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PHYSICOCHEMICAL CHARACTERISTICS, ANTI-NFLAMATORY PROPERTIES AND MINERAL CONTENT OF INTEREST POST STROKE OF *FICUS MUCUSO* WELW. EX FICALHO (MORACEAE)

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Keywords:

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ABSTRACT: *Ficus mucuso* Welw. ex Ficalho (Moraceae) is a plant used to treat the after-effects of strokes in Côte d'Ivoire. The aim of the present work is to characterize the physicochemical properties of the stem bark of this plant drug, evaluate its anti-inflammatory properties and determine the presence of minerals of interest in post-stroke treatment. Minerals play a crucial role in stroke prevention and management. Physicochemical tests revealed that *Ficus mucuso* stem bark has a pH of 5.81, a fairly low average water content (7.98%), a relatively high average dry matter content (92.02%), an ash content of 9.21%, and is rich in iron, potassium, magnesium and calcium, important minerals in stroke prevention and management. EAq has the highest concentrations of Potassium (160±11.3 mg/L) and Calcium (11±1.05 mg/L), while EEt has the highest concentrations of Magnesium (2.3 ± 0.12 mg/L) and Iron (1.38 ± 0.04 mg/L). *Ficus mucuso* stem ecore extract also has anti-inflammatory activity. Aqueous and ethanolic extracts of *Ficus mucuso* stem bark induce inhibition of protein denaturation at concentrations 0.097 and ≥ 0.39 mg/mL at 100%. *Ficus mucuso* should be integrated into the process of establishing an Improved Traditional Medicine for the care of populations in the prevention of stroke and its treatment and the reduction of the risk of recurrence.

INTRODUCTION: Minerals play a fundamental role in maintaining good health. Elements such as calcium, magnesium, potassium, iron and zinc are essential to the proper functioning of the body [1]. They contribute to strong bones, muscle and nerve function, energy production and a strong immune system. Minerals play a crucial role in stroke prevention and management [2, 3]. Among them, magnesium stands out for its neuroprotective effect, reducing brain damage by regulating calcium influx into neurons and limiting excitotoxicity, a key mechanism of post-stroke damage. According to recent studies [4], magnesium supplementation with anti-hypertensive treatment has a beneficial effect, and therefore a protective role against the onset of ischemic stroke.

Potassium helps regulate blood pressure, thereby reducing the risk of recurrence [5]. Iron plays an essential role in many physiological processes, including erythropoiesis, immunity and oxidative metabolism. Iron deficiency and excess have been associated with stroke risk in observational studies.

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Iron deficiency and anemia are common comorbidities in elderly patients with cardiovascular disease and have a significant impact on muscle function and physical performance. They lead to reduced strength, early fatigue, poor quality of life, prolonged hospitalization and impaired survival. Anti-inflammatory vitamins and minerals are needed to reduce the development of fatty deposits in the vessel, prevent the risk of recurrence and improve the health of the nervous system. An observational study of early-rehabilitation ischemic and hemorrhagic stroke patients in a hospital setting demonstrated that iron deficiency and anemia were independently associated with lower functional capacity after acute stroke and poor functional outcomes after rehabilitation [6]. Magnesium is an essential mineral and cofactor for over 300 enzymatic reactions in the body. High dietary intakes of each of these minerals have been reported to reduce the risk of hypertension, which is the main risk factor for modifiable stroke [7]. Plants are important sources of minerals that are very useful in phytotherapy and could be so in the treatment of stroke sequelae. In Côte d'Ivoire, many plants and improved traditional medicines are used to treat the after-effects. The aim of this study is to carry out a physicochemical characterization, evaluate the anti-inflammatory properties of *Ficus mucuso* stem bark and determine the presence of minerals of useful interest in post-stroke treatment.

MATERIAL AND METHODS:

Extraction of Aqueous and Ethanolic Extracts: The work was carried out from March to May 2024.Stem bark of *Ficus mucuso* Welw. ex Ficalho (Moraceae) was used as plant material. They were harvested, cleaned with water and air-dried, protected from light, at 25°C for three weeks. They were then crushed and ground to a fine powder using a grinder. Aqueous extraction was carried out according to the method of Zihiri *et al*.[8]. 100 g of bark powder was added to 2 L of distilled water, boiled for 45 min. The decoctate was filtered successively through a square of sterile cloth, onto cotton and onto a fold of filter paper (Whatman® 2 mm paper). The filtrate was dried for 24 h in an oven at 60°C to obtain a dry aqueous extract. The modified method of Olakunle *et al*. [9] was used for ethanolic extraction. 100 g of powder was macerated in 1.5 L of 70% ethanol, then homogenized under magnetic stirring for 72 h. The filtrate obtained by the same procedure was dried in an oven at 40°C for 24 h. The different extraction yields were calculated in relation to the initial mass (100g) of *Ficus mucuso* bark powder for three trials. They were determined using the following formula [10]:

$$R\ (\%) = (m/M) \times 100$$

R: yield, m: mass of extract after solvent evaporation in (g), M: mass of starting plant material in (g).

