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Nutritional Profile of *Annona Muricata* and *Ageratum Conyzoides* Based Herbal Teas for the Management of a Precancerous Lesion: Atypical Hyperplasia of the Mammary Gland

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ABSTRACT

The consumption of herbal teas is of paramount importance due to their health benefits, including slowing down cell proliferation, particularly the development of new vessels and inactivation of carcinogenic products through antioxidant effects. This study aimed at assessing the nutritional composition of an antiproliferative tea beverage made up of *Annona muricata* and *Ageratum conyzoides*. These two plants, taken individually, and in combination in different proportions, showed unusual inhibitory activity on mammary gland cell proliferation. Five herbal tea samples were obtained by mixing A.muricata and A.conyzoides in different proportions: 100/0; 75/25; 50/50; 25/75 and 0/100 with the respective codifications: $C_{100}R_h$; $C_{75}R_h$ C50R_h; $C_{25}R_h$ and C_0R_h. Lipid, protein and carbohydrate content of the different formulations were determined using standard reference methods. K, Ca, Mg and Na content was determined using atomic absorption spectrophotometry. The data were analyzed and the results showed that Annona muricata ($C_{100}R_h$) and *Ageratum conyzoides* (C_0R_h) leaf extract contained 25.07 g/100 g dry mass and 12.07 g/100 g dry mass (protein), 21.57 g/100 g dry mass and 22.41 g/100 g dry mass (lipids) and 0.61 g/100g dry mass and 0.23g/100 g dry mass (soluble carbohydrates). Of the three intermediate herbal tea combinations ($C_{75}R_h$, $C_{50}R_h$, $C_{25}R_h$), $C_{75}R_h$ (75% *A.M* and 25% *A.C*) had the highest protein content (17.65 g/100g dry mass), the lowest soluble carbohydrate content (0.33 g/100g MS), the highest sodium (63.03 mg/100g MS) and potassium (467.09 mg/100g MS) content and the lowest calcium content (18.19 mg/100g MS). These relatively high levels in nutrient composition have allowed for the projection of the necessary dietary adjustments when treating patients with atypical breast hyperplasia with herbal teas.

Keywords: Atypical Hyperplasia; Mammary Gland; *Annona Muricata*; *Ageratum Conyzoides*; Nutritional Value; Herbal Tea; Precancerous Lesion

Abbreviations: AMH: Atypical Mammary Hyperplasia; WHO: World Health Organization; IARC: International Agency for Research on Cancer; GOD: Glucose Oxidase; H2O2: Hydrogen Peroxide; POD: Presence of Peroxidase; GH: Growth Hormone

Introduction

Hyperplasia, a term for an abnormally large volume of a tissue or organ due to an increase in the number of its cells [1], is characterized by the proliferation of epithelial cells of a specific tissue. It becomes atypical when the proliferating cells are abnormal or in an unusual way. When this abnormal proliferation attacks the cells that line the ducts or lobules of the breast it is called Atypical Mammary Hyperplasia (AMH) [2]. The prevalence of AMH is increasing every year and it is one of the leading breast disorders in women of childbearing age [3]. AMH is considered a potential precancerous lesion that can progress to breast cancer if left undiagnosed and untreated [4,5]. Breast cancer is a heterogeneous disease in which normal cells of the mammary gland adopt a malignant phenotype and proliferate indefinitely and uncontrollably, destroying breast tissue [6]. According to a report by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC), approximately 22 million people will be annually affected by cancer by 2030: 8 million more than in 2012 [7]. In 2021, cancer was the leading cause of death worldwide, accounting for about 10 million deaths: the most common in terms of new cases being breast cancer with 2.26 million cases [8]. In Cameroon, the figures are even more alarming and breast cancer is the most common in terms of prevalence and mortality. According to several studies, 70-80% of patients are diagnosed at advanced stages and nearly 2000 women die from it each year [9]. In addition to the delay in diagnosis, other risk factors for cancer include gender, age, family history, reproductive risk factors and obesity [10]. Obesity is particularly characterized by an abundance of adipose tissue where oestrogen synthesis takes place, and it plays a major role in both abnormal cell proliferation in the mammary gland and in the uterus, and that can progress to malignancy and metastasis [11]. There is an association between high fat, protein and carbohydrate diets and the increased risk of breast cancer through increased oestrogen production, hyperinsulinaemia and insulin resistance. High fat and protein diets play a role in the increase in oestrogen production by adipose tissues [12,13].

