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Nutritional Composition and Clinical Tolerance of a Food Plant in Female Wistar Rats: The Case of *Moringa oleifera* Species

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Abstract: *Moringa oleifera* L. is a plant used throughout the world in many fields, including agriculture, health and nutrition. *Moringa oleifera* leaves are consumed throughout West Africa, particularly in Côte d'Ivoire by vulnerable populations. With a view to enhancing the value of the *Moringa oleifera* plant, a contribution to nutritional composition and clinical tolerance in rats force-fed an aqueous extract of this plant was studied. The study was carried out experimentally on 12 adult female Albino Wistar rats divided into two (2) batches of six (6) rats. The first batch received distilled water by gavage (twice a day for 4 days), while the second received the aqueous extract of *Moringa oleifera* at 0.2 g/mL by gavage. Nutritional composition indicated protein, lipid, carbohydrate, ash and dietary fiber contents of 26.12±0.18%; 6.8±0.13%; 14.37±0.26%; 9.5±0.67% and 43.2±0.519% respectively. Acidity and pH values were also 1.1±0.0 meq/100 g and 5.3±0.00 respectively. After observation, all animals survived for the duration of the experiment and showed no adverse clinical signs. This shows that the aqueous extract of *Moringa oleifera* is not only rich in nutrients, but is also clinically tolerated by rats. These results open up interesting prospects for the use of the *Moringa oleifera* plant in the form of an alicament that could be an effective means of combating public health diseases in breastfeeding women.

Keywords: *Moringa oleifera*, Clinical Signs, Female Rats, Nutritional Composition

1. Introduction

Leafy vegetables are generally used as a means of combating public health problems such as non-communicable diseases and malnutrition. Indeed, the WHO recommends regular consumption of culinary preparations based on leafy vegetables. *Morinaga oleifera*, a leafy vegetable in the Moringaceae family, is a species cultivated in India and Sri Lanka, and is now acclimatized in almost all tropical regions. It is a small, fast-growing melliferous tree and behaves like an invasive plant **(Parotta and John, 2013)**, resisting drought. Many humanitarian programs use *Moringa oleifera* leaves as a natural means of combating malnutrition in infants and nursing mothers **(Kumar, 2004)**. According to Indian tradition, *moringa* leaves can cure over 300 diseases. As a result, the dry leaves with their well-balanced nutritional profile are used in numerous culinary preparations and applications around the world **(Atakpama *et al*,. 2023)**. To make the most of their benefits, *Moringa oleifera* leaves are used in powder form. *Moringa* powder is packed with nutrients such as vitamins A and E, minerals and proteins. In addition to its nutritional potential, *Moringa* (leaf) has demonstrated health benefits. In phytotherapy, *Moringa oleifera* leaf powder is indicated for stimulating the immune system, reducing fatigue and lowering blood pressure **(Mughal, 1999; Ministère de la santé du Congo, 2009)**. It is also a plant for combating type 2 diabetes, overweight and obesity **(Kouakou *et al*., 2020)**. The tree is an important source of food in developing countries, where poor nutrition is a major concern. In the Western world, the dried leaves are sold as a dietary supplement in powder or capsule form **(Torrens, 2022)**. The *Moringa oleifera* species can be used to combat malnutrition and malaria **(Tété-Bénissan *et al*., 2012; Atakpama *et al*., 2023)**. The *Moringa oleifera* species, in addition to its effect on the nutritional quality of milk, also acts on the quantity of milk produced in lactating women **(Ann and Eli, 2021)**. As a result, studies on the physicochemical and biochemical parameters that underpin its many therapeutic uses deserve to be sought. Ignorance of the toxicological properties of plants used in traditional medicine also exposes people to the real risks of therapeutic accidents **(Maiga *et al*., 2005)**. This oral use could lead to intoxication, hence the interest in conducting a study of clinical tolerance in rats when taking aqueous extracts of the *Moringa oleifera* plant orally. Our work aims to achieve this goal through a nutritional composition and clinical tolerance test. This study is being carried out with a view to developing *Moringa oleifera* as an alicament to combat public health diseases in nursing mothers.

2. Materials and methods

Harvesting the plant

Moringa oleifera plant material was harvested from spontaneous flora at the Bingerville site (southern Côte d'Ivoire) from March to May, the period and site when the *Moringa oleifera* plant is available. It was then dried and processed for aqueous extraction for animal experimentation.