Physico-chemical Characterization:

Measurement of pH, Moisture Content, Dry Matter and Ash Content: The physico-chemical characteristics were carried out in accordance with the standards of the Association Française de Normalisation (2000). pH was determined by direct measurement using a pH meter. Moisture content (H%) was determined using the oven-drying process, which consists in drying a precise mass of fresh sample at 105°C until a stable weight is obtained. The moisture content (H%) is calculated using the following formula:

$$H\ \% = (Pf - Ps) / Pf \times 100$$

With: Pf: fresh sample weight (g), Ps: dry sample weight (g). Dry matter (Ms%) is what is obtained when water is removed from a product.

$$MS\% = 100\% - H\%$$

Total ash was determined by incinerati²ng a sample of *Ficus mucuso* crude extract at 550°C in an electrically heated muffle furnace to virtually constant mass. The percentage by mass of crude ash is expressed by the following formula:

$$\text{Ash content } \% = [(M1-M2) / (M1-M0)] \times 100$$

M0: mass in grams of incineration capsule, M1 and M2: mass in grams of incineration capsule loaded with test sample and ash respectively. All tests were performed in triplicate to obtain an average value.

Quantification of Minerals of Interest: Chemical composition was analyzed using a HACH DR3900 VIS bench-top spectrophotometer. The minerals of interest were Calcium (Ca), Potassium (K), Magnesium (Mg) and Iron (Fe). 1g of each extract

was diluted in 100 mL of distilled water, then the samples were directly introduced into a sample holder ranging from 5 mL to 10 mL, highly transparent to X-rays. Depending on the minerals to be analyzed, specific reagents (EGTA, ferrover, hach 1432198, 1432298, 1432399) were added to the solution for a specific waiting time. The wavelengths corresponding to each mineral are programmed on the spectrophotometer before the samples are measured.

Study of Anti-inflammatory Activity: The anti-inflammatory activity of a substance can be assessed by measuring its ability to inhibit the thermal denaturation of ovalbumin *in-vitro* (%I). The test is based on the controlled destabilization of ovalbumin under the effect of heat 11. The reaction mixture is prepared from 0.2 mL egg albumin, 2.8 mL phosphate-buffered saline [PBS: 137 mMNaCl (8.0 g/L), 2.7 mMKCl (0.2 g/L), 10 mM Na2 HPO4 (1.44 g/L), 1.76 mM KH2 PO4 (0.24 g/L); pH 6.8] and 2 mL distilled water or extract at different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.12 mg/mL, 1.56 mg/mL, 0.78 mg/mL, 0.39 mg/mL, 0.195 mg/mL and 0.097 mg/mL). The mixture was then incubated for 15 minutes at 37°C and heated for 5 minutes at 70°C. Absorbance is measured at absorption wavelengths using a UV-visible spectrophotometer at 630 nm, after cooling. Diclofenac sodium (10 mg/mL and 1 mg/mL) is the positive control, while distilled water is the negative control.

The assay is performed in triplicate and data are presented as mean ± standard deviation. The rate of inhibition of thermal denaturation of ovalbumin is calculated from the relationship:

$$\% \text{ inhibition} = [(\text{Absorbance of extract} / \text{Absorbance of control}) - 1] \times 100$$

When %I<0 (Hyperchromic effect), the product is said to be inactive; when 0<%I<46.7±19.1, the product is weakly active; when 46.7±19.1<%I<75, the product is active and if 75<%I<100, it is very active.

Statistical Processing: Data were processed using Excel 2019 statistical software. Bioassay results were expressed as mean ± SD.

RESULTS:

Yields and Physico-Chemical Characteristics of the Different Extracts: The yield of *Ficus mucuso* stem bark extracted from ethanol is slightly higher (19.9%) than that from distilled water, which is around 19.1%. Both extracts have a powdery appearance. The ethanolic extract (EEt) is dark brown in color, while the aqueous extract (EAq) is light brown **Table 1, Fig. 1**. The tests show a Ph of 5.81, a fairly low average water content (7.98%), a relatively high average dry matter content (92.02%), and an ash content of 9.21%. These contents are inversely proportional and ensure greater plant stability and consequently longer storage life.

