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Congolese edible caterpillars, valuable sources of bioactive compounds with human health benefits

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Abstract

Insects are part of the regular diet of more than two billion people around the world and are not only delicacies. Insects provide great opportunities to replace meals but can have important additional benefits as well. In the Democratic Republic of the Congo (DRC), caterpillars are the most consumed insects, and they are consumed by more than 70% of the population throughout the year. The aim of this research was to report the microscopic features, mineral micronutrients, chromatographic fingerprints, antioxidant activities and peroxidase inhibition of edible Congolese caterpillars of the genus *Cinabra, Imbrasia* and *Gonimbrasia* from DRC. Microscopic analysis showed the presence of characteristic features, specific to each host plant of caterpillars, such as palisade cells, stomata, trichomes, sclereids, fibres, vessels, pollen and starch grains. Phytochemical screening by chromatographic techniques revealed the presence of phenolic acids, flavonoids and terpenes as major secondary metabolites. Elemental analysis on dry matter showed that studied caterpillars are insects containing significant amounts of micronutrients such as copper, magnesium, manganese, selenium and zinc. *Gonimbrasia belina* had the highest selenium, magnesium and zinc content (0.12 g/100 g, 0.17 g/100 g and 0.011 g/100 g, respectively) than *Cirina forda, Cinabra hyperbius, Imbrasia truncata* and *Imbrasia* sp., *C. forda* and *Imbrasia* sp. had the highest copper content (0.003 g/100 g). *C. forda* had the highest manganese content (0.006 g/100 g). All aqueous extracts displayed high radical-scavenging activities with IC_{50} values ranging from 10 to 80 µg/ml. Extracts showed the best cellular antioxidant activities on reactive oxygen species-induced chemiluminescence using L012 on human leucocytes 60 monocytes related to their IC_{50} values less than 0.5 µg/ml. In specific immuno-extraction followed by enzymatic detection of myeloperoxidase assay, all extracts of caterpillars exhibited a dose-dependent inhibitory effect on myeloperoxidase activity in the range concentrations of 1 to 20 µg/ml excepted extracts of *Imbrasia epimethea*, *Imbrasia* sp. and *I. truncata*. Our results showed that insects are not only valuable source of lipids, proteins and micronutrients such as selenium but also are sources of phytochemicals with therapeutic benefits.

Keywords: antioxidant activity, *Imbrasia* sp., insects, myeloperoxidase, selenium

1. Introduction

Insects are part of the regular diet of more than two billion people around the world, and are not only delicacies. Insects are traditional food and it is difficult to estimate the percentage of people eating them because their frequency of consumption is poorly documented. In Democratic Republic of Congo (DRC), caterpillars are the most consumed insects and they are especially popular during the rainy season (Van Huis *et al.,* 2013). Insects have important health benefits and could contribute to food security and improvement of nutrition.

Caterpillars constitute among the world's most diverse groups of edible insects for the abundance in lipids, protein and micronutrients. In the DRC, the consumption of insects

including caterpillars is part of people's eating habits and it is estimated that 80% of the Kinshasa human population consumes at least one species of insects five days per month, with 66.4 to 154 g of insects being consumed per person per day, depending on insect order (Nsevolo *et al.,* 2016; Van Huis, 2003). Caterpillars are obtainable at markets everywhere during the wet season, and make quite a considerable contribution to the animal protein of the diet of Congolese people. For example, more than 70% of the Kinshasa's population consume caterpillars throughout the year. The main supply provinces of Kinshasa are the former provinces of Equateur (64%) and of Bandundu (24%) (Van Huis, 2003).

Four main species of caterpillars are consumed (Supplementary Figure S1): *C. forda* (Westwood, 1892), *I. epimethea* (Drury, 1773), *I. truncata* (Aurivillius, 1908), *Imbrasia* sp. Many caterpillars are used for food by peoples in the Kwango and Kwilu provinces which are generally considered to be poor in protein resources. The caterpillars *C. forda* called '*Mingolo, Mikwati*' are not only an important source of proteins for local consumption, but also provide substantial income to these deprived areas such as Kahemba. The trade of caterpillars from Kahemba to large urban centres such as Kinshasa and Kikwit provides a source of income for the inhabitants of the region, particularly the most vulnerable severely affected by konzo. Konzo is a distinct neurological disease with the selective upper motor neuron damage associated with oxidative damage, induced by cyanide poisoning through the ingestion of poorly processed bitter cassava under malnutrition conditions (Kapepula *et al.,* 2017a). In some countries, diets deficient in essential amino acids and micronutrients, are supplemented with insects for the missing nutrients. In the DRC, for example, lysine-rich caterpillars (*I. epimethea, I. truncata* and *Nudaurelia oyemensis*) supplement lysine-poor staple proteins (Akhtar and Isman, 2018; Kodondi *et al.,* 1987). Insects represent a good alternative source of quality lipids, proteins and nutrients, and are known for their good nutritive value due to their high protein content. The protein quality of insects is promising regarding availability and digestibility (Akhtar and Isman, 2018; Orkusz, 2021; Rumpold and Schluter, 2015). Many manuscripts in the literature report on chemical composition and biological activities of edible insects; in contrast, relatively little is known about the caterpillars for which few data are reported. However, Nino *et al.* (2021) reported the identification of phenolic compounds including flavanols in insects like the mulberry white caterpillar (*Rondotia menciana*). Phenolic compounds have been long recognised to have several bioactivities including antioxidant, anti-inflammatory, antidiabetic, antimicrobial and anticancer, cardiovascular protection, and neuroprotection (Nino *et al.,* 2021). The aim of this research was to report the microscopic features, mineral micronutrients, chromatographic fingerprints, antioxidant activities, and peroxidase inhibition of edible Congolese caterpillars by microscopic, phytochemical, elemental analysis.

