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RESEARCH ARTICLE

IN VITRO CYTOTOXICITY, HEMOLYTIC EFFECT AND MINERAL PROFILE ASSESSMENT OF EXTRACTS FROM TWO ANTIMALARIAL PLANTS IN CÔTE D'IVOIRE: *MOMORDICA CHARANTIA* AND *PARQUETINA NIGRESCENS*

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ABSTRACT

The aim of this study was to contribute to a better understanding of the biological and chemical properties of *Momordica charantia* and *Parquetina nigrescens* by assessing their potential toxicity, hemolytic activity, and mineral composition. Cytotoxicity tests performed on Vero and RD cell lines revealed that *P. nigrescens* exhibited a dose-dependent response, with mean IC_{50} values of 51.70 ± 3.8 µg/mL (24 h), 187.23 ± 6.4 µg/mL (48 h), and 137.40 ± 5.2 µg/mL (72 h). *M. charantia* showed comparable cytotoxicity at 24 h (73.81 ± 4.1 µg/mL) and 48 h (74.39 ± 3.9 µg/mL), but significantly increased cytotoxicity at 72 h (24.40 ± 2.3 µg/mL), suggesting a delayed action mechanism. The hemolytic activity assessment showed very low hemolysis (<1%) for both extracts, indicating good erythrocyte tolerance up to 200 µg/mL. Mineral analysis revealed high levels of calcium (3132.69 ± 102.7 mg/kg), zinc (807.93 ± 27.6 mg/kg), iron (108.97 ± 9.1 mg/kg), and magnesium (198.30 ± 12.5 mg/kg) in *P. nigrescens*, supporting its potential as a complementary mineral source. In comparison, *M. charantia* displayed lower concentrations, particularly in iron (6.81 ± 0.7 mg/kg) and magnesium (11.76 ± 1.2 mg/kg), but remained a moderate source of potassium (307.36 ± 15.4 mg/kg). In conclusion, *P. nigrescens* stands out for its rich mineral profile and moderate cytotoxicity, whereas *M. charantia* demonstrates increased long-term cytotoxicity, along with good hemolytic tolerance. These findings support further pharmacological investigations to enhance the therapeutic potential of these medicinal plants.

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INTRODUCTION

Malaria remains one of the leading causes of morbidity and mortality worldwide, with approximately 249 million cases and 608,000 deaths reported in 2022, 95% of which occurred in sub-Saharan Africa [(1)].This disease, caused by *Plasmodium* parasites and transmitted by *Anopheles* mosquitoes, particularly affects children under five years of age and pregnant women in endemic regions. Despite significant progress made through the introduction of artemisinin-based combination therapies (ACTs), prevention campaigns, and more recently, vaccination, malaria control still faces numerous challenges. Among these are the emergence of parasite resistance and limited access to modern healthcare, especially in rural areas[(2,3)]. In this context, medicinal plants continue to play a central role in malaria management in Africa. It is estimated that about 80% of the continent's population relies on traditional medicine to treat various ailments, including malaria[(4,5)].In West Africa, numerous plant species have been used for generations for their therapeutic properties, and several are currently under scientific investigation to validate their efficacy and safety[(6,7)].In Côte d'Ivoire, ethnobotanical studies have highlighted a wide diversity of plants traditionally used for malaria treatment in both rural and urban settings [(8,9)].Among the most frequently cited species by traditional healers are *Momordica charantia* (Cucurbitaceae) and *Parquetina nigrescens* (Apocynaceae). *M. charantia* is known for its antioxidant, antidiabetic, antiparasitic, and immunomodulatory properties[(10,11)].*P. nigrescens* is traditionally used in the treatment of fevers, anemia, and digestive disorders. Several studies have also reported its hematopoietic and antioxidant effects[(12,13)].However, despite the widespread use of these two species in traditional medicine, scientific data regarding their toxicity, effects on erythrocytes, and mineral composition remain limited. Previous studies have mainly focused on crude extracts and their antiparasitic activity, without systematically evaluating their potential harmful effects on normal human cells, particularly cell lines and erythrocytes [(14,15,16)]. Furthermore, the mineral content of these plants is still poorly documented, although certain elements may contribute to the efficacy or toxicity of herbal treatments[(17,18)].The lack of data

on these parameters represents a barrier to the safe valorization of these medicinal plants. It is therefore essential to simultaneously evaluate their biological activity, safety profile, and mineral content, whether beneficial or potentially toxic. This study aims to deepen the understanding of the biological and chemical properties of *Momordica charantia* and *Parquetina nigrescens* through the evaluation of their potential toxicity, hemolytic effect, and mineral composition.