Polyphenols are good in slowing down cell proliferation; they are particularly good in the development of blood vessels and in inactivating carcinogenic products through their antioxidant activities. Plants, taken in form of herbal tea, are used by about 80% of the world's population for their curative virtues [14,15]. They can help female populations to reduce the risk of developing gynaecological cancers. *Annona muricata* and *Ageratum conyzoides* are two plants from the Annonaceae and Asteraceae families respectively, which have shown convincing results in previous studies. Nutritionally, *Annona muricata* leaves are rich in protein (25 ± 0.06 g/100g), lipid (21.22 ± 1.01 g/100g), carbohydrate (16.62 ± 0.09 g/100g), Potassium (363.05 mg/kg), Calcium (11183.50 mg/kg), Sodium (694.86 mg/kg), Magnesium (9619 mg/kg) and Iron (139.50 mg/kg) [16,17]. *Ageratum Conyzoides* leaves contain crude protein (24.53 ± 0.104), lipid (3.78 ± 0.069), carbohydrate (36.81 ± 0.006), Sodium (88.50 ± 0.346), Potassium (139.10 ± 0.006), Calcium (220.60 ± 0.173), Magnesium (110.13 ± 0.115), Iron (22.73 ± 0.289), Zinc (43.50 ± 0.173) and Phosphorus (380.13 ± 0.173) [18]. The rich nutritional composition of these plants is responsible for their medicinal effects, including antibacterial, antiproliferative and even anticancer activities. Referring to their antiproliferative and anticancer effects, *Annona muricata* has shown convincing results on breast cells with 98% efficacy in previous studies [19]. Adebayo, et al. [20] demonstrated that the in vitro anticancer activity of aqueous extracts of *Ageratum Conyzoides* is more effective on lung, uterine, breast and liver cells. Based on these results, a combination of the two plants in the form of formulas proved to have more interesting properties leading to promising conclusions on slowing down AMH (Publication in progress). The main objective of this work was to evaluate the nutritional properties of formulations made by combining *Annona muricata* and *Ageratum Conyzoides*.

Methodology

Plant Material

Annona muricata and *Ageratum Conyzoides* were collected in Yaoundé, Centre region in October and November 2019 between 5:30 and 6:30 am. They were identified by comparison at the National Herbarium of Cameroon against the numbers 6575 SRFK (YA) and 18681/SRF/Cam (YA) respectively. The collected plants were weighed and spread out for 24 hours on large trays in a room (20-21°C) for wilting.

Preparation of Extracts

The wilted leaves were dried at 40°C in a ventilated oven for 24 hours. Once dry, they were milled using a blender and then sieved using a 150 µm mesh size sieve. The obtained powders were infused in boiling water in the ratios of 1/5 and 1/6 g/mL for *Annona muricata* and *Ageratum Conyzoides* respectively for 2 hours, stirring every 30 minutes. The solution was filtered, and the residue was rinsed twice. The obtained solution was freeze-dried, and a mixing plan used in industrial research and development studies [21,22] helped to have five combinations of the freeze-dried extracts in the proportions 100/0; 75/25; 50/50; 25/75 and 0/100 corresponding to $C_{100}R_h$; $C_{75}R_h$; $C_{50}R_h$; $C_{25}R_h$ and C_0R_h respectively (C for *Annona muricata* and Rh for *Ageratum Conyzoides*).

Crude Protein Content

Crude protein content was determined according to the method described by Devani and al (1989) [23]: it is a spectrophotometric method based on the conversion of total organic nitrogen to ammonium sulphate.

Carbohydrate Content

The determination of soluble carbohydrates was carried out using the Chronolab assay kit based on the oxidation of glucose to gluconic acid catalysed by glucose oxidase (GOD). The hydrogen peroxide

(H2O2) produced is detached using a chromogenic oxygen acceptor, phenol ampirone in the presence of peroxidase (POD).

Lipid Content

Total lipids were extracted with Soxhlet according to the Russian method as described by Bourely, (1982) [24] based on the differential solubility of lipids in organic solvents (hexane) at high temperatures about 12 hours.

