Phytochemical screening and cytotoxicity studies of *Chrysophyllum pruniforme* Pierre ex Engl. barks

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ABSTRACT

Background: *Chrysophyllum pruniforme* of family sapotaceae is a plant used in traditional medicine in Gabon. **Materials and Methods:** In this study, *C. pruniforme* barks were subjected to phytochemical screening and cytotoxicity investigations. Different concentrations of aqueous and total phenolic extract were tested on mice and on human erythrocytes. **Results:** Phytochemical screening of *C. pruniforme* barks revealed the presence of flavonoids, saponins, and tannins, reducing sugars, polyphenols and traces of anthraquinones. When tested *in vitro*, aqueous and the phenolic extracts showed hemolytic activities on human erythrocytes with phenolic compounds being more cytotoxic than aqueous extracts. *In vivo* study of toxicity, allowed to determine the LD_{50} at 90 mg/kg for the doses of 50, 150 and 250 mg/kg of body weight. **Conclusion:** These data indicate in one hand that *C. pruniforme* is rich in phenolic compounds and that the aqueous and total phenolic extracts could be considered as toxic for mice and maybe potentially toxic to humans in the other hand.

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INTRODUCTION

In sub-Saharan Africa, traditional phytotherapy is an alternative to modern chemical- and industrial-based medicines and is widely used in rural and even urban areas. This is essentially due to the prohibitive cost of pharmaceutical-based medicine and the low incomes of a major part of the population. In addition, the efficacy of many of these traditional and plant-based medicines is proven. In Africa, for example the leaves of *Trichilia emitica* (Miliaceae), a plant used in traditional medicine in Mali, activated the complement system and induced the proliferation of T and B-lymphocytes.[1] In Asia and particularly in China and Japan, *Bupleurum falcatum* (Umbelliferae), a Sino-Japanese plant, has shown a biological activity on gastric diseases.[2] Ultimately, western and pharmaceutical-based medicines were initially developed from phyto-molecules and later, took advantage of applied and industrial chemistry. Either obtained from plant decoctions or after extensive purification of phytochemical compounds, plant-derived medicines have been proven to be effective in the prevention or treatment various infectious diseases (bacterial, fungal parasitic and viral infections) and non-infectious diseases. These secular treatments include body-washes, decoctions, massages, ingestions, etc. These local and traditional phytotherapies tend to be promoted by local and international organizations. However, the lack of valuable and complete knowledge on the nature and recipes of these medicines is one of the major concerns of local politics, physicians, and scientists. From a public health angle, it is crucial to gain knowledge on these plant-based medicines prepared and prescribed by practitioners, particularly in terms of toxicity, composition, specific efficacy on the disease and relay the findings to practitioners of this alternative medicine for the protection and the security of the patients. In this study, our objective was to investigate, *in vitro* (on erythrocytes) and *in vivo* (on mice), the effects of *Chrysophyllum pruniforme* [synonym: *Donella pruniformis* (Pierre ex Engl.)],[3] a sapotaceae plant widely spread from West to East Africa and Central Africa (from Uganda, Tanzania, Sierra Leone, Congo and Gabon), and often used by traditional

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practitioners. In Central Africa, particularly in Congo and Gabon, *C. pruniforme* bark infusion is used to heal coughs. In the present study, we have performed a phytochemical analysis *C. pruniforme* bark extract and we have evaluated the cytotoxicity of different extracted-fractions (water and ethanol fractions and polyphenol-enriched fraction) on human erythrocytes and on mice.

MATERIALS AND METHODS

Plant identifications and barks collection

The botanical identification of *C. pruniforme* was carried out by botanical experts from the Gabonese National Herbarium (voucher number 1359 S.R.F). Bark collection was carried out after local authorities authorized our investigation protocol [Figure 1].

