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Galactogogue Effects of Two Annual Leafy Vegetables *Euphorbia Hirta* and *Manihot Esculenta* in Adult Female Wistar Rats

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Abstract There are very few solutions available to combat galloping hypogalactia. The aim of this study was to evaluate the galactogogue effect of *Euphorbia hirta* and *Manihot esculenta* in adult female Wistar rats with a view to using them as lactogenic plants. The nutrient content of these leafy vegetables and the plasma concentration of prolactin in treated female rats were calculated using standard methods. The results recorded for *Euphorbia hirta* and *Manihot esculenta* were respectively: protein (14.41±0.02%; 26.12±0.18%), carbohydrate (35.43±5.30%; 14.37±1.3%), lipid (3.8±0.13%; 6.8±0.13%), dietary fibre (37.16±1.55%; 43.2±0.51%), ash (9.2 ± 0.13%; 6.2± 0.11%), polyphenols (368.66±50.75 mg EAG/ g DM; 375.5±4.78 mg EAG/ g DM), flavonoids (290.66±48.36 mg EQ/ g DM; 283 78± 3.9 mgEQ/ g DM). Furthermore, plasma prolactin concentrations were similar in female rats treated with dry aqueous extracts of *Euphorbia hirta* (15.22± 0.67 ng/mL), *Manihot esculenta* (12.52 ± 0.57 ng/mL) and galactogil (15.15 ± 0.07 ng/mL), however, the said plasma prolactin concentrations recorded were significantly different from those of control female rats treated with distilled water (5.18 ± 0.78) ng/mL. This suggests the presence of a lactogenic active ingredient in *Euphorbia hirta* and *Manihot esculenta*. These two leafy vegetables could help improve milk production in nursing mothers. However, further studies are needed to assess the lactogenic power of the leafy vegetables studied in combination.

Keywords: *Nutrients, Euphorbia hirta, Manihot esculenta, prolactin*

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1. Introduction

Malnutrition can be defined as an excess or insufficient intake of one or more nutrients [1]. According to the WHO and UNICEF, child malnutrition accounts for 30% to 50% of deaths among children under the age of 5 worldwide. It is linked to qualitative and quantitative deficiencies in the diet of young children. This pathology leads to irremediable delays in physical and cognitive development, particularly when it occurs during gestation and during the first two years of life. While there are many reasons for early cessation of breastfeeding, the one most frequently cited, which accounts for almost a third of early weanings, is the mother's perception that she cannot produce enough milk to allow her baby to grow optimally [2]. This problem is complex and may be due to biological causes such as stress, fatigue, Caesarean delivery, prematurity or multiple births, or to a lack of confidence in the mother's ability to breastfeed or difficulties in managing breastfeeding [3].

In the face of infant malnutrition, the World Health Organisation (WHO) recommends exclusive breastfeeding for up to six months because of the recognised long-term benefits for the infant. However, the prevalence of breastfeeding remains low worldwide, particularly in high-income countries such as France, the United Kingdom and the United States, where less than 20% of mothers still exclusively breastfeed their infants for 6 months [4,5].

Advice from a healthcare professional, support from family and friends, and a suitable environment are therefore alternatives to the problems of insufficient milk production. However, despite these precautions, the problem may persist and taking galactogenic compounds, i.e. those that stimulate milk production, is one way of helping mothers who are having real difficulties breastfeeding [3].