MATERIALS AND METHODS

Harvesting plants and preparing extracts: Fresh leaves of *Parquetina nigrescens* (Wennberg) Bullock, *Momordica charantia* L., were collected in Agboville (Southeastern Ivory Coast) from march 2022 to April 2022 (Figure 1). After identification at the National Center for Floristics (NCF)at Felix Houphouët-Boigny University and a sample deposit at the herbarium, plant samples were air dried in shade at room temperature and ground into powder. Two times 100g of the powder were macerated respectively in 1L of distilled water and 1L of 70% ethanol hydroalcoholic solvent using a blender. Macerates were filtered twice on hydrophilic cotton and once on Whatmann filter paper. Filtrates were evaporated through rotary vacuum evaporator and dried in an oven at 45 °C for 48 h to obtain aqueous and hydroethanolic extracts which were stored at 4 °C for further use.

a

b

Figure 1. Pictures of fresh leaves from selected plants. a- *Momordica charantia* (Cucurbitaceae) ; b- *Parquetina nigrescens* (Apocynaceae)

Cytotoxicity assessment: The cytotoxicity of *Momordica charantia* and *Parquetina nigrescens* extracts was evaluated *in vitro* using two cell lines: Vero cells (kidney fibroblasts from *Chlorocebus sabaeus*) and RD cells (human rhabdomyosarcoma). These cell lines are commonly employed in toxicity studies due to their stability, reproducibility, and sensitivity to bioactive compounds[19,20].Cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 200 mM L-glutamine, and 1% streptomycin. Incubation was performed in flasks at 37 °C in a humidified atmosphere containing 5% CO_2. Once confluence was reached, the cells were detached, counted, and seeded into 96-well plates at a density of 1×10^4 cells/well. After 24 hours of adhesion, the cells were exposed to different concentrations of plant extracts (ranging from 1.56 to 100 µg/mL). Each experimental condition was tested at three distinct incubation times : 24 h, 48 h, and 72 h. All experiments were conducted in biological triplicates. Cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 200 mM L-glutamine, and 1% streptomycin. Incubation was performed in flasks at 37 °C in a humidified atmosphere containing 5% CO_2. Once confluence was reached, the cells were detached, counted, and seeded into 96-well plates at a density of 1×10^4 cells/well. After 24 hours of adhesion, the cells were exposed to different concentrations of plant extracts (ranging from 1.56 to 100 µg/mL). Each experimental condition was tested at three distinct incubation times: 24 h, 48 h, and 72 h. All experiments were conducted in biological triplicates.

***In vitro* hemolysis test:** The *in vitro* hemolysis assay was performed to ensure that the observed antiplasmodial activity in the biological tests was not due to a nonspecific action of the extracts on human erythrocyte membranes. Indeed, apparent antiparasitic activity may result from red blood cell destruction, thereby preventing the normal development of intraerythrocytic parasites[21]. A volume of 5 mL of whole blood was collected from a healthy volunteer into an EDTA tube (50 mM) as an anticoagulant, following informed consent. The blood sample was kept on ice to maintain cellular integrity. It was then washed three times with phosphate-buffered saline (PBS, pH 7.4). Each wash involved centrifugation at 2200 rpm for 15 minutes at 4 °C, followed by careful removal of the supernatant. After the final wash, the optical density (OD) of the packed red blood cells was adjusted to 1.5 at 550 nm using a spectrophotometer, as described by Elizondo-Luevano et al. (2024)[22].A 10% (v/v) erythrocyte suspension was then prepared in PBS at room temperature. The tested extracts were dissolved in PBS, a physiologically compatible solvent, to prepare stock solutions at concentrations of 100 µg/mL and 200 µg/mL. The final solvent volume in each mixture did not exceed 1% to avoid solvent-induced hemolysis. For each test, 10 µL of extract solution was added to 190 µL of the 10% erythrocyte suspension in microtubes, for a final volume of 200 µL. All samples were prepared in triplicate. Two types of controls were included: a negative control (red blood cells in PBS alone, representing 0% hemolysis) and a positive control (red blood cells incubated with 1% Triton X-100, representing 100% hemolysis). The mixtures were incubated at room temperature for 1 hour under gentle magnetic stirring. After incubation, the tubes were centrifuged at 2200 rpm for 5 minutes, and 150 µL of the supernatant was transferred to a 96-well plate for spectrophotometric reading. Absorbance was measured at 540 nm using an ELISA reader. The percentage of hemolysis was

calculated using the formula described by Takebayashi et al. (2010) [23]and Balouiri et al. (2016)[24].