Extraction of plant phytochemical compounds

Different mixtures of phytochemical compounds were independently extracted (100 grams per liter; 24 hours; room temperature) after vigorous stirring with water (fraction A) and 100% ethanol (fraction B). In addition, polyphenol-enriched fraction (fraction C) was extracted from 50 g of material (in 800 mL of 80% acetone; 24 hours; room temperature). Acetone solvent was removed by using a rotavapor. The remaining aqueous solution was successively washed twice with dichloromethane and with ethyl acetate (to remove flavonoids, the pellet containing polyphenols were frozen and lyophilized).[4]

Phytochemical screening

Fraction A and B were subjected to chemical-based screening to determine their composition (secondary metabolites). Different tests were conducted in order to determine the chemical composition of each fraction.

Identification of saponins

1 gram of Fraction A and B each were collected separately, homogenized in 3 mL of water for 15 seconds, and stored at room temperature for 15 minutes. Occurrence and persistence of foam (more than 1 cm height) indicated the presence of saponins.[5]

Anthraquinones test

1 gram of initial ground material was mixed with 2 mL of chloroform. The supernatant was collected and aqueous KOH at 10% (v/v) was added to the test tube. After agitation, occurrence of a reddish coloration indicated the presence of anthraquinones.[6]

Polyphenols test

Reaction to ferric chloride ($FeCl_3$) was used to reveal the presence of polyphenol. 2 mL of fraction A and

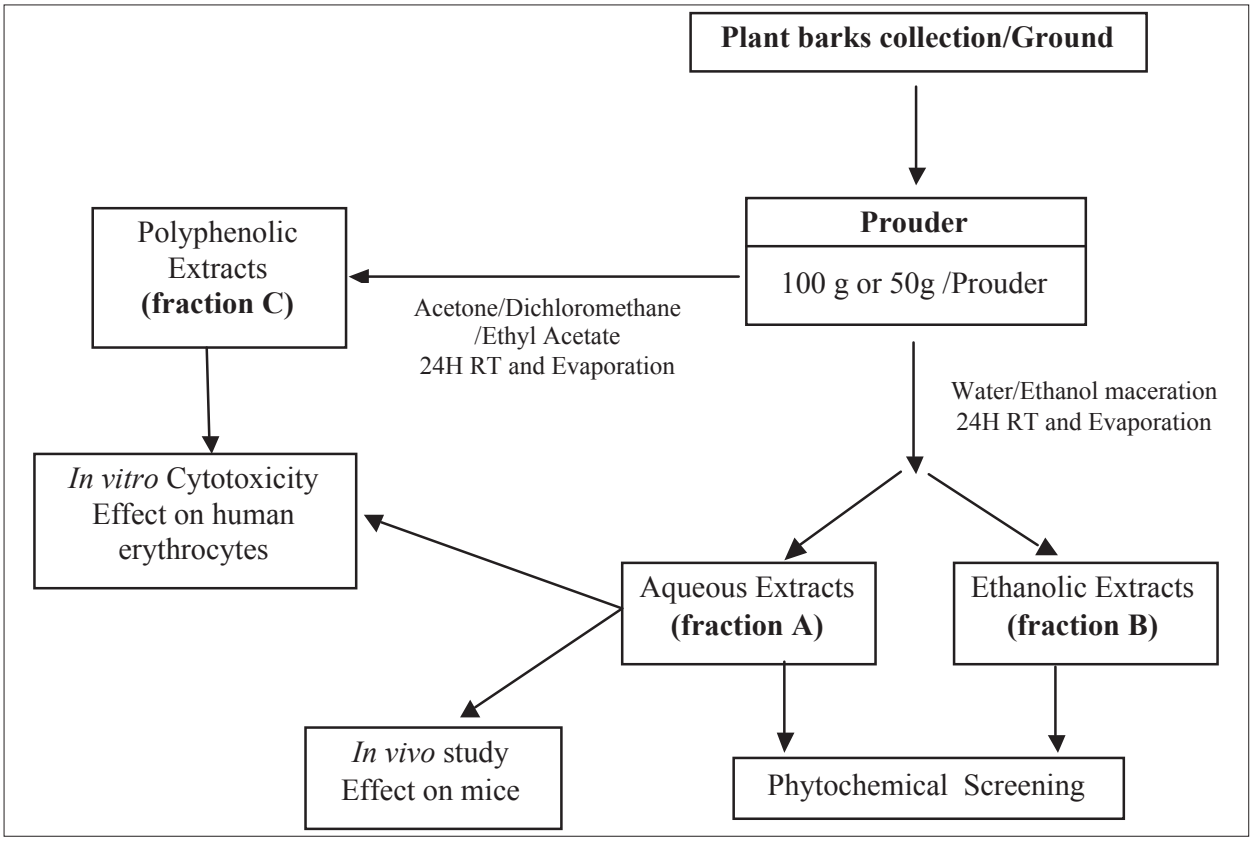

Figure 1: Study design the investigations protocol Barks collection from *Chrysophillum pruniforme*