2. Materials and methods

Insect materials

Samples of wood-smoked caterpillars were taken at different periods in districts of DRC from September to April. The samples of edible caterpillars (minimum 400 g for each) were collected from the markets of the areas of DRC that are Katanga, Kinshasa, Equateur, Kwango and Tshikapa in 2017 and 2018 (Table 1). The identity of the caterpillars was confirmed by biologists from the University of Kinshasa (DRC) and Professor Malaisse François from the University of Liège. The dried caterpillars were rinsed once with water to remove smoke residue and then oven dried at 40 °C. They were finely ground in a high-speed mill (Retsch ZM 100 Model; Retsch GmbH, Haan, Germany), sieved at 180 µm particle size and kept in hermetic flasks.

Table 1. Studied Congolese caterpillars.

Code	Scientific names	Origins	Vernacular names
C1	*Gonimbrasia belina* (Westwood, 1894)	Katanga, June 2018	*Binkubala*
C2	*Cinabra hyperbius* (Westwood, 1881)	Katanga, June 2018	*Binkubala*
C3	*Cirina forda*	Kahemba (Kwango), February 2018	*Masese*
C4	*Cirina forda*	Kahemba (Kwango), September 2017	*Makoso*
C5	*Cirina forda*	Kenge (Kwango), February 2018	*Mingolo*
C6	*Imbrasia epimethea*	Equateur, December 2017	*Benkenzo*
C7	*Imbrasia truncata*	Equateur, December 2017	*Mbinzo*
C8	*Cirina forda*	Tshikapa, February 2018	*Massamba*
C9	*Imbrasia* sp.	Equateur, September 2018	*Mboyo*
C10	*Imbrasia truncata*	Equateur, September 2018	*Mbinzo*

Chemicals and reagents

All reagents and solvents used were of analytical and high-performance liquid chromatography (HPLC) grade and purchased from Merck VWR (Leuven, Belgium), Sigma-Aldrich (Bornem, Belgium), Wako Chemicals GmbH (Neuss, Germany), Roche (Mannheim, Germany) and Calbiochem, EMD Millipore (Billerica, MA, USA).

Microscopic analysis

Microscopic preparations and observations were made using lactic acid reagent (European Pharmacopeia reagent) according to Bahati *et al.* (2017). Observations and pictures were made with a Zeiss Primo Star microscope coupled to a camera (DP 200) (Carl Zeiss Microscopy GmbH, Jena, Germany).

Preparation of extracts

Aqueous extracts were prepared by the infusion of 10 g of powders of caterpillars with 150 ml of boiling water (100 °C) for 10 min. Infusions were cooled at room temperature before filtration and the evaporation of the solvent was performed by lyophilisation (Apparatus: Christ Alpha 1-4 LSC®; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The extracts were then weighed and kept in dark hermetic flasks at 4 °C.

Phytochemical analysis

Analytical thin layer chromatography (TLC) of 10 µl of solution from 100 mg/ml of methanolic and ethyl acetate extracts was carried out on normal phase Silica gel 60 F_{254} plates (Merck, Darmstadt, Germany), using different eluents for the identification of phytochemicals (Wagner *et al.,* 2013). Analytical separation on high-performance liquid chromatography with diode-array detection (HPLC-DAD) was carried out on a Hypersil ODS®RP18 column (Thermo Fisher Scientific, Tournai, Belgium) according to Kapepula *et al.* (2017b).