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Determination of physicochemical and biochemical parameters

Moisture content was determined in an oven (MEMMERT 854 SCHWABACHW, Germany) using the **AOAC method (1975)**. Ash content was obtained by weighing the residue of the sample incinerated at 550°C **(AOAC, 1990)**. *Moringa oleifera* protein content was estimated using the Kjeldahl method **(AOAC, 1990)**. Total lipids were extracted using the SOXHLET method **(AOAC, 1990)**. Total carbohydrates and energy value were determined using the calculation method recommended by **FAO (2002)**. Dietary fiber was determined using the **AOAC (1990)** method. Titratable acidity and pH were determined according to the method described by **AOAC (1990)**.

Aqueous extraction of Moringa oleifera plant

Extraction was based on the solubilization of dietary fibers with distilled water **(Adepo, 2013)**. Ground plants were subjected to aqueous extraction, with a quantity of 100 g of plant material for 1.5 L of distilled water. The whole was heated at 80°C for 45 min. The homogenate, cooled for 5 min, was filtered through a small-mesh poplin cloth. To the residue obtained, 1 L of distilled water was added. The whole was heated at 80° C for 30 min and then filtered. The total filtrate constituted the aqueous extract. It was freeze-dried to obtain the dry aqueous extract.

Animal experimentation

Laboratory animals

The experiments were conducted on twelve (12) adult female rats of the Albino Wistar strain. Their average weight was 255 ± 5 grams, and they were divided into two (2) homogeneous batches of 6 rats in metabolic cages. During this period, water and pellets were served. After this period, the rats were weighed again and divided into two homogeneous batches.

Experimentation itself.

The experiment lasted four days during the test period. The two (2) batches of rats, L1 and L2, received by gavage, twice a day, respectively 1 gram of freeze-dried aqueous extract of *Moringa oleifera* dissolved in 5 milliliters of distilled water and 5 milliliters of distilled water, which constituted the negative control for four (4) days. Force-feeding took place at 8 a.m. in the morning and at 4 p.m. in the evening. During the experimental phase, parameters related to clinical signs in female rats were evaluated: survival, variation in body mass, variation in color of sense organs, stool, urine and sleep disturbances.

Statistical analysis

All analyses were performed in triplicate. Nutritional composition and serum parameters were analyzed by descriptive analysis using Statistica version 7.1 software.

3. Results and discussion

Table 1: Nutritional composition of *Moringa oleifera* species

Parameters	Average content
Moisture (g/100g F M)	70.5±1
Dry matter (%)	29.5
Ash (g/100g D M)	9.5±0.67
Carbohydrates (g/100g D M)	14.37±0.26
Protein (g/100g D M)	26.12±0.18
Lipids (g/100g D M)	6.8±0.13
Dietary fiber (g/100g D M)	43.2±0.51
Energy value (kcal/100g)	171.96±4.29
pH value	5.3±0.00
Acidity (meq/100 g)	1.1±0.00

F M: fresh matter
D M: dry matter

Table 2: Clinical safety parameters studied

Parameters related to clinical signs	Answers
Survival	-
Body mass	-
Color of sense organs	-
Skin reaction	-
Sleep disorders	-
Stool and urine color and texture	-

- There is no variation for the parameter concerned

Biochemical analysis reveals that fresh *Moringa oleifera* leaves contain a high water content. However, the recorded content remains lower than those of **Ndong *et al.* (2007)**, who found values of over 73% for fresh *Moringa oleifera* products (leaves, flowers and pods). Moisture content is an important parameter in food preservation. Thus, the relatively high moisture content of the fresh *Moringa oleifera* leaves analyzed (70.5±1%) would be a disadvantage for preservation, as the shelf life of a product is closely related to its water content **(Atalar and Dervisoglu, 2015)**. Among the elements making up the physicochemical parameters is pH. The pH determined (5.3) is lower than that found by **Bardi (2015)**, who measured a value of 7.10. Nevertheless, this pH of 5.3 can be considered as a biological pH indicating that any decoction from *Moringa oleifera* species can be consumed orally without irritation linked to the acid factor related to *Moringa oleifera* based culinary preparations, as the pH tolerated by the body is 5 to 9 **(Silvia, 2012)**. The high protein content (26.12±0.8%) recorded is relatively identical to that found by **Bardi (2015)** who, according to his results, obtained a content of 25%. The high protein content was reported according to the study results (39.69±0.01%) of **Barichella *et al.* (2018)**.