Mineral Content

The minerals Ca, Na, Mg and K were analysed according to the method described by Horwitz, (2000) [25] based on the separation of the minerals from the sample matrix by wet digestion of the organic matter. The separated minerals were diluted in acid and their content determined by atomic absorption spectrophotometry.

Statistical Analysis

Statistical analyses were performed using IBM SPSS 20.0 software for Windows. The data were analysed by 1-way ANOVA and Tukey's multiple range test was used to determine the differences among samples. Significant levels were defined as probabilities of 0.05 or less. The obtained results were presented as mean ± standard error of the mean (SEM). Excel 2016 software was used for graphical representations.

Results and Discussion

Crude Protein, Fat and Soluble Carbohydrate Content

Table 1 shows the macronutrient content of the different combinations (formulations). Protein content ranged from 25.07 g/100g DM to 12.01 g/100g DM: fat content from 22.42 g/100g DM to 20.65 g/100g DM and carbohydrate content from 0.63 g/100g DM to 0.23 g/100g DM with statistical significant difference ($p< 0.05$) between the protein content of the different herbal tea combinations. *Ageratum Conyzoides* leaf tea C0Rh had the lowest protein content (12.01 g/100 g DM) compared to *Annona muricata* leaf tea C100Rh (25.07 g/100 g DM) and the combinations $C_{75}R_h$, $C_{50}R_h$, $C_{25}R_h$ of 17.65 g/100 g; 17.30 g/100 g and 16.42 g/100 g DM respectively. A significant difference ($p< 0.05$) was also observed between the lipid content of the different combinations. *Ageratum Conyzoides* leaf tea C0Rh had the highest lipid content (22.41 g/100 g DM) compared to *Annona muricata* leaf tea $C_{100}Rh$ (21.57 g/100g DM). Of the three combinations, the lipid content of the $C_{50}R_h$ herbal tea combination was the highest (22.42 g/100g DM) followed by the $C_{75}R_h$ and $C_{25}R_h$ formula. *Ageratum Conyzoides* leaf tea C0Rh had the lowest soluble carbohydrate content 0.23 g/100 g DM, followed by $C_{75}R_h$, $C_{100}R_h$, $C_{25}R_h$and $C_{50}R_h$.

Table 1: Protein, lipid, and soluble carbohydrate contents (g/100g DM) of herbal tea formulations based on *Ageratum Conyzoides* and *Annona muricata* leaves.

	$C_{100}R_h$	$C_{75}R_h$	$C_{50}R_h$	$C_{25}R_h$	C_0R_h
Protein	25.07 ± 0.006^e	17.65 ± 0.006^d	17.30 ± 0.006^c	16.42 ± 0.006^b	12.01 ± 0.006^a
Lipid	21.57 ± 0.006^c	21.13 ± 0.006^b	22.42 ± 0.006^d	20.65 ± 0.006^a	22.41 ± 0.006^d
Soluble carbohydrates	0.61 ± 0.0057^c	0.33 ± 0.0057^b	0.63 ± 0.0057^c	0.61 ± 0.0057^c	0.23 ± 0.0057^a

Note: Means assigned with different letters in the same rows are significantly different ($p<0.05$). $C_{100}R_h$: 100% *Annona muricata* leaf powder; $C_{75}R_h$: 75% *Annona muricata* leaf powder and 25% *Ageratum conyzoides* leaf powder; $C_{50}R_h$: 50% *Annona muricata* leaf powder and 50% *Ageratum conyzoides* leaf powder; $C_{25}R_h$: 25% *Annona muricata* leaf powder and 75% *Ageratum conyzoides* leaf powder; C_0R_h: 100% *Ageratum conyzoides* leaf powder.

