

Phytochemical Screening and Anti-anemic Activity of *Manihot esculenta* Crantz Leaves

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Abstract Background: *Manihot esculenta* Crantz is a plant species used in the treatment of anaemia in Benin and Africa. The aim of this study was to valorise the flora of Benin by evaluating the antioxidant and antianemic activities of the aqueous macerate of *Manihot esculenta* Crantz leaves after phytochemical screening. **Methodology:** Phytochemical screening of the plant material was carried out by differential tube staining and precipitation reactions. Total phenolic compounds were determined by the Folin-Ciocalteu method, while the DPPH trapping test was used to assess antioxidant activity. The acute oral toxicity test was performed in vivo in Wistar rats at a dose of 2000 g/kg body weight. Biochemical parameters (serum creatinine, ASAT and ALAT transaminases) and haematological parameters (blood leukocytes) were used to assess this toxicity. *Manihot esculenta* Crantz, macerated in water, was administered by gavage to anaemic Wistar rats at a dose of 300 mg/kg body weight to assess its anti-anaemic activity. **Principals findings:** The leaves of *Manihot esculenta* Crantz contain gallic tannins, flavonoids, coumarins, mucilages, anthocyanins, triterpenes and leuco-anthocyanins. The presence of phenolic compounds was confirmed by the content of $10.70 \pm 0.082 \text{ mgEAG/gES}$ obtained after their determination by spectrophotometer. 3.54 mg/mL is the concentration of the aqueous macerate of *Manihot esculenta* leaves, which was able to scavenge 50% of the DPPH in solution, thus demonstrating its antioxidant potential. No deaths were observed and there were no significant changes in body weight, serum creatinine, transaminases (ALAT and ASAT) or blood leukocyte levels. The Lethal Dose 50 (LD_{50}) of *Manihot esculenta* leaves is greater than 2000g/kg body weight in rats when taken alone. Aqueous maceration of *Manihot esculenta* Crantz at a dose of 300 mg/kg was able to correct haemorrhage-induced anaemia from day 7, in contrast to untreated anaemic rats whose anaemia was compensated from day 12. **Conclusion:** The leaves of *Manihot esculenta* Crantz are a potential candidate in the search for bioactive molecules with antioxidant and anti-anaemic activities.

Keywords: Anemia, DPPH, toxicity, *Manihot esculenta*, Wistar rats

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1. Introduction

Anaemia is a major public health problem affecting mainly young children, pregnant and postpartum women, adolescents and menstruating women. It is characterised by a lower than normal number of red blood cells or haemoglobin in the red blood cells. Globally, anaemia is estimated to affect 40% of children aged 6 to 59 months, 37% of pregnant women and 30% of women aged 15 to 49 years. The main causes are nutritional iron deficiency, thalassaemia, sickle cell disease and malaria [1]. Africa and South-East Asia are the most affected regions, with high prevalence rates. Benin has not been spared, with 15.11% of deaths attributed to the disease between 2014

and 2018 [2]. In Africa, nearly 80% of the population uses traditional medicine to treat themselves. The use of traditional health systems is mainly due to their cultural, social and relative economic proximity, but also to equitable access to health care [3]. Among the many plant species used by the local population to treat anaemia are *Justicia secunda* [4], *Moringa oleifera* [5], *Sorghum bicolor* [6], *Cocos nucifera* [7], *Manihot esculenta*...

Manihot esculenta Crantz (Euphorbiaceae), more commonly known as cassava, is one of the most important staple crops in tropical and semi-arid regions. The main part of the plant is its starchy tuberous root, while the leaves are also used for various medicinal purposes: rheumatism, fever, headache, loss of appetite, diarrhoea, diabetes, heart disease, prostate problems, allergies, gastrointestinal problems and blood disorders, etc. [8,9]. In Benin, cassava leaves are

widely available after harvest but have almost no market value [10]. This study aims to valorise the flora of Benin by providing scientific evidence of the efficacy of *Manihot esculenta* in the treatment of anaemia by evaluating its anti-anaemic potential.