Cell free antioxidant assays

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity were performed on aqueous dry extracts according to the method described by Kapepula *et al.* (2017b). The radical cation ABTS•+ was generated by persulfate oxidation of ABTS. A mixture of ABTS (0.7 mM) and sodium persulfate (2.45 mM) was realised and kept overnight at room temperature in the dark to form a radical cation ABTS•+, for a further use. Then after, the solution was diluted 200 times with methanol and 1,980 ml was transferred into a 5-ml tube at which 20 µl of the tested compound was added at the final concentration ranging from 10 µg/ml to 100 µg/ml. The resulting solution was mixed and stored at room temperature in the dark for 30 min. The radical scavenging activity of each compound was evaluated by measuring the absorbance decrease of the radical cation at fixed wavelength located at 734 nm.

For DPPH assay, samples (20 µl for each concentration ranging from 10 µg/ml to 100 µg/ml) were analysed after mixing with DPPH• solution (1,980 µl) for 30 min in the dark. The decrease of absorbance at 517 nm was compared to the control and standard with a Spectrophotometer Hewlett-Packard 8453 (Hewlett-Packard, Waldbronn, Germany). Gallic acid was used as positive control, ABTS•+ and DPPH• scavenging activity of extracts were expressed in IC_{50} values.

Cellular and enzymatic assays

Measurement of cellular antioxidant activity

The reactive oxygen species (ROS) production by activated monocytes HL-60 was measured by L012-enhanced chemiluminescence (CL) assay. To HL-60 suspensions (25×10^4 cells/well) distributed in a 96-well microtiter plate (White Combiplate 8, Thermo Labsystems, Vantaa, Finland), are added 2 ul of the stock solution of infusion extracts at the final concentrations of 0.1, 0.5, 1 and 2 µg/ml; in comparison with standards (gallic acid and quercetin) used at 10^{-4} M, 10^{-5} M and 10^{-6} M, respectively. After 5 µl of $CaCl_2$ (10^{-3} M), 5 µl of horseradish peroxidase (HRP) (25 ug/ml), 20 µl of L012 (10^{-4} M) and, just before CL measurement, 15 µl of PMA (5.10^{-7} M, final concentration) 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 (Ngombe *et al.,* 2019a).

Peroxidase inhibition

Measurement of active myeloperoxidase

This measure was performed using the Specific immuno-extraction followed by enzymatic detection (SIEFED), a licenced method developed by Franck *et al.* (2013) for the specific detection of equine and human myeloperoxidase (MPO). This method was used for the screening of phytochemicals or extracts that could modulate the activity of MPO (Franck *et al.,* 2013). The method proceeded in three steps. Firstly, MPO was extracted from a solution or a biological sample by specific immobilised antibodies (immune-extraction step). Secondly, a series of washings eliminated un-specifically bound compounds or interfering substances. Thirdly, MPO enzymatic activity was revealed by using H_2O_2 (10 µM) as substrate, Amplex Red (40 µM) as fluorogenic electron donor, and nitrite (10 mM) as enhancer

of the reaction. The activity of MPO transforms Amplex red into a highly fluorescent compound, resorufin, and after incubation (30 min at 37 °C in the dark), the fluorescence emission was read (Fluoroscan Ascent FL, λ excitation, 544 nm; λ emission, 590 nm (Thermo Fisher Scientific, Tournai, Belgium)). The control assay set as 100% MPO activity was performed with purified MPO in the presence of dimethyl sulfoxide (DMSO).

Inhibition of horseradish peroxidase oxidant (or oxidatic) activity

HRP oxidant activity was evaluated using the luminol-based chemiluminescence technic described previously (Ngombe *et al.,* 2019b). This method evaluates the modulatory effect of extracts on HRP catalytic activity using L-012, a luminol-based chemiluminescent probe. Two microliters of extract solutions at final concentrations of 0.1, 1 and 10 µg/ml were added to 163 µl of phosphate buffer, 5 µl of HRP (1 mg/ml), 10 µl of L-012 and 20 µl of H_2O_2. In each assay, three wells were loaded without plant extracts but with DMSO and were taken as control (100% CL response). To measure the basic CL response at the absence of activation, three other wells without plant extract did not receive H_20_2 (NA, not activated). The CL response was monitored for 30 min at 37 °C with a Fluoroscan Ascent spectrophotometer (Thermo Fisher Scientific) and expressed as the integral value of the total CL emission.

Mineral content

The determination of mineral content was carried out by the Inductively Coupled Argon Plasma Optical Emission Spectrometry (ICP-EOS) described previously (Florent *et al.,* 2020).

Cell toxicity

Cell toxicity on the HL-60 cells was assessed using the MTS test used previously (Ielciu *et al.,* 2018) with slight modifications. 96-well cell culture microplates were seeded with 250,000 cells per well (100 µl of cellular suspension). Thereafter, cells were incubated with 2 µl of aqueous samples (concentrations ranging between 0.5, 1.2 and 10 ug/ml). Cell toxicity was assessed by adding 10 µl of MTS solution and measuring absorbances at 450 nm each hour during three hours on a Multiscan. Cisplatin (5.10^{-3} M) was used as a positive control, water and DMSO as negative controls.