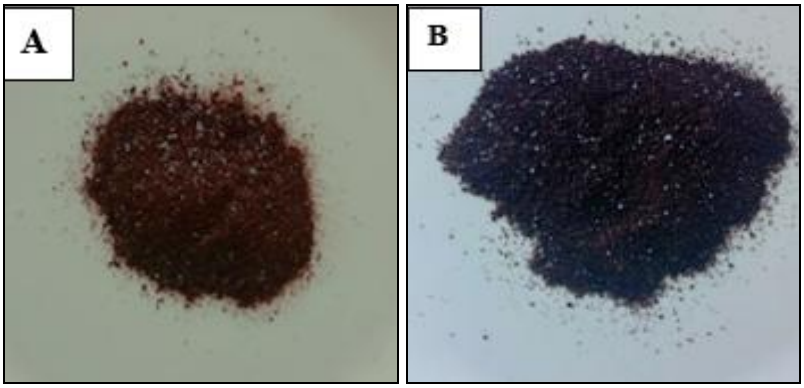

FIG. 1: POWDER OF *FICUS MUCUSO* AQUEOUS AND ETHANOLIC EXTRACTS AFTER EXTRACTION (A: AQUEOUS EXTRACT; B: ETHANOLIC EXTRACT)

TABLE 1: YIELD, COLOR AND ASPECT OF AQUEOUS AND ETHANOLIC EXTRACTS OF *FICUS MUCUSO*

Extracts	Weight obtained (g)	Yield (%)	Color	Aspects
EAq	19,1 ± 047	19,1	Brown	Powdery
EEt	19,9 ± 056	19,9	Dark brown	Powdery

EAq: Aqueous extract; EEt: Ethanolic extract.

Minerals of Interest: The element scan determined the presence and levels of potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) in the plant extracts analyzed. These levels are presented in **Table 2.** EAq has the highest concentrations of potassium (160 mg/L) and calcium (11 mg/L), while EEt has the highest concentrations of magnesium (2.3 mg/L) and iron (1.38 mg/L). EAq has 160 mg/L of Potassium, compared with 13 mg/L for EEt, a 12-fold increase. Calcium in EAq is 10 times higher than in EEt. In contrast to the previous two minerals, the concentration of Magnesium in EEt is three times higher than in EAq.

TABLE 2: MINERAL COMPOSITION OF *FICUS MUCUSO* STEM ECORCE POWDER

Extracts	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Iron (mg/L)
EAq	160 ± 11,3	11 ± 1,05	0,6 ± 0,03	0,984 ± 0,08
EEt	13 ± 0,75	0,1 ± 0,07	2,3 ± 0,12	1,38 ± 0,04

EAq: Aqueous extract EEt: Ethanolic extract.

Anti-inflammatory Activity: The results of the *in-vitro* anti-inflammatory test on the aqueous and ethanolic extracts of *Ficus mucuso* are shown in **Table 3.** The table shows the percentage inhibition of protein denaturation in egg white. Diclofenac sodium at 0.1 mg/mL is weakly active (30.59%), while diclofenac sodium at 1 mg/mL is highly active (102.98%). Inhibition percentages for aqueous extracts at 3.12 mg/mL and ethanolic extracts with concentrations ranging from 0.39 to 3.12 mg/mL were over 100%. EEt at 0.195 mg/mL was active and that at 0.097 mg/mL was weakly active, while EAq was highly active at 0.097 mg/mL, active at concentrations of 0.195 and 0.78 mg/mL and weakly active at 0.39 mg/mL. This study shows that aqueous and ethanolic extracts of *Ficus mucuso* stem bark are anti-inflammatory at concentrations of 0.097 and ≥ 0.39 mg/mL respectively **Fig. 2.**