2. Materials

Plant material

Fresh *Manihot esculenta* leaves harvested in the centre of Benin, Collines department, Dassa-Zoumè commune. The plant material was dried for 14 days at room temperature. It was then ground into powder using an electric grinder.



Figure 1. Aerial part of *Manihot esculenta* Crantz

Animal Materials

Wistar albino rats aged 8-12 weeks with an average body weight of 145 g. They have been acclimatised to the normal conditions of animal husbandry.



Figure 2. Wistar albino rats

3. Methodology

Phytochemical screening

Secondary metabolites were identified by differential staining and precipitation reactions of the major chemical groups contained in the leaves of *Manihot esculenta* Crantz using the classical method [11,12,13]. Alkaloids,

catechic tannins, gallic tannins, flavonoids, anthocyanins, leuco-anthocyanins, quinone derivatives, saponosides, triterpenoids, steroids, cyanogenic derivatives, coumarins, reducing compounds, anthracene derivatives and heterosides were the metabolites studied in this study.

Extraction

Extraction was carried out by maceration, in accordance with the traditional use of *Manihot esculenta* leaves. Based on extraction techniques reported in the literature [13], 750 mL of distilled water was added to 75 g of powder. The filtrate obtained was evaporated to dryness at 40°C using a Rotavapor (Heidolph Laborota 4000 efficient) connected to a water cooler (Julabo FL 300). The dry extract obtained was weighed and the extraction yield was calculated using the following formula :

$$\text{Yield} = (\text{Mass of dry extract})/(\text{Mass of plant matter}) \times 100$$

Once the phenolic compounds had been identified in the qualitative test, they were assayed. 125 µL of sample was mixed with 625 µL of Folin-Ciocalteu reagent. After incubation for 5 minutes, 500 µL of sodium carbonate Na_2CO_3 was added to the reaction medium. The mixture was vortexed and incubated in the dark for 2 hours. The absorbance was read at 765 nm using a spectrophotometer. The content of phenolic compounds was derived from calibration ranges established with gallic acid (0-10 mg/ml) and is expressed as mg gallic acid equivalent (GA) per gram dry extract [14,15].

Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to assess antioxidant activity. It is based on the reduction of the DPPH free radical in the presence of a hydrogen donor. The absorbance was read at 517 nm [16]. The percentage of DPPH scavenging by extracts is calculated according to the following relationship

$$\text{PA} = [(\text{Ab} - \text{As})/\text{Ab}] \times 100$$

Where PA : Percentage of Absorbance ; Ab : Absorbance of the blank; As: Absorbance of the sample.

From the graph of the percentage reduction versus extract concentration, determine the IC_{50} , which corresponds to the concentration of extract that has trapped 50 % of the DPPH radical in solution. The test is carried out in duplicate.

Acute oral toxicity of an aqueous extract of *Manihot esculenta* leaves

The toxicity test was performed as recommended by the Organisation for Economic Co-operation and Development guideline 423 for the testing of chemicals [17] and according to the acute oral toxicity protocol described in the literature [18,19]. Two groups of three multiparous, non-pregnant female Wistar rats, aged 8 to 12 weeks and weighing an average of 140 g, were used. The first group consisted of control rats fed physiological water, while the second group was fed *Manihot esculenta* aqueous macerate. Physiological water and aqueous extract (2000 mg/kg body weight) were administered to the rats by gavage. The rats were observed for four hours after gavage and then daily for 14 days. At the beginning and end of the experiment, the rats were weighed and blood was collected by orbital puncture. Serum creatinine and transaminases (ASAT and ALAT) were measured to

assess kidney and liver function. Immune function was assessed by white blood cell count.

Anti-anaemic activity

Four groups of male and female Wistar rats, with an average body weight of 147 g, were used to evaluate the anti-anaemic activity of *Manihot esculenta* leaves macerated in water: a positive control group (anaemic and gavaged with physiological water); a negative control group (non-anaemic and gavaged with physiological water); a reference control group (anaemic and gavaged with Vitafer reference drug); and a test group (anaemic and gavaged with the test extract at a dose of 300 mg/kg body weight). All groups were fed from day 2 to day 15. Anaemia was induced in rats on day 0 by orbital puncture at a rate of 4 mL blood per rat of 150 g body weight. Blood was collected from each rat by tail vein puncture on days 0, 2, 7, 10 and 15 to measure haemoglobin levels and to assess the efficacy of the extract under investigation [18].