Statistical analysis

Each concentration was tested in triplicate in each assay, and at least three different assays were performed. All results were expressed as mean values±standard deviation (SD). GraphPad 7.0 (GraphPad Software, San Diego, CA, USA) was used to perfom the statistical analysis and the IC_{50} values were calculated under application of the function 'log (inhibitor) vs normalised response-variable slope' with the concentrations converted in decimal logarithms. One-way analysis, Two-way analysis (ANOVA) and Sidak multiple comparisons test were used with $P<0.05$ such as the level of statistical significance.

2. Results and discussion

Botanical microscopic features

Powders of the caterpillar's species treated with lactic acid reagent showed the specific botanical microscopic characters related to their host plants. Microscopic analysis showed the abundance of spheric starch granules, calcium oxalate cluster crystals, stomata (anomocytic stomata), epiderm of polygonal cells, lignified and crystalline fibres, scalariform vessels, isolated sclereids, fibrous sclereids, glandular and non-glandular trichomes, pollen grain, upper epidermis with part of the underlying palisade, fibrovascular tissues (Supplementary Figures S2 and S3).

Botanical features characterised correspond to histological elements of nourishing plants. Identified microscopical features were characteristic of leaves and flowers. Chewing insects, such as caterpillars and beetles, consume large portions of plant tissues, which are partially digested. Some of the plants are listed to be the nourishing plants for Congolese edible caterpillars. Among these species we cited *Burkea africana, Erythrophleum africanum, Hymenocardia acida, Dialium englerianium, Swartia madagascariensis, Pterocarpus angolensis, Gasciana gosweileri, Ochna* sp. (Leleup and Daems, 1969). *C. forda*, called '*Makoso*' in Kwango local langage for example, are found in wooded savannah of the Kwango, '*Mikoso*' (Kipende) or '*Mikwatshi*' (Kitshok). According to the typical ecology of regions of DR Congo, previous studies have listed different botanical families of host plants for Congolese edible caterpillars (Ashton *et al.,* 2011; Lunga, 2017). Latham (2008) reported the existence of 50 host plant species of caterpillars in Kongo Central (DRC) (Latham, 2008). Lisingo *et al.* (2010) listed more than 20 host plant species of caterpillars in the Kisangani and Tshopo district in DRC (Lisingo *et al.,* 2010). The determination of microscopic details of host plants of caterpillars in the future could be vital for the knowledge the origin of caterpillars.

Phytochemicals

Chemical analysis performed by chromatographic techniques showed the presence of phytochemicals such as phenolic compounds (Supplementary Figure S4) and terpenoids probably from host plants. By comparing with standards used, the rutin was identified in *G. belina*, *C. hyperbius* and *C. forda* (Supplementary Figure S5) and

gallic acid in all caterpillars. Menthol was also identified in all caterpillars such as terpenoids. Phytochemicals are known to be specific to plants. Few studies reported the presence of secondary metabolites in insects and cardenolides of *Asclepias* sp. were already identified in monarch butterflies (*Danaus plexippus*) (Bruneton, 2015). Secondary metabolites like phenolic compounds have been found not only due to the insect absorption and metabolization of the plant-derived phenolic present in their diet, but also from the ability of insects to synthesise phenolic compounds *de novo* through the sclerotization process (Andersen, 2010; Nino *et al.,* 2021). The great majority of phenolic compounds found in insects are attributed to herbivore feeding behaviour and their biotransformation capacity of host plant metabolites. Several phenolic compounds have been isolated from insects, for example tricin, luteolin, apigenin, orientin, iso-orientin, vitexin, isovitexin, kaempferol, kaempferol-3,7-di-*O*-glucoside, quercetin, quercetin-3-*β*-*O*-glucoside, isorhamnetin, myricetin, catechin, ferulic acid, sinapic acid (Nino *et al.,* 2021). The chemical composition of secondary metabolites would depend on the way that the plants are nourished. The feeding plants of caterpillars differ from one region to another. Concerning *C. forda* from Kahemba, it feeds in February on *E. africanum,* namely '*Mukoso*' in the local language, and in September on *B. Africana,* namely '*Musese*' in the local language (Leleup and Daems, 1969). In Kongo Central, another area, *C. forda* is the host tree of *Crossopteryx febrifuga*. Further studies to identify, isolate and characterise the different major secondary metabolites of caterpillars in comparison with their host plants should be conducted.

