouithys-Mickalad, A., Frédérick, M., *et al.* (2017) Comparison of Metabolic Profiles and Bioactivities of the Leaves of Three Edible Congolese Hibiscus Species. *Natural Product Research*, **31**, 2885-2892. <https://doi.org/10.1080/14786419.2017.1305382>
- [7] Franck, T., Mouithys-Mickalad, A., Robert, T., Ghitti, G., Deby-Dupont, G., Neven, P. and Serteyn, D. (2013) Differentiation between Stoichiometric and Anticatalytic Antioxidant Properties of Benzoic Acid Analogues: A Structure/Redox Potential Relationship Study. *Chemico-Biological Interactions*, **206**, 194-203. <https://doi.org/10.1016/j.cbi.2013.09.009>
- [8] Gurav, S.S. and Gurav, N.S. (2014) *Indian Herbal Drug Microscopy*. Springer, Berlin. <https://doi.org/10.1007/978-1-4614-9515-4>
- [9] Odeja, O., Obi, G., Ogwuche, C.E., Elemike, E.E. and Oderinlo, Y. (2015) Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Senna occidentalis* (L.) Leaves Extract. *Clinical Phytoscience*, **1**, 6. <https://doi.org/10.1186/s40816-015-0007-y>

- [10] Manikandaselvi, S., Vadivel, V. and Brindha, P. (2016) Studies on Physicochemical and Nutritional Properties of Aerial Parts of *Cassia occidentalis* L. *Journal of Food and Drug Analysis*, **24**, 508-515. <https://doi.org/10.1016/j.jfda.2016.02.003>
- [11] Singh, V.V., Jain, J. and Mishra, A.K. (2017) Determination of Antipyretic and Antioxidant Activity of *Cassia occidentalis* Linn Methanolic Seed Extract. *Pharmacognosy Journal*, **9**, 913-916. <https://doi.org/10.5530/pi.2017.6.143>
- [12] Khan, A.A., Rahmani, A.H., Aldebasi, Y.H. and Aly, S.M. (2014) Biochemical and Pathological Studies on Peroxidases: An Updated Review. *Global Journal of Health Science*, **6**, 87-98. <https://doi.org/10.5539/gjhs.v6n5p87>
- [13] Shiba, Y., Kinoshita, T., Chuman, H., Taketani, Y., Takeda, E., Kato, Y., Kawai, Y., *et al.* (2008) Flavonoids as Substrates and Inhibitors of Myeloperoxidase: Molecular Actions of Aglycone and Metabolites. *Chemical Research in Toxicology*, **21**, 1600-1609. <https://doi.org/10.1021/tx8000835>
- [14] Gau, J., Furtmüller, P.G., Obinger, C., Prvost, M., Van Antwerpen, P., Arnhold, J. and Flemmig, J. (2016) Flavonoids as Promoters of the (Pseudo-)Halogenating Activity of Lactoperoxidase and Myeloperoxidase. *Free Radical Biology and Medicine*, **97**, 307-319. <https://doi.org/10.1016/j.freeradbiomed.2016.06.026>
- [15] Ray, R.S. and Katyal, A. (2016) Myeloperoxidase: Bridging the Gap in Neurodegeneration. *Neuroscience and Biobehavioral Reviews*, **68**, 611-620. <https://doi.org/10.1016/j.neubiorev.2016.06.031>
- [16] Malle, E., Furtmüller, P.G., Sattler, W. and Obinger, C. (2007) Myeloperoxidase: A Target for New Drug Development? *British Journal of Pharmacology*, **152**, 838-854. <https://doi.org/10.1038/sj.bjp.0707358>
- [17] Arauz, J., Ramos-Tovar, E. and Muriel, P. (2016) Redox State and Methods to Evaluate Oxidative Stress in Liver Damage: From Bench to Bedside. *Annals of Hepatology*, **15**, 160-173.
- [18] Kavishe, R.A., Koenderink, J.B. and Alifrangis, M. (2017) Oxidative Stress in Malaria and Artemisinin Combination Therapy: Pros and Cons. *The FEBS Journal*, **284**, 2579-2591. <https://doi.org/10.1111/febs.14097>