Statistical analysis

Graphs were generated using Graphpad software. Results are presented as mean $\pm$ two standard errors of the mean ($M \pm 2ESM$). Means were compared using the Mann-Whitney test for toxicity and Dunn's multiple comparison test for efficacy. The significance level was set at 5%.

4. Results and Discussion

Phytochemical screening

The table below shows the metabolites identified following phytochemical screening.

Table 1. Phytochemical screening of *Manihot esculenta* leaves

Metabolites	Results
Catechic tannins	-
Gallic tannins	+
Flavonoids	+
Anthocyanins	+
Leuco anthocyanins	+
Alkaloids	-
Reducing compounds	+
Mucilages	+
Saponosides	-
Cyanogenic derivatives	-
Triterpenes	+
Steroids	-
Coumarin	+
Quinone derivatives	-
Free anthracenics	-
C -heteroside	-
O -heteroside	-
Cardiotonic derivatives	-

+: present; -: absent

Table 1 shows that the leaves of *Manihot esculenta* contain gallic tannins, flavonoids, coumarins, mucilages, anthocyanins and leuco-anthocyanins. Quinone derivatives, cyanogenic derivatives and cardiotonic derivatives are absent. Mohammad's team also identified

phenolic compounds in the leaves of *Manihot esculenta* in 2018 [20]. Coumarins, triterpenes and phenolic compounds have been reported as bioactive compounds in *Manihot esculenta* [11]. The presence of these metabolites makes the plant material studied a potential candidate in the search for bioactive compounds with antioxidant activity and would justify its use by the population.

The extraction yield of the aqueous macerate of *Manihot esculenta* leaves was $11.2 \pm 0.06\%$. This shows the richness of the plant material in polar compounds.

The total content of phenolic compounds was 10.70 ± 0.082 mgEAG/g dry extract. This content demonstrates the effective presence of phenolic compounds in the leaves of *Manihot esculenta* and confirms the results of the phytochemical screening. The aqueous macerate is richer in total phenolic compounds than the hexanic (5.35 ± 0.66 mg GAE/g) and chloroformic (7.52 ± 0.09 mg GAE/g) extracts, according to the work of Mohammad and colleagues [20]. On the other hand, the content of 13.47 ± 0.56 mg GAE/g obtained with ethyl acetate is closer to the value obtained in this study [20]. This could be explained by the extraction capacity of the solvent, which depends, on the one hand, on its affinity with the phytochemicals and, on the other hand, on its polarity (Dah-Nouvlessounon *et al.*, 2015) [22].

Antioxidant activity

Expressed as 50% DPPH inhibitory concentration (IC_{50}). 3.54 mg/mL is the concentration of *Manihot esculenta* aqueous extract that was able to scavenge 50% of the DPPH in solution. This IC_{50} is close to that of ascorbic acid (reference antioxidant), which was 3.22 mg/mL in the present study. It is close to that obtained by Al-Rofaai's team (2.638 mg/mL) [23]. This result provides evidence that *Manihot esculenta* leaves contain bioactive molecules capable of scavenging DPPH. Their consumption is therefore recommended to slow down the effect of oxidants on the body. The antioxidant activity of ethyl acetate (0.19 mg/mL), chloroform (1.39 mg/mL) and hexane (1.74 mg/mL) extracts is higher than that observed in this study [21]. A large body of research has demonstrated the biological activity of many secondary metabolites. Phenolic compounds are recognised as potent antioxidants with the ability to prevent and repair damage caused by free radicals. The anti-free radical activity observed in the present study would be due, on the one hand, to the presence of phenolic compounds (identified by phytochemical screening and confirmed by spectrophotometric assay) in the plant material studied, through their ability to reduce and decolorise the DPPH radical by releasing protons to it, but also to the synergistic effect of the different metabolites highlighted in *Manihot esculenta* [24,25,26].