B were prepared and mixed with 1–2 drops of ferric chloride (solubilized in 2% ethanol). Occurrence of a dark blue or green coloration indicated the presence of polyphenols.[5]

Tannins test

1.5 grams of initial ground material was mixed with 10 mL of methanol 80%, agitated for 15 minutes, and the soluble fraction was transferred into test tubes. Occurrence of tannins was tested by addition of $FeCl_3$ (1% in water). Occurrence of dark blue coloration indicated the presence of gallic tannins, whereas, brown green coloration indicated the presence of catechic tannins.[6]

Sugars reduction

2 mL of fraction A and B were prepared and mixed with 5 mL of Fehling's solutions (I and II) and boiled in a water bath for five minutes. Occurrence of a brick-red precipitate indicated the presence of free reducing sugars.[7]

Cytotoxicity on human erythrocytes

The cellular toxicity of *C. pruniforme* extracts was evaluated on blood samples from healthy donors. In whole blood or on erythrocytes clots, the degree of erythrocytes destruction can be used to evaluate the cytotoxicity of different fractions. We performed our assay as follows: Blood was collected from donors into an ethylenediaminetetraacetic acid (EDTA) tubes.

Whole blood experiment

Briefly, the initial number of erythrocytes was determined by pipetting 10 µL of blood and numbering using a cell counting glass system (Malassez cells). Since the cytotoxicity assays were performed with 400 µL of blood, the dilution factor (40) was systematically reported to ensure an accurate report of the number of lyses cells. 10–40 µL of fraction A and C were added to blood samples (to 400 µL). Samples were incubated for 45 minutes at 37°C. Observation and counting were performed under light microscope (40 ×).

Table 1: Phytochimical screening of aqueous and ethanolic extracts of *C. pruniforme*

Compounds	*Chrysophyllum pruniforme*	Ethanolic extracts
	Aqueous extracts	
Flavonoids	+	+
Saponins	+	–
Catechic tannins	+	+
Gallics tannins	–	–
Reducing sugars	++	++
Phenolic compounds	++	++
Anthraquinons	traces	traces

Statistic analysis

Statistical analysis was done using the software Prism 4. Non-linear regression Analysis LOGEC 50 was used to compare extract haemolytic activity, a P value of $P < 0.05$ was considered as statistically significant.

Acute toxicity studies (*in vivo* assay)

Experiments were carried out according to the World Health Organization (WHO) guidelines on the evaluation of the medicinal plants cytotoxicity. The lethal dose (LD_{50}) was determined according to the Schorderet method (1992).[8] Briefly, eight mice (22–30 g) divided into four groups were used to perform this experiment. Group one, two, three, and four received orally and respectively water (control), 50, 150, and 250 mg/mL of each of the fraction A. Mice were kept into the nursery for a month and the number of deaths among the different groups reported.

RESULTS

To our knowledge, this is the first report on *C. pruniforme* phytochemical screening and cytotoxicity. Our investigations show that *C. pruniforme* is particularly rich in reducing sugars and phenolic compounds. The plant also contains flavonoids, saponins, and catechic tannins, [Table 1]. However, cytotoxicity study [Figure 2] showed that *C. pruniforme* extracts were hemolytic. *C. pruniforme* aqueous extracts showed hemolytic activities of 0%, 3%, 7%, 20%, 33%, and 47% for the concentrations of 1.25, 2.5, 3.75, 5, 7.5, and 10 mg/mL, respectively, whereas total phenolic extracts showed hemolytic activities of 7%, 79%, 99.6%, 99.9%, and 100% for the concentrations of 1.25, 2.5, 3.75, 5, 7.5, and 10 mg/mL, respectively. The hemolytic activity of total phenolic extracts was significantly high compared to the one of aqueous extracts ($P < 0.0001$) [Figure 2].