In addition, during the first six months of life, infants

who are not breastfed have a mortality risk 14 times higher than those who are exclusively breastfed [6], the main cause of which is insufficient milk production [7]. In addition, breast milk is associated with better performance in intelligence and cognitive development tests. It improves intelligence from birth to adulthood [8]. It also reduces the risk of overweight, obesity and diabetes in children and later in adults [9]. Breast milk plays a vital role in the physiology, growth and protection of newborn babies against disease. It is a complete, balanced and economical food. With the aim of boosting the production of breast milk in some mothers, research has been carried out into ways and means of stimulating milk secretion, using sustainable, natural solutions. The results of nutritional research and surveys carried out by Salifou *et al.* [10] and Adepo *et al.* [7] on the use of lactogenic plants have shown that the leaves of *Euphorbia hirta* (Euphorbiaceae) and *Manihot esculenta* (Euphorbiaceae) have lactogenic properties. Scientific proof is needed to confirm this claim. The aim of this work is therefore to evaluate the effect of *Euphorbia hirta* and *Manihot esculenta* leaves on the lactation of female rats with a view to using them as lactogenic plants. Specifically, the physico-chemical and nutritional parameters, and phytochemical compositions of the leaves of these two plants will be determined. The plasma concentration of prolactin in rats gavaged with dry aqueous extracts of the leaves of these two plants will be also determined.

2. Materials and Methods

2.1. Plant Material

The plant material used consists of *Euphorbia hirta* and *Manihot esculenta* leaves harvested in Bingerville in the Abidjan District (Côte d'Ivoire). This site, with an average annual temperature of around 26°C, is ideal for the growth of these plants.

2.2. Animal Material

Animal material consisted of 14 week-old adult female rats of the Wistar strain (*Rattus norvegicus*) with an average body weight of 255 ± 5 g. They were pre-selected at four weeks to avoid mating. Maintenance and feeding conditions were carried out according to standards (Alison, 2004). The rats were housed in groups of 6 in stainless steel metabolic cages and placed in conditions to isolate them from stress and insomnia, as stress and insomnia can inhibit prolactin secretion (WHO, 1989). In addition, solid and liquid metabolic waste was evacuated daily to avoid microbial contamination. Hygiene and feeding conditions are key factors in assessing the impact of plant extracts.

2.3. Treatment of Manihot Esculenta and Euphorbia Hirta Leaves

One batch of 3 kg of each leafy vegetable was harvested. Each harvested batch was divided into 3 samples of identical weight, with 1 kilogram for each sample. Samples of each vegetable were dried in a clean, dry place away from direct sunlight and powdered. The two leafy vegetable powders obtained after grinding were used to determine physico-chemical and nutritional parameters.

2.4. Preparation of *Manihot Esculenta* and *Euphorbia Hirta* leaf Extracts

Extraction is based on the solubilization of pectin in distilled water [11,12]. One hundred (100) grams of leaf powders of *Manihot Esculenta* and *Euphorbia hirta* were dissolved in 1.5 L of distilled water. The whole was heated at 80 to 90 ° C for 45 min. The resulting homogenate was cooled for 5 min and then filtered through a small mesh poplin cloth. To the residue obtained, one (1) L of distilled water was added. The whole was heated at 80 ° C for 30 min then filtered again. The total filtrate constituting the aqueous extract was freeze-dried to obtain a dry aqueous extract.

2.5. Determination of Physico-chemical and Nutritional Parameters of the Two Leafy Vegetables

The moisture content was determined in an oven (MEMMERT 854 SCHWABACHW, Germany) using A.O.A.C method [13]. The ash content was obtained by weighing the residue of the sample incinerated at 550°C [14]. The protein content of *Euphorbia hirta* and *Manihot esculenta* was estimated using the Kjeldahl method [14]. Total lipids were extracted using the SOXHLET method [14]. Dietary fiber was determined using A.O.A.C method [14]. Total carbohydrates and energy value were determined using the calculation method recommended by FAO [15].

2.6. Determination of Phytochemical Compositions of the Two Leafy Vegetables

Flavonoid content was assessed using the method of Meda *et al.* [16]. Polyphenol content was determined by the Folin-ciocalteu reaction using the method described by Singleton and Brown [17].