Acute oral toxicity

The results of the acute oral toxicity test on the aqueous macerate of *Manihot esculenta* leaves are presented in the following table.

Comparison of the values obtained on Day 0 and Day 14 showed no significant difference in body weight, creatinemia, transaminases or leukocyte counts. Thus, there was no disturbance in the body condition, renal,

hepatic or immune functions of the rats. These results indicate the absence of acute oral toxicity when cassava leaves are administered as a single dose of 2000 mg/kg body weight. This implies that the LD₅₀ is greater than 2000 mg/kg body weight. These results show that the aqueous extract of *Manihot esculenta* is non-toxic to the liver and kidney, justifying its use in the pharmacopoeia and in cooking. The same observation was made by Al-Rofaai's team, who found that the LD₅₀ was greater than 5000 mg/kg body weight [23].

Table 2. Physical, biochemical and haematological results of the acute oral toxicity test on extracts of *Manihot esculenta*

Parameters	Day 0	Day 14	P value	Statistical difference
Weight (g)	140 ± 30	183 ± 25	0.2	Not significant
Serum creatinine (mg/L)	12 ± 2	14 ± 2	0.4	Not significant
Transaminases ASAT (U/l)	76 ± 14	33 ± 10	0.1	Not significant
Transaminases ALAT (U/l)	48 ± 9	20 ± 5	0.1	Not significant
Leukocytes	8.6 ± 1.6	7.4 ± 2.4	0.4	Not significant

Anti-anaemic activity of *Manihot esculenta* leaf extract

Figure 3 shows the evolution of haemoglobin levels before and after induction of anaemia in rats.

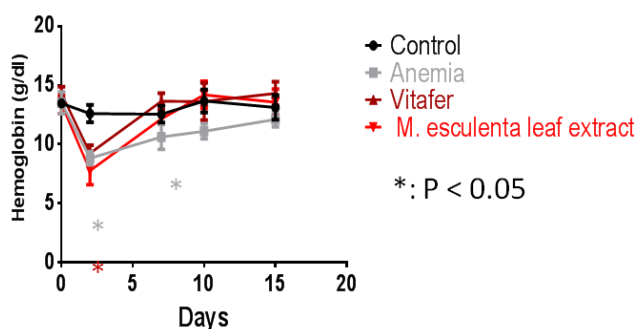


Figure 3. Evolution of haemoglobin levels in groups of rats

At Day 0, haemoglobin levels ranged from 13.4 ± 0.7 to 14.3 ± 0.5 g/dl in the different groups of rats. It decreased significantly on day 2, from 7.8 ± 1.0 to 9.2 ± 0.6 g/dl, following the haemorrhage that characterises anaemia in the rats. The haemoglobin level then increased progressively in the various anaemic groups and was no longer significantly different from its Day 0 value from Day 7 in the groups treated with *Manihot esculenta* extract or Vitafer, indicating compensation for the anaemia. In the untreated anaemic group, the anaemia was not compensated until Day 12. The metabolites present in *Manihot esculenta* leaves are responsible for the observed anti-anaemic potential. These results are similar to those of Senou *et al*, (2016), who studied the anti-anaemic activity of *Sorghom bicolor* [6], as well as Agbogba *et al*, (2019), on the anti-anaemic activity of *Psorospermum febrifugum* [18].

5. Conclusion

This study evaluated the acute toxicity, anti-radical and

anti-anaemic activities of the aqueous macerate of *Manihot esculenta* leaves after phytochemical screening. Several secondary metabolites such as gallic tannins, flavonoids, coumarins, mucilages, anthocyanins, leuco-anthocyanins and reducing compounds were identified. They don't contain toxic compounds (cyanogenic derivatives, quinone derivatives) and have good antioxidant properties. They aren't toxic when taken orally as a single dose. The evaluation of the anti-anaemic activity showed satisfactory results, comparable to those of Vitafer, which is sold in pharmacies and used in medicine to treat anaemia. What if eating manioc leaves prevented anaemia and the whole range of diseases caused by oxidants? We'll have to rethink our culinary art.

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