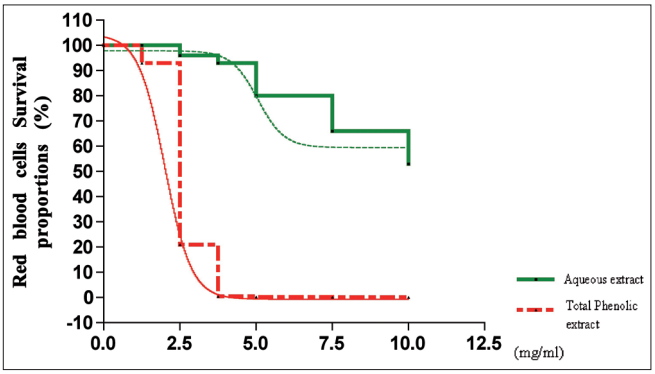

Figure 2: Hemolytic activity of aqueous and phenolic extracts on human erythrocytes. Aqueous and phenolic extracts effect on human erythrocytes was assayed at six different concentrations: 1.25, 2.5, 3.75, 5, 7.5, and 10 mg/mL. Aqueous extract hemolytic activity started with the concentration of 2.5 mg/mL. At concentration 10 mg/ml, aqueous extract led to 47% of erythrocytes lyses. Phenolic extract hemolytic activity started with the concentration of 1.25mg/mL. At concentration 2.5 mg/ml, phenolic extracts achieved 79% erythrocytes lyses

In vivo study, we see the death of two mice, 1 hour after the administration of 50 mg/mL of the aqueous extracts to the second group. The administration of 150 mg/mL to third group resulted in the death of six mice. 250 mg/mL killed all mice of the fourth group after 24 hour of treatment. This experience shows the toxic effects of *C. pruniforme*.

DISCUSSION

Plants rich in flavonoids and tannins are used in traditional medicine for the treatment of microbial infections and as hemostatic agents.[9-12] Phytochimical screening shows that *C. pruniforme* bark is rich in phenols, flavonoids, tannins, and saponins. We can, therefore, suggest *C. pruniforme* as a potential herbal medicine.

Several studies have shown that saponins have hemolytic effects on the erythrocytes.[13-15] Our study shows that the aqueous extracts present hemolytic effect on human erythrocytes. This could be explained by the presence of saponins on *C. pruniforme* bark. However, the polyphenol compounds are not known to have hemolytic properties, we can extrapolate that total phenolic extracts contain more than polyphenols. They may also be associated with other plant components such as carbohydrates and proteins.[16] This would explain the observed hemolytic effect; these compounds could be probably associated with saponins. The reduced hemolytic activity of the aqueous extracts when compared to total phenolic extract, maybe to an extent, due to the protective effect of flavonoids which have been reported in literature to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, antitumor, and antioxidant activities.[17] Another possible explanation of the differences observed may be the facts that compound in phenolic extract are concentrated.

The cytotoxic or toxic effects of *C. pruniforme* raise the security issue of *C. pruniforme* consumption. Population should be advised regarding the potentials risks (anemia and intoxication) of *C. pruniforme* consumption. *In vivo* studies on mice showed that the LD_{50} was 90 mg/kg, which might suggest that this plant is probably toxic. As *C. pruniforme* shows hemolytic activities, we can suggest this plant as a potential candidate for the treatment of polyglobulies, hence, further investigation is required. In future studies, we will separate the acid phenolic and tannins from this plant. Identify the molecules and redo the same tests to confirm or reverse phenolic compounds that have effectively a hemolytic effect.

CONCLUSION

C. pruniforme shows a hemolytic effect on human erythrocytes. These effects are observed on the aqueous extracts and are more pronounced on the total phenolic extracts. As the empirical use of *C. pruniforme* might be to an extend detrimental for health, methods for safe use of its extracts, the plant possible side effect and associated symptoms should be determined and communicated to all the population.

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