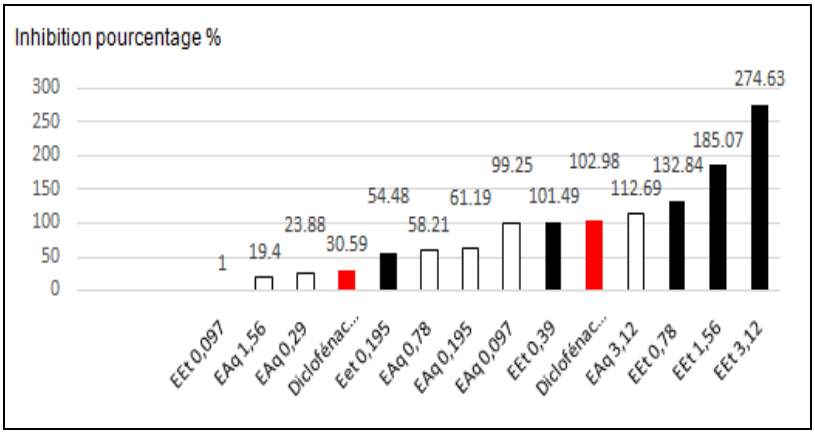

FIG. 2: PERCENTAGE INHIBITION OF AQUEOUS, ETHANOLIC AND DICLOFENAC EXTRACTS AT DIFFERENT CONCENTRATIONS. %I<0 (Hyperchromic effect), inactive product; 0<%I<46,7±19,1 weakly active product; 46,7±19,1<%I<75, active product and if 75<%I<100, very active product; EAqFm: aqueous extract of *Ficus mucuso*. EEtFm: ethanolic extract of Fm: *Ficus mucuso* n=3, m ± esm; T =Temoin Distilled water.

DISCUSSION: The data obtained show that the yield of extraction of the crude extract of *Ficus mucuso* stem bark from ethanol is slightly higher (19.9%) than that from distilled water, which is around 19.1%. This may be explained by the fact that ethanol better concentrates the active compounds present in our plant. Variations in extraction yields can be attributed to the different solubility of compounds in extraction solvents [12]. In addition to the extraction method, other factors can influence compound extraction efficiency. These include the nature and volume of the solvent used, pH, temperature, extraction time and sample composition, sample collection, etc. [13]. Mineral quantification, on the other hand, shows that the aqueous extract has the highest concentrations of potassium (160 mg/L) and calcium (11mg/L), while the EEt has the highest concentrations of magnesium (2.3 mg/L) and iron (1.38 mg/L). These results could guide the choice of extraction solvent in further studies or in the development of various treatments for stroke victims. The aqueous extract

is very rich in potassium, an important mineral compound in the treatment and recurrence of stroke [1, 5]. High potassium intake is associated with a lower risk of stroke in women [2]. Ethanolic extract is rich in magnesium. In scientific studies [3], magnesium intake was associated with a reduced risk of stroke. *Ficus mucuso* stem bark powder has a relatively low water content (7.98%) and a high dry matter content (92.02%). These contents are inversely proportional, giving the plant greater stability and hence longer storage life. This justifies the fact that the plant from Mali and Burkina is of good quality on our markets. This study also shows that *Ficus mucuso* stem extract also has anti-inflammatory activity. Aqueous and ethanolic extracts of *Ficus mucuso* stem bark induce inhibition of protein denaturation at concentrations 0.097 and ≥ 0.39 mg/mL at 100%. Thus, the transformation of fibrinogen into fibrin and of the various factors involved in thrombolytic and anticoagulant effects could be explained by possible denaturation. These results corroborate those of Kumari [11], who showed that *Rhizophora mucronata* leaves 400 mg dose-dependently inhibited egg protein denaturation by 97.56%. In the case of stroke, in addition to the damage to the brain, the immune system itself causes further damage after the blood supply has been restored. Brain cells undergo stress, leading to an inflammatory reaction. This has a negative effect on brain tissue [14]. *F. mucuso* extract could provide a means of containing this inflammation, thereby minimizing its long-term sequelae.

CONCLUSION: The aim of the present work was to characterize the physicochemical properties of *Ficus mucuso* stem bark, to determine its anti-inflammatory properties and the content of minerals of interest in post-stroke treatment. Physicochemical tests revealed that *Ficus mucuso* stem bark has a pH of 5.81, a fairly low average water content (7.98%), a relatively high average dry matter content (92.02%), an ash content of 9.21%, and is rich in iron, potassium, magnesium and calcium, important minerals in stroke prevention and management. *Ficus mucuso* stem bark extract also has anti-inflammatory activity. Aqueous and ethanolic extracts of *Ficus mucuso* stem bark were found to induce inhibition of protein denaturation at concentrations 0.097 and ≥ 0.39 mg/mL at 100%. *Ficus mucuso* should be integrated into the process of establishing an Improved Traditional Medicine for the care of populations in the prevention of stroke and its treatment and the reduction of the risk of recurrence.

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