Nevertheless, this lower protein content (26.123±0.8%) compared with the value of (39.69 ± 0.01%) would be justified by a variable concentration of mineral nitrogen in the soil at harvesting sites **(CNRA, 2008)**. This high content has led to the species being used in a number of research studies. For example, the *Moringa* species is used in the fight against malnutrition **(Rouamba, 2013)** due to the significant presence of proteins and mineral elements **(Atchibri *et al.*, 2012)**. The minerals contained in ash are important for the proper functioning of the body. The ash content of *Moringa oleifera* leaf samples was 9.5±0.67%. *Moringa oleifera* could therefore be considered an important source of nutritional intake, especially minerals. In general, minerals are the basis for stimulating the action of the enzymes involved in prolactin secretion, since minerals are the enzymes' metal cofactors. *Moringa oleifera* could provide a means of combating mineral deficiencies. Among food plants, according to the literary review, leafy vegetables are rich in water, minerals, vitamins and dietary fiber. They have a low energy value, thanks to their high fiber content and low lipid concentration. Due to their nutritional qualities especially in dietary fiber, leafy vegetables should be present at every meal because they can be used in the fight against digestion-related pathologies and non-communicable diseases such as hypertension, type 2 diabetes, overweight and obesity **(National Nutrition Program, 2017)**. Dietary fiber has an impact on reducing obesity and in weight reduction **(Gruffat, 2018)**. In fact, dietary fibers possess the satietogenic effect i.e. they have a satiating effect ideal for weight regulation. *Moringa oleifera* is rich in dietary fiber, estimated at 43.2±0.51%. These results are in line with those of authors who consider that leafy vegetables are generally rich in dietary fiber. Dietary fibres are non-digestible carbohydrate residues and are present in high proportions in *Moringa oleifera* species. Consumption of the fiber-rich *Moringa oleifera* species would be significantly beneficial to health. Made up of insoluble and soluble fibers, dietary fibers contribute to healthy digestion by increasing intestinal transit. It also helps combat cardiovascular disease by reducing bad cholesterol levels. It also helps combat type 2 diabetes by significantly reducing intestinal sugar absorption **(Haroun, 2017)**. *Moringa* is one of the less expensive annual leafy vegetables with a health value that is easily found among local products in Côte d'Ivoire. This study shows that *Moringa* leaves can contribute to the body's well-being by providing essential nutrients. The nutritional richness of the *Moringa* plant has led to its use in a number of oral administration studies relating to early childhood performance in both urban and rural environments. Nevertheless, a clinical tolerance study is important, given the possible presence of toxicity in wild plants **(Maïga *et al.*, 2005)** and in food. Indeed, *Moringa oleifera* is a wild plant **(Adjatin, 2006)**, hence the need for a tolerance study. Clinical signs are a medical examination by sight or touch, or visible manifestations noted during a medical examination. The life parameter is the first tool used to assess the tolerance test **(Ouattara *et al.*, 2014)**. In the study conducted, all test animals survived 1 gram of the product. Also, the study on the parameter related to body mass did not vary. Indeed, the body mass of female rats given the aqueous extract and those given distilled water was not significantly different. This second parameter also makes it possible to assess clinical tolerance to the aqueous extract of the *Moringa oleifera* plant. It is an important indicator for toxicity studies **(Senou *et al.*, 2017)**. Faeces analysis did not reveal any significant difference over the duration of the animal experiment. Insomnia and redness of the sensory organs, symptomatic disorders of clinical observation in rats, were absent throughout the experiment. These results are in line with those of previous studies described in the literature, indicating that there is no evidence of food allergy with the *Moreinga oleifera* plant species **(Omarjee and Grégoire-Krikorian, 2017)**. In fact, although widely prescribed in traditional medicine and in many countries, *Moringa oleifera* has been the subject of a number of studies, both in terms of its acute and chronic toxicity on laboratory animals, and its good tolerance in humans. In addition, one study indicates that *Moringa oleifera* is widely consumed as a food source, and oral administration of dried *Moringa oleifera* leaf powder up to 2000 mg/kg resulted in no change in clinical signs or pathology **(Moodley, 2017)**.

4. Conclusion

The results of the nutritional composition study show that the contents recorded are significant in terms of protein, carbohydrates, ash and dietary fiber. The pH and acidity values also constitute biological data. These results show that the *Moringa oleifera* plant has a relatively high nutritional value and could be used to combat protein-energy malnutrition, with clinical tolerance.

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