The protein content of *A. muricata* in the present study was approximately equal to 25 ± 0.06 g/100g DM, same value obtained by Usunobun and al. (2012) [26] in Nigeria; higher than 24.3 ± 0.1 g/100g DM and 15.74 ± 1.01 g/100g DM obtained by Rosemary and al. (2017) [27] and Ogbonna and al. (2019) [28] respectively. The protein content of *A. conyzoides* was lower than 14.73 g/100g DM and 15.67 g/100g DM respectively obtained by Agbafor and al. (2015) [29] and Agunbiande and al. (2012) [30]. These differences could be explained by various parameters such as the age of the plant before harvesting, the application of chemical or natural fertilizers on the harvesting land, the nature of the soil [31], and the different treatments carried out on the plant material such as drying. The combination of 75% *A. muricata* and 25% *A. conyzoides* tea had the highest protein content after the 100% *A. muricata* formula at 17.65 g/100 g DM. According to the *Agence Nationale de Sécurité Sanitaire de l'Alimentation de l'Environnement et du Travail* (ANSES) the average daily protein intake of a woman is 0.9 g/kg body weight. This means that a woman weighing 60 kg should have a protein intake of 54 g per day. The average protein content of 100 g of herbal tea dry matter (average of the five contents obtained) is 17.69 g/100g. Therefore, consumption of 100g of herbal tea per day provides 5.08 g of protein, thereby contributing approximately 10% of the protein RDA for a 60 kg woman. The protein intake of the tea (depending on the quantity) can be subtracted from the food intake. A high protein intake can lead to a rapid proliferation of tumour cells due to the stimulation of growth hormone (GH), which itself promotes the synthesis of IGF 1, another factor in the proliferation of tumour cells [32]. A lipid-rich diet has a higher energy density than one rich in other macronutrients. The role of lipids has been discussed in the development of overweight and obesity. When fatty acids are involved in energy production, the

polyunsaturated fatty acids are oxidised, leaving the saturated fatty acids to accumulate in adipose tissue, where they trigger abundant oestrogen production [32].

As breast cancer prevention is a major public health objective, the quantification of lipids in our herbal tea samples was of paramount importance. The lipid content of *A. muricata* $C_{100}R_h$ tea (21.57 g/100g DM) was slightly higher than 21.22 ± 1.01 g/100 g DM obtained by Usunobun et al. (2015) [33] in Nigeria. That of *A. conyzoides* C0Rh tea (22.41 g/100g DM) was significantly higher than 5.67 g/100 g DM obtained by Agubiande, et al. (2012) [30]. The combination of 75% *A. muricata* and 25% *A. conyzoides* $C_{75}R_h$ had an average lipid content of 21.13 g/100 g DM. ANSES recommends an average daily lipid intake of 1 g/kg body weight for a woman. This means that a 60 kg woman should consume 60 g of fat per day. The average fat content of 100g dry mass is 21.63g, contributing 36% of a 60kg woman's requirement. The fat intake from the tea (depending on the amount) can be subtracted from the food intake as too much fat can lead to increased fatty tissue in the body which is a source of abundant oestrogen production and thus increases the risk of developing cancer of the primary oestrogen targets [34]. Carbohydrates, when consumed in large quantities and in absence of balanced caloric expenditure, can be considered as risk factors for several diseases such as cancer. The combination of 50% *A. muricata* and 50% *A. conyzoides* ($C_{50}R_h$) showed the highest soluble carbohydrate content 0.63g/100g DM followed by the combination of 25% *A. muricata* and 75% *A. conyzoides* ($C_{75}R_h$) 0.61g/100g DM which is not very advantageous in this case. The soluble carbohydrate content of the combination of 75% *A. muricata* and 25% *A. conyzoides* ($C_{75}R_h$) was 0.33g/100g DM, which would be an advantage for the management of people at risk.

Mineral Contents (Na, Ca, K, Mg)

Table 2 shows the mineral (Na, K, Mg and Ca) content of the herbal tea formulations based on *Ageratum conyzoides* and *Annona muricata* leaf powder. Minerals have various roles in cell proliferation. Of the three intermediate combinations, the tea combination with 75% *Annona muricata* and 25% *Ageratum conyzoides* (C75Rh) had the highest Na content (63.03 mg/100 g DM), followed by C50Rh (56.53 mg/100g DM) and C25Rh (48.46 mg/100g DM). This C75Rh combination also had the highest K content (467.09 mg/100 g DM). Sodium is frequently ingested in food in the form of naturally occurring sodium chloride (table salt). It is stimulated by growth factors and ensures the alkalinisation of the intracellular environment, a process essential for the activation of DNA synthesis and thus the initiation of the mitotic cycle. Potassium is nutritionally important for pH regulation and the proper functioning of carbohydrate and protein metabolism. Most people with cancer generally have too much sodium, not enough potassium, and a pH of 4, which leads to tissue damage and weakening of organs [35]. The potassium content of the tea would regulate the pH of the body by filling the potassium deficiency in people at risk. The combination of 75% *Annona muricata* and 25% *Ageratum conyzoides* herbal tea had the lowest calcium content of 18.19 mg/100 g DM; calcium is known to be a macro element required for hormone release [36]. Hypercalcaemia would lead to the deposition of calcium salts in the ducts of the breast, exposing the woman to precancerous connections [37].