Mineral content

Insects contain some micronutrients that are important for animal health and development (Orkusz, 2021; Rumpold and Schluter, 2015). ICP-EOS mineral analysis on the dry matter showed the presence of caterpillars in copper, magnesium, manganese, selenium and zinc (Table 2). The concentration of these elements varied significantly ($P<0.05$) between analysed samples. *G. belina* was the caterpillar with high concentrations of minerals. For selenium, *G. belina* contained high concentration followed by *I. truncata*, *C. forda* and *C. hyperbius*. *C. forda* and *Imbrasia* sp. which did not contain selenium. *C. forda* (C4 and C5) had the high concentration of manganese. The concentrations of selenium in caterpillars are comparable to those of other foods of animal origin such as chicken, eggs, fish and meat (Navarro-Alarcon and Cabrera-Vique, 2008). The selenium content of foods from both plant and animal origins tends to be greatly influenced by the local soil selenium environment. Mineral micronutrients of edible insects are influenced by the diet of insects. Osasona and Olaofe (2010) evaluated the mineral content of *C. forda* from Nigeria and showed that it contained micronutrients such as magnesium and zinc whose concentrations are far below those of our study. Copper and manganese were not detected in this species (Osasona and Olaofe, 2010). Another study on mineral content of *C. forda* from Nigeria equally showed that this species contained manganese, magnesium and zinc such as detected micronutrients with the concentrations relatively close to our results (Akinnawo, 2000). Previous studies showed that the mopane worm (*G. belina*), contains significant amounts of micronutrients like cupper, iron, magnesium, manganese and zinc (Lategan, 2019; Orkusz, 2021). Obtained results on studied samples showed that edible caterpillars are the source of mineral micronutrients such as copper, magnesium, manganese, selenium and zinc. Selenium is an essential trace element for all living organism. Insects participate in the biological cycling of selenium in terrestrial and fresh-water ecosystems and are shown to possess higher potential to bioaccumulate selenium (Golubkina *et al.,* 2014; Kieliszek, 2019). Selenocysteine is the form of selenium found in animal foods and is from their selenoproteins (Kieliszek, 2019; Rayman, 2012). Selenium is considered to play an important role in insect physiology. Edible Congolese caterpillars could be excellent sources of selenium. Caterpillars were identified as an important source of protein for local

Table 2. Micronutrient contents of Congolese caterpillars expressed in g/100 g of dry matter (mean, n=3).

	Selenium	Zinc	Magnesium	Manganese	Copper
Gonimbrasia belina	0.12	0.011	0.17	0.003	0.002
Cinabra hyperbius	0.02	0.007	0.11	0.004	0.002
Cirina forda (C4)	0.04	0.006	0.15	0.006	0.003
Cirina forda (C5)	not detected	0.007	0.03	0.006	0.003
Imbrasia truncata (C10)	0.06	0.007	0.09	0.004	0.002
Imbrasia sp.	not detected	0.006	0.08	0.004	0.003
Egg[1]	0.025	0.002	0.02	not reported	0.0002
Chicken meat[1]	0.015	0.001	0.02	not reported	0.0001

[1] Data from Kieliszek (2019) and Thai food composition database (2015; Mahidol University, Institute of Nutrition, Salaya, Thailand).

consumption of the rural population of Kahemba, the most affected by konzo. Bumoko *et al.* (2015) have shown that deficiency in essential trace elements, notably selenium, is associated with greater motor impairment in children with konzo. It is likely that selenium deficiency contributes to the pathogenesis of konzo through mechanisms that are responsible for oxidative damage (Bumoko *et al.,* 2015). For preventing konzo, a disease that occurs in the context of malnutrition, interventional trials may benefit from a combination of dietary supplementation and processing methods to remove cyanogenic compounds from cassava prior to human consumption (Bumoko *et al.,* 2015; Kapepula *et al.,* 2018). Consumption of edible insects could provide significant amounts of copper, iron, magnesium, manganese, selenium, and zinc.

In the DRC, the health benefits of caterpillars were investigated for the management of malnutrition and anaemia. Cereal made with caterpillars and used as a micronutrient-rich supplement to complementary feedings have shown beneficial effects in infants aged between 6 and 18 months (Bauserman *et al.,* 2015a,b).

Antioxidant activities

The radical scavenging activity performed with *in vitro* biochemical methods, namely ABTS and DPPH assays, showed that aqueous extracts from caterpillars have the ability to scavenge free radicals with IC_{50} values comprised between 10.7±1.4 µg/ml and 78.5±14.3 µg/ml (Table 3). The IC_{50} values showed that the caterpillars *C. hyperbius*, *Imbrasia* sp. and *I. truncata* are the most active. All aqueous extracts from caterpillars exhibited higher scavenging activities. Radical-scavenging activities of caterpillars are comparable with those of Congolese traditional vegetables. *Manihot glaziovii* was the most active vegetable with 12.4 µg/ml and 20.5 µg/ml such as IC_{50} values in ABTS and DPPH assays, respectively (Kapepula *et al.,* 2018). For the one species from different areas or harvested during different periods, a significant difference of antioxidant activity was observed: *Imbrasia forda* (C3 and C4) from Kahemba are more active than *I. forda* from Kenge (C5) and from Tshikapa (C8). For DPPH assay, *I. forda* from Kahemba harvested in February is more active than the one harvested in September and *I. truncata* from Equateur harvested in December 2018 (C10) is more active than the one harvested in September 2017(C7). Bioactivities of natural products vary largely with the chemical composition, which depends on the geographical area of collection. The obtained results showed that the chemical composition in phytochemicals of caterpillars related to phenological stages of the insects or the host plants would be the basis of the differences observed in radical-scavenging activity.