2.7. Animal Experimentation

Four (4) groups of six (6) female rats were orally treated with different solutions at a rate of 5 mL per rat twice a day for 4 days. Treatments were given in the morning at 8 a.m. and in the evening at 4 p.m. [12]. Group 1 (control) received distilled water. Group 2 received *Euphorbia hirta* aqueous extract (0.2 g/mL). Group 3 received *Manihot esculenta* aqueous extract (0.2 g/mL). Finally, group 4 (reference control) received galactogil (0.2 g/mL). On the 5th day, blood was collected from all the rats and centrifuged at 3000 rpm for 10 min using a JOUAN centrifuge. The serum obtained was used for the determination of prolactin.

2.8. Determination of Prolactin in Rat Serum

Prolactin was determined according to the method of

Djiane and Kelly [18]. The assay principle combines the Sandwich enzyme immunoassay method with a final fluorescence detection. Prolactin concentrations were calculated automatically using a VIDAS-type immunoanalyzer.

2.9. Statistical Processing

Quantitative data were collected using Excel spreadsheets. A NEWMAN-KEULS test with a classification criterion at the 5% threshold was carried out to assess the significant difference between the means. The test was performed using Statisticat version 7.1 software.

3. Results and Discussion

Table 1 of the biochemical analysis shows the water content of the leaves of the two leafy vegetables *Euphorbia hirta* and *Manihot esculenta* to be 59.06 ± 0.08% and 66.26 ± 3.26% respectively. On the whole, these leafy vegetables have a relatively low moisture content compared with the average of 50%. Moisture content is an important parameter in the preservation of foodstuffs. The relatively high moisture content of the leaves would be a disadvantage for preservation, as the shelf life of a food product is closely linked to its moisture [19]. Moisture content is a quality criterion and an important indicator of a food's suitability for preservation. Thus, the low water content observed in this study would have an advantage on the storage life of foods in powder form by allowing long preservation through inhibition of the proliferation of microorganisms likely to spoil the food product [20]. The lipid content of the nutritional composition of leafy vegetables was assessed at 3.8 ± 0.13% and 5.93 ± 0.31% respectively. The levels observed are broadly similar for *Euphorbia hirta* and *Manihot esculenta* leafy vegetables. In view of the results in Table 1, the lipid content remains low compared with the other contents. Nevertheless, the presence of lipids of plant origin can be an advantage in reducing bad cholesterol levels, which is a health indicator, especially with the presence of unsaturated fatty acids, which are fundamental components of the lipids obtained. This advantage may constitute a natural means of combating possible cardiovascular diseases that could be encountered in people suffering from the aforementioned disease. The results indicate protein macromolecule contents of 14.41 ± 0.02% and 29.09 ± 0.11%. The leaf is the most widely used part of the plant, consumed in sauces as a simple vegetable, with moderate medicinal use and less use in animal feed. The high rate of use of this leaf can be explained by awareness of its nutritional virtues, which was raised by the Maritime Red Cross in the prefecture of Yoto. The other organs, such as the seeds, stems and roots, are used in the treatment of infectious diseases, digestive infections, haematology, neurology and gynaecology. With regard to ash, the rate obtained in this study was statistically identical for *Euphorbia hirta* 9.2 ± 0.13% and higher than that obtained for the *Manihot esculenta* plant 6.2 ± 0.11%. The minerals contained in ash are important for the proper functioning of the body, as they are used in catabolic reactions involving carbohydrate, lipid and protein molecules, as minerals are the metal cofactors for enzymes. The dietary fiber values obtained for the three leafy vegetables were 37.16 ± 1.55% and 43.83 ± 1.53% respectively. The values recorded are generally high, although those for the *Manihot esculenta* species differed significantly from those for the *Euphorbia hirta* species. In addition, the importance of dietary fiber containing water-soluble fiber such as pectin is thought to be due to its anti-diarrhoeal and anti-cancer properties, due to its detoxifying properties [21]. The presence of non-water-soluble fiber is also thought to improve digestibility and facilitate intestinal transit [22]. Fiber consumption reduces the risk of cardiovascular disease, colon cancer and obesity. The lipid content of the three vegetables was 3.8 ± 0.13%, 6.8 ± 0.13% and 5.93 ± 0.31% respectively. The richness in dietary fiber and protein is in line with that found by National Nutrition Policy in Côte d'Ivoire.