Mineral profile analysis: The mineral content of *Momordica charantia* and *Parquetina nigrescens* extracts was determined using inductively coupled plasma optical emission spectrometry (ICP-OES), following standardized AOAC protocols (1990) as described by Poitevin et al. (2010)[25]. This analysis was performed in two main steps: sample mineralization and quantification of the elemental content. An exact mass of 0.3 g of each dried extract was weighed into a porcelain crucible. The samples were ashed at 600 °C for 5 hours in a muffle furnace until a uniform white ash was obtained. After cooling to room temperature, 5 mL of 1 N nitric acid (HNO_3) was added to each crucible. The mixtures were evaporated to dryness on a heated sand bath. The resulting residue was then treated with 5 mL of 1 N hydrochloric acid (HCl) and subjected to a second thermal digestion at 400 °C for 30 minutes. After another cooling step, 10 mL of 0.1 N HCl was added, and the mixture was transferred into a 50 mL volumetric flask. The crucible was carefully rinsed three times with 10 mL of 0.1 N HCl to recover any remaining residues, and the rinses were added to the same flask. The volume was then adjusted to the mark using the same acidic solution. The final solution was filtered through a 0.36 µm syringe filter to remove suspended particles. The resulting mineralized solutions were analyzed using ICP-OES (Agilent 5100 ICP-OES) to quantify the concentrations of iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), copper (Cu), potassium (K), sodium (Na), and manganese (Mn). Quantification was performed according to the protocols described by Skoog et al. (2014)[26]

Statistical analysis: All data were expressed as mean ± standard deviation (SD) from three independent replicates (n = 3). To compare the effects of different extract concentrations on cell viability and hemolysis, one-way analysis of variance (ANOVA) was performed. All statistical analyses were conducted using GraphPad Prism version 8.01 (GraphPad Software Inc., San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

RESULTS

Cytotoxity evaluation: The cytotoxicity of hydroethanolic extracts from *Parquetina nigrescens* and *Momordica charantia* was assessed on Vero and RD cell lines according to the ISO 10993-5:2009 standard, which classifies extracts into four toxicity levels ranging from highly cytotoxic to non-cytotoxic (Table 1). At 24 h, both extracts were moderately cytotoxic, with a more pronounced effect observed for *P. nigrescens* (IC_{50} = 51.70 µg/mL) compared to *M. charantia* (IC_{50} = 73.81 µg/mL), although this difference was not statistically significant (p > 0.05). At 48 h, *P. nigrescens* exhibited weak cytotoxicity (IC_{50} = 187.23 µg/mL), showing a significant improvement, whereas *M. charantia* remained stable and moderately cytotoxic (IC_{50} = 74.39 µg/mL). At 72 h, *M. charantia* approached the cytotoxicity threshold (IC_{50} = 24.40 µg/mL), while *P. nigrescens* remained weakly cytotoxic (IC_{50} = 137.40 µg/mL) with no significant variation observed (Table 2).

Hemolysis Assay: Both extracts (*Parquetina nigrescens* and *Momordica charantia*) exhibited hemolytic activity below 1% at concentrations of 100 and 200 µg/mL, indicating a very low hemolytic potential. At 200 µg/mL, *P. nigrescens* induced hemolysis of 0.57 % ± 0.06, while *M. charantia* showed 0.53 % ± 0.05 (Table 3).

Table 1. Cytotoxicity classification of the extracts based on IC_{50} values in normal cell lines

CI_{50} (µg/mL)	Interpretation
<20	Highly cytotoxic
20-100	Moderately cytotoxic
100-1000	Slightly cytotoxic or well tolerated
>1000	Non-cytotoxic

Table 2: Cytotoxicity of *P. nigrescens* and *M. charantia* extracts expressed as IC_{50} (µg/mL) at different exposure times

	Parquetina nigrescens	*Momordica charantia*
	IC_{50} (µg/mL)	
24 H	51,70	73,81
48 H	187,23	74,39
72 H	137,4	24,4

Table 3. *In vitro* hemolytic effect of hydroethanolic extracts of *Parquetina nigrescens* and *Momordica charantia* on human erythrocytes

	Extract (µg/mL)	Absorbance (540 nm)	% Hémolysis
P. nigrescens	100	0,0166± 0,0012	0,44± 0,05
	200	0,02± 0,0015	0,57± 0,06
M. charantia	100	0,0176± 0,0010	0,35± 0,04
	200	0,0216± 0,0014	0,53± 0,05
PBS		0,003± 0,0003	0± 0,00
Triton X-100		2,306± 0,030	100± 1,20

PBS: Phosphate-buffered saline, used as a negative control with no expected hemolytic effect. Triton X-100: 1% non-ionic detergent, used as a positive control to induce complete hemolysis.