Table 3. IC_{50} values (µg/ml) of aqueous extracts from caterpillars on ABTS and DPPH assays (mean±SD, n=6).[1]

	IC_{50} (µg/ml)[2]	
Samples	**ABTS assay**	**DPPH assay**
Gonimbrasia belina	$14.6±1.0^{a}$	$54.3±3.1^{d}$
Cinabra hyperbius	$10.7±1.4^{ab}$	$26.6±2.0^{de}$
Cirina forda (C3)	$15.8±1.7^{a}$	$57.0±5.9^{ef}$
Cirina forda (C4)	$16.6±1.4^{a}$	$78.5±1.3^{defg}$
Cirina forda (C5)	$20.7±2.3^{abc}$	$68.8±10.4^{eh}$
Imbrasia epimethea	$16.6±1.6^{a}$	$43.5±2.1^{ghi}$
Imbrasia truncata (C7)	$12.1±0.8^{ac}$	$52.3±2.7^{egj}$
Cirina forda (C8)	$36.4±3.8^{a}$	$73.6±8.6^{eijk}$
Imbrasia sp.	$10.8±1.2^{ac}$	$21.7±2.4^{dfghjk}$
Imbrasia truncata (C10)	$13.3±1.5^{ac}$	$26.6±3.0^{dfghijk}$
Gallic acid	0.7±0.1	1.1±0.1

[1] ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate; DPPH = 2,2-diphenyl-1-picrylhydrazyl; SD = standard deviation.
[2] IC_{50} values present significant differences (a-c for ABTS test and d-k for DPPH test).

Using one-way analysis (ANOVA), for ABTS assay, significant differences ($P<0.0001$) were observed in the radical-scavenging activities between all extracts and *C. forda* from Tshikapa (C8), *C. hyperbius* and *C. forda* (C5), *C. forda* (C5) and *I. truncata, Imbrasia s*p. For DPPH, significant differences were observed between *G. belina* and *C. hyperbius, C. forda* (C4), *Imbrasia* sp., *I. truncata* (C10); and between the different extracts in a specific way.

No significant correlation was found between the micronutrient contents and the antioxidant capacities (ABTS Pearson correlation coefficients: +0.199, $P=0.801$; +0.059, $P=0.981$; -0.284, $P=0.586$; +0.714, $P=0.111$ and +0.456, $P=0.367$ for selenium, zinc, magnesium, manganese and cooper, respectively/DPPH Pearson correlation coefficients: +0.193, $P=0.808$; +0.109, $P=0.837$; +0.177, $P=0.737$; +0.682, $P=0.136$ and +0.459, $P=0.359$ for selenium, zinc, magnesium, manganese and cooper, respectively).

In addition to conventional cell-free antioxidant assays, the assessment of the antioxidant and anticatalytic potential in cell models is relevant. In the present work, the lucigenin dependent chemiluminescence (CL) test was used to evaluate the ROS extracellular production by stimulated neutrophils (Derochette *et al.,* 2013). Lucigenin is considered to be a more specific probe for the detection of superoxide anions directly produced by NADPH oxidase activity (Li *et al.,* 1998).

The addition of increasing concentrations of the caterpillars aqueous extracts (0.1-2 µg/ml) resulted in a dose-dependent decrease of ROS produced by PMA-activated monocytes HL-60 in comparison to the water control (Supplementary Figure S6). Our results showed that the cellular antioxidant

activity of infusion extracts is significantly higher in the following order: *Imbrasia* sp. > *C. hyperbius* > *G. belina* > *I. truncata* (C10) > *C. forda* (C3) > *C. forda* (C4) > *I. epimethea* > *I. truncata* (C7) > *C. forda* (C5) and *C. forda* (C8). Significant differences were observed in the inhibitory effect of different extracts (two-way analysis, ANOVA). These results showed that extracts from caterpillars have an inhibitory effect on the extracellular ROS production in HL-60.