Table 1. Nutritional composition (%) of two leafy vegetables

Parameters (g/100g)	*Euphorbia hirta*	*Manihot esculenta*
Humidity	59,06 ± 0,08 a	66,26 ± 3,26 b
Proteins	14,41 ± 0,02a	29,09 ± 0,11b
Lipids	3,8 ± 0,13a	5,93 ± 0,31b
Carbohydrates	35,43 ± 5,30a	14,89 ± 0,01b
Ashes	9,2 ± 0,13a	6,2± 0,11b
Dietary fiber	37,16 ± 1,55a	43,83±1,53b

Each result is the mean of three trials. Letters represent statistical significance. Mean values on the same line followed by different letters are significantly different at p<0.05 according to the Newman-Keuls test.

Table 2 shows the convergence of high levels of the respective secondary metabolites in the two species *Euphorbia hirta* and *Manihot esculenta*, which are as follows; polyphenols (368.66±50.75 mg EAG/g DM; 381±4.24 mg EAG/g DM), flavonoids (290.66±48.36 mg EQ/ g DM; 283 ± 1.41 mg EQ/g DM). One of the major originalities of plants lies in their capacity to produce highly diversified natural substances. In addition to the classic primary metabolites (carbohydrates, proteins, lipids, nucleic acids), phytochemical compounds such as flavonoids and phenols are found in *Manhiot esculenta* [23] and *Euphorbia hirta* [24]. The flavonoids most commonly used as dietary supplements are vanillin and vanillic acid, resveratrol, ellagic acid, curcumin and quercetin [25]. Flavonoids, particularly coumarins, are anti-coagulant and anti-platelet aggregation agents [26]. A clinical study has demonstrated the anticancer activity of quercetin when administered intravenously to cancer patients.

Table 2. Composition of polyphenols (mg EAG/gMS) and flavonoids (mg EQ/gMS) in two leafy vegetables

Parameters	*Euphorbia hirta*	*Manihot esculenta*
Polyphenols	368,66±50,75 a	381±4,24 a
Flavonoids	290,66±48,36 a	283 ± 1,41a

Each result is the mean of three trials. Letters represent statistical significance. Mean values on the same line followed by different letters are significantly different at p<0.05 according to the Newman-Keuls test.

Furthermore, Table 3 shows the plasma prolactin concentrations of female rats treated with aqueous extracts of *Euphorbia hirta*, *Manihot esculenta*, distilled water (Negative Control) and Galactogil (Positive Control)

estimated at 15.22± 0.67; 12.52 ± 0.57; 5.18 ± 0.78 and 15.15 ± 0.07 ng/ml respectively. These results suggest the presence of a lactogenic active principle in the two species studied, albeit with significance in *Euphorbia hirta*.

Table 3. Plasma concentration of prolactin in adult female rats tested and controls

Products	Water	Aqueous extract of *Euphorbia hirta*	Aqueous extract of *Manihot esculenta*	Galactogil
Prolactin levels (ng/ml)	$5,18_a$ ± 0.78a	15,22± 0,67b	12,52 ± 0,57b	$15,15_c$ ± 0,07b

4. Conclusion

The results of this study show that leafy vegetables generally have a high nutritional value, although they are specifically richer in protein, dietary fiber and minerals. Evaluation of the lactogenic power of the plants *Euphorbia hirta* and *Manihot esculenta* through the plasma concentration of prolactin confirms the lactogenic power of the plants studied. It would be advisable to encourage the population to consume leafy vegetables, as they contribute significantly to prolactin production and could promote the production of breast milk quantitatively and qualitatively through the presence of dietary fiber and the nutritional richness of the plants studied.

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