Mineral content: Analysis of the extracts revealed that calcium (Ca) is the predominant mineral in both plants, with a slightly higher concentration in *Parquetina nigrescens* (3132.68 mg/kg) compared to *Momordica charantia* (2968.72 mg/kg), suggesting a potential role in supporting bone health (Figure 1). Zinc (Zn) also stood out with a markedly higher level in *P. nigrescens* (807.92 mg/kg) than in *M. charantia* (120.49 mg/kg), thereby enhancing its immunostimulant and antioxidant profile. The concentrations of potassium (K), sodium (Na), and magnesium (Mg) were relatively similar between the two species, with a slight predominance in *P. nigrescens*. Lastly, iron (Fe) content was significantly higher in *P. nigrescens* (108.96 mg/kg vs. 6.8 mg/kg), which could contribute to anemia prevention (Figure 2).

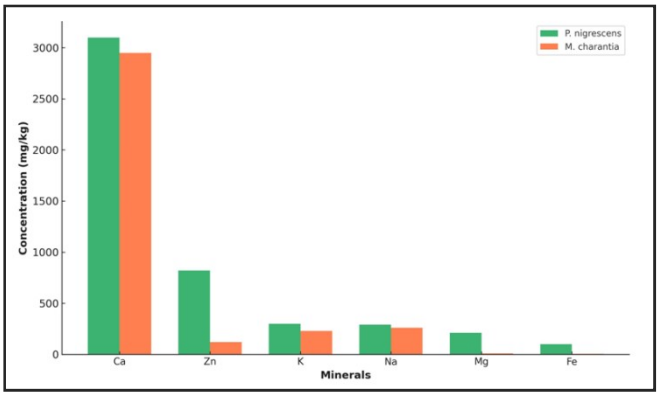

Figure 2. Major mineral composition of *P. nigrescens* and *M. charantia* Extracts

DISCUSSION

The evaluation of the cytotoxicity of hydroethanolic extracts of *Parquetina nigrescens* and *Momordica charantia* revealed contrasting profiles, highlighting the dynamic nature of secondary metabolite effects over time. According to ISO 10993-5:2009[27], which provides threshold values for interpreting the toxicity of substances based on their 50% inhibitory concentration (IC_{50}), our extracts can be generally considered as moderately to slightly cytotoxic, with time-dependent variations. At 24 hours, both extracts exhibited moderate cytotoxicity (*P. nigrescens*: IC_{50} = 51.70 µg/mL;*M. charantia*: IC_{50} = 73.81 µg/mL). This level of activity may be attributed to the presence of phenolic compounds, alkaloids, or saponins, which are known to interact with cell membranes and induce moderate cytotoxic effects at these concentrations [28, 29]. However, a marked difference was observed at 48 hours. The increase in the IC_{50} value for *P. nigrescens* (187.23 µg/mL) suggests a reduction in cytotoxic effect, possibly due to metabolization, degradation of unstable active constituents, or the induction of cellular defense mechanisms.[30] This could also reflect an adaptive response by Vero and RD cells to prolonged exposure to less stable metabolites. In contrast, *M. charantia* maintained a relatively constant level of toxicity at 48 hours (IC_{50} = 74.39 µg/mL), indicating a certain stability of active compounds in the extracellular medium. Components such as momordicine and cucurbitacins, well-documented in this species, are known for their chemical stability and prolonged effects on cell proliferation and membrane integrity [31, 32]. At 72 hours, the trend was clearly reversed: *M. charantia* became more cytotoxic (IC_{50} = 24.4 µg/mL), approaching the cytotoxicity threshold (20 µg/mL), while *P. nigrescens* remained slightly cytotoxic (IC_{50} = 137.4 µg/mL). This late-stage increase in *M. charantia* toxicity could result from progressive intracellular accumulation of bioactive compounds or the formation of active secondary metabolites via enzymatic degradation[33]. Moreover, the Vero and RD cells used as cytotoxicity assay models differ in metabolic activity, membrane permeability, and oxidative defense capacity[30]. Future studies should therefore assess the extent of cellular damage using more specific tools, such as propidium iodide staining, MTT assay coupled with microscopic observation, and reactive oxygen species (ROS) quantification. Our findings suggest that the interpretation of plant extract cytotoxicity depends not only on concentration and chemical composition but also on the duration of exposure. Thus, beyond dose-dependency, the time factor plays a critical role in evaluating the toxic potential of plant-based extracts.