Aqueous extracts from caterpillars contain various phytochemicals. Caterpillars contain high concentrations of good quality lipids and proteins (Nsevolo *et al.,* 2016; Rumpold and Schlüter, 2015). Fat acid profile of lipids extracted from caterpillars include saturated, monounsaturated, polyunsaturated fatty acids and tocopherol (Fogang Mba *et al.,* 2019; Rumpold and Schlüter, 2015; Womeni *et al.,* 2009). The high inhibitor effect on ROS production of caterpillar extracts can be attributed to hydrophilic and lipophilic compounds. Lipophilic compounds such as carotenoids are known to be powerful scavengers of superoxide anions (Galano *et al.,* 2010). As antioxidants, polyunsaturated fatty acids act as ROS scavengers or inhibitors of ROS production (Richard *et al.,* 2008). For proteins, Mouithys-Mickalad *et al.* (2020) showed that proteinaceous extracts have the ability to inhibit neutrophils ROS production. These authors reported that black soldier fly larvae proteins could suppress oxidative damage resulting from inflammatory cell activity such as neutrophils (Mouithys-mickalad *et al.,* 2020). Nevertheless, phytochemicals contribute to the total antioxidant capacity of the caterpillar extracts. Previous studies have shown the interesting antioxidant capacities of insect extracts related to their phenolic compounds (Nino *et al.,* 2021).

Peroxidasic inhibition

Peroxidases are involved in protective mechanisms, but some of them can also lead to deleterious effects such as lipoprotein oxidation, carcinogenesis and necrosis of the liver (Ray and Katyal, 2016). MPO is a peroxidase released during degranulation of neutrophils and monocytes. MPO permits the microbicide action of neutrophils by the production of hypochlorous acid. However, unlike its beneficial activity, MPO also contributes to the development of many disorders. MPO and its metabolites are biological markers for infectious, non-infectious and neurodegenerative diseases (Khan *et al.,* 2014; Ray and Katyal, 2016).

The results of the MPO direct technique showed that all infusion extracts of caterpillars exhibited an inhibitory effect on MPO activity in the range concentrations of 1 to 20 µg/ml excepted extracts of *I. epimethea* and *I. truncata* (C7). Caterpillars *I. epimethea* and *I. truncata* (C7) showed an activator effect on MPO activity (Supplementary Figure S7).

In MPO SIEFED technique, all infusion extracts of caterpillars exhibited a dose-dependent inhibitory effect on MPO activity in the range concentrations of 1 to 20 µg/ml excepted extracts of *I. epimethea*, *Imbrasia* sp. and *I. truncata* (C10) (Supplementary Figure S8). All extracts except that of *G. belina* and *C. forda* (C5) showed a slight prooxidant effect at concentrations of 1 ug/ml. These inhibitory effects were significantly higher and indicated a better interaction between molecules of caterpillar extracts and the active site of the enzyme.

While the SIEFED indicates the ability of a ligand to bind to the enzyme active site and the reversibility of this interaction, the MPO direct analysis informs about a global interaction of compounds of the extract sample with the intermediate form (compound I:CpI) of MPO without being able to distinguish between the stoichiometric reducing action on the MPO peroxidase cycle and the anti-catalytic one. In the experiment models, the active form of myeloperoxidase (MPO-Fe (III) reacts with hydrogen peroxide to form oxyferryl π cation radical (CpI form). CpI form converts back into MPO-Fe (III) coupled with chloride ion, transforming into hypochlorous acid. The SIEFED and the MPO direct analysis allow one to investigate the interaction between MPO and its potential modulators of its activity such as phytochemicals. The assays evaluate the capacity of caterpillar extracts to modulate the back reduction of the Cp I form to MPO-Fe (III) (Franck *et al.,* 2013; Nyssen *et al.,* 2018). Aqueous extracts from caterpillars contain various phytochemicals like polyphenols and terpenes. Polyphenols by interacting directly with MPO, via low-energy bonds, could modify the structure of the enzyme, clutter the active site, or compete with substrates for binding at the active site. For flavonoids, the requirement of the pyrogallol group of the B ring is important for the inhibitory activity on MPO. The inhibitory activity on MPO is more pronounced with compounds having the pyrogallol group on the B-ring, the C2-C3 double bond in the C-ring, and the hydroxyl groups in the 3', 4', and 5' (Boly *et al.,* 2011). For phenolic acids, the number of OH groups and the elongation of the carboxylic group seems to be essential for the inhibition of MPO activity, probably by facilitating the interaction with the MPO active site or structure (Franck *et al.,* 2013). Other molecules like lipophilic compounds have a better interaction with the hydrophobic pocket at the entrance of the active site of MPO.

These two techniques are therefore complementary. Indeed, the absence of effect on MPO activity in the SIEFED technique does not mean the phytochemicals do not interact with the enzyme. The inhibitory effect of caterpillar extracts indicates that caterpillars are the potential inhibitors of MPO. Therefore, the inhibition effect

of MPO activity may represent an attractive therapeutic opportunity in various diseases associated with excessive or protracted activation of PMNs (Nyssen *et al.,* 2018). The obtained results showed that the chemical composition in phytochemicals of caterpillars extracts would be the basis of differences in MPO inhibitory activities observed. The antioxidant and anti-inflammatory activities obtained using cellular and enzymatic models (neutrophils, MPO and HRP) indicate that consumption of caterpillars can potentially prevent or reduce the inflammatory process associated with oxidative damage.