The evaluation of the anti-hemolytic activity revealed percentages below 5% for the extracts of *Parquetina nigrescens* and *Momordica charantia* at the tested concentrations. This threshold, generally accepted as indicative of the absence of significant lytic effects on erythrocytes, suggests that both extracts are well tolerated by red blood cells *in vitro*. This property is of particular interest in an antimalarial context, where the disease is often associated with hemolytic anemia and alterations in erythrocyte membrane stability. Therefore, the absence of notable hemolysis is a valuable indicator of the safety profile of substances with potential therapeutic interest. These findings are consistent with those reported by Imaga et al. (2010)[34], who observed no significant hemolytic effect of *P. nigrescens* on human erythrocytes at concentrations below 500 µg/mL. Similarly, Obiandu et al. (2020)[35] demonstrated that the aqueous extract of *M. charantia* did not induce hemolysis in human red blood cells, even at high doses. Furthermore, Ranasinghe et al. (2012)[36] emphasized that the integrity of red blood cell membranes is a key parameter for the preliminary evaluation of the toxicity of phytotherapeutic compounds, particularly in hematological or infectious diseases such as malaria. The mineral analysis of the extracts revealed a significantly higher content of essential macro- and microelements in *Parquetina nigrescens* compared to *Momordica charantia*, a difference confirmed by statistically significant analysis. *P. nigrescens* was particularly rich in calcium (3132.69 mg/kg), zinc (807.93 mg/kg), potassium (307.36 mg/kg), magnesium (198.30 mg/kg), and iron (108.97 mg/kg), key elements supporting physiological functions of the body, especially in the context of malaria. These results are consistent with the findings of Kayode & Yakubu (2017)[37] and Adase et al. (2022)[38], who emphasized the nutritional richness of *P. nigrescens*, particularly in calcium, iron, and zinc, minerals essential for red blood cell production, immune function, and tissue repair. Calcium and magnesium are also involved in muscle contraction, nerve transmission, and electrolyte balance, which are often impaired in cases of malaria or anemia. In contrast, *M. charantia* exhibited much lower concentrations of most of these elements, especially magnesium (11.76 mg/kg) and iron (6.81 mg/kg), which could limit its nutritional contribution in formulations aimed at providing complementary therapeutic support. However, traces of phosphorus, copper, and manganese were detected in both extracts, indicating a varied but less concentrated mineral composition in *M. charantia*. The high zinc and iron content of *P. nigrescens* is of particular relevance in malaria management. Iron plays a crucial role in erythropoiesis, while zinc contributes to immune response modulation and resistance to parasitic infections[39]. In addition, the associated presence of potassium and magnesium may support ionic balance and reduce metabolic complications related to severe infections.

CONCLUSION

The findings of this study indicate that the hydroethanolic extracts of *Parquetina nigrescens* and *Momordica charantia* exhibit promising biological potential, with moderate to low cytotoxicity depending on exposure time, good hemolytic tolerance, and distinct mineral profiles. *Parquetina nigrescens* stands out for its richness in essential minerals such as iron, zinc, calcium, and magnesium, key elements in supporting physiological functions, particularly in the context of malaria. The relative stability of *M. charantia*'s cytotoxic activity over time, along with its lack of hemolytic effect, also makes it a plant of interest. These results suggest that both extracts could be considered for inclusion in phytotherapeutic formulations with antimalarial or nutritional purposes, pending further *in vivo* studies and deeper investigations into their mechanisms of action.

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GLOSSARY

ACTs: artemisinin-based combination therapies
AOAC: Association of Official Analytical Chemists
EDTA: Ethylenediaminetetraacetic acid
ELISA: Enzyme-Linked Immunosorbent Assay
FBS: Fetal Bovine Serum
IC_{50}: Inhibitory Concentration 50
ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry
MEM: Minimum Essential Medium
NCF: National Center for Floristics
OD: Optical Density
PBS: Phosphate-Buffered Saline

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