Edible insects contain ample protein, healthy fatty acids, minerals, vitamins and phytochemicals and represent a valuable resource that may promote human health. Our study demonstrates that Congolese caterpillars contain phytochemicals such polyphenols and terpenes; also mineral micronutrients to quantities close to those of eggs and meat. Phytochemicals play numerous biological properties. Flavonoids and phenolic acids are a group of phenolic compounds or secondary metabolites that are widely distributed in higher plants and are part of our daily diet. It has been reported that flavonoids and phenolic acids exhibit a wide variety of therapeutic effects, including anti-inflammatory, antioxidant, antiviral, antibacterial, anticarcinogenic, antituberculosis, vasodilatory, and antiallergic activities. When ingested in the diet, they may exert a protective effect against behavioural and neuronal damage (Kapepula *et al.,* 2018). Terpenes present attractive biological properties such as analgesic, anti-inflammatory and anticonvulsant activities.

Such as the consumption of cereals fortified with edible insects improved minerals status and growth in infants, Congolese caterpillars constitute excellent source of minerals, especially of the children of Kahemba, the most affected by konzo. Previous studies reported that Children with konzo had low levels of selenium, copper, and zinc (Bumoko *et al.,* 2015). When incorporated in the diet of Kahemba's population, caterpillars can provide significant amounts of minerals such as copper, magnesium, manganese, selenium and zinc.

By their phytochemical, micronutrient contents and bioactivities, caterpillars could be used as a diet supplement with antioxidant properties in the context of pathologies associated with oxidative damage such as konzo.

Cytotoxicity effect of caterpillars

In our study, MTS assay was used to determine the general cytotoxicity of aqueous extracts from caterpillars in HL-60 cells. The results obtained using the MTS test showed that at the tested concentrations (1, 5 and 10 µg/ml) none of the samples decrease the viability of HL-60 cells after three hours of incubation (Supplementary Figure S9). On the other hand, there was a slight increase in cell viability for some extracts at different concentrations (*G. belina*, *C. forda*, *I. epimethea*, *I. truncata*) compared to the control. Data obtained from the present study clearly indicate that aqueous extracts from caterpillars are not cytotoxic to human leukaemia compared to cisplatin used as a positive control.

4. Conclusions

This study reported the botanical microscopic features, chromatographic fingerprints, micronutrients content, antioxidant activities and the inhibition peroxidasic activity of edible Congolese caterpillars. Microscopic analysis showed the presence of characteristic botanical features, specific to each nourishing plant of caterpillars. Phytochemical screening by TLC and HPLC-DAD revealed the presence of phenolic acids, flavonoids and terpenes as major secondary metabolites. Aqueous extracts from studied caterpillars displayed high ABTS and DPPH radical-scavenging activities and showed the best cellular antioxidant activities on ROS-induced chemiluminescence using L012 on HL 60 monocytes. Caterpillars exhibited a dose-dependent inhibitory effect on MPO activity. Our results showed that insects are not only a valuable source of protein and micronutrients such as selenium but also an excellent source of antioxidants. However, the *in vitro* tests should be supplemented in the future by *in vivo* evaluations. This could contribute to demonstrate the nutritional and therapeutic benefits of caterpillars.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2022.0072.

Figure S1. Edible Congolese caterpillars.

Figure S2. (A) Stomata; (B) epiderm cells; (C) fragments of fibres; and (D) fragments of fibrous sclereids with crystals from host plants of caterpillars (40×).

Figure S3. (A) Unicellular non glandular trichome; (B) cells of upper epidermis containing crystals of calcium oxalate; (C) pollen grains; and (D) sclereids from host plants of caterpillars at 40×.

Figure S4. TLC chromatogram of methanolic extracts from caterpillars.

Figure S5. HPLC chromatogram of the methanolic extract of caterpillar C2: *C. hyperbius.*

Figure S6. Effects of quercetin and aqueous extracts from caterpillars on the L012-chemiluminescence (CL) response produced by PMA activated monocytes HL60.

Figure S7. Effect of gallic acid and aqueous extracts from caterpillars on MPO activity measured by direct MPO assay.

Figure S8. Effect of gallic acid and aqueous extracts from caterpillars on MPO activity measured by SIEFED.

Figure S9. Effect of cisplatin and aqueous extracts from caterpillars in MTS assay on HL60 measured by spectrophotometry.

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Conflict of interest

The authors declare no conflict of interest.

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