Table 2: Content of mineral elements (Na, K, Mg and Ca) (mg/100g DM).

	$C_{100}R_h$	$C_{75}R_h$	$C_{50}R_h$	$C_{25}R_h$	C_0R_h
Na	36.92 ± 0.01^a	63.03 ± 0.01^e	56.53 ± 0.001^d	48.46 ± 0.001^b	50.04 ± 0.001^c
K	271.85 ± 0.001^b	467.09 ± 0.001^e	238.25 ± 0.001^a	328.19 ± 0.001^d	279.63 ± 0.001^e
Mg	18.78 ± 0.001^b	27.53 ± 0.001^c	37.38 ± 0.001^d	4.87 ± 0.001^a	51.64 ± 0.001^e
Ca	176.88 ± 0.001^c	18.19 ± 0.001^a	570.56 ± 0.001^e	382.29 ± 0.001^d	98.44 ± 0.01^b

Note: Means assigned with different letters in the same rows are significantly different ($p<0.05$). $C_{100}R_h$: 100% *Annona muricata* leaf powder; $C_{75}R_h$: 75% *Annona muricata* leaf powder and 25% *Ageratum conyzoides* leaf powder; $C_{50}R_h$: 50% *Annona muricata* leaf powder and 50% *Ageratum conyzoides* leaf powder; C25Rh: 25% *Annona muricata* leaf powder and 75% *Ageratum conyzoides* leaf powder; C_0R_h: 100% *Ageratum conyzoides* leaf powder.

This low Ca content would be an asset as it would solve the problems of mammary calcifications, since high concentrations of calcium salts puts the individual at risk of developing benign tumours. The Magnesium content of the combination of *Annona muricata* and *Ageratum Conyzoides* herbal tea was 27.53 mg/100 g. Magnesium is a mineral required in hormone metabolism. It is also important for the release and action of insulin [38]. The higher the amount of magnesium in women at increased risk of cancer, the more it will lead to hyperinsulinemia, a factor that promotes the development of cancerous disease. According to WHO, the recommended daily intake of sodium is not more than 2000 mg/day [39]. Consumption of 100 g of $C_{75}R_h$ herbal tea meets 3.15% of the daily sodium requirement. For potassium, the European Food Safety Authority (EFSA) recommends an intake of 3500 mg/day for adult women (EFSA, 2016). $C_{75}R_h$ herbal tea meets 13.34% of the daily potassium requirement. The intake of potassium will promote the proper functioning of carbohydrate and protein metabolism. As for magnesium, the recommended intake for adults is 6 mg/kg/day [40] and the lowest magnesium content was that of the $C_{25}R_h$ formulation (4.87mg/100g DM). Finally, for calcium, the recommended nutritional intake for adults is 900 mg, for women over 55 and the elderly the requirement is 1200 mg/day. The highest calcium content was found in $C_{50}R_h$ (570.56 mg/100g DM) and the

lowest in $C_{75}R_h$ (18.19 mg/100g DM). Calcium is known to be a macro element necessary for the release of hormones. The higher its content in the body, the more calcium salts will be deposited in the ducts of the breast exposing the woman to precancerous connections, the $C_{75}R_h$ formulation with a content of 18.19 mg/100g DM would be an asset for women at risk.

Conclusion

The objective of this study was to study the nutritional profile of herbal teas made from leaf powder of *Annona muricata* and *Ageratum Conyzoides* leaves combined in various proportions for the treatment of atypical hyperplasia. It was found that of the five combinations, the one with 75% *Annona muricata* and 25% *Ageratum Conyzoides* presented a better nutritional profile because of its low nutrient content (proteins and carbohydrates) and medium mineral content (Na, K and Ca). These results suggest a real asset for the dietary management of young women at risk of developing breast cancer or not. Furthermore, due to the high content of phenolic compounds in the $C_{75}R_h$ combination, its antiproliferative activity on mammary precancerous lesions induced in Wistar rats was studied and it showed an inhibitory effect on the disordered proliferation of mammary gland cells. As this work is in the process of being published, there are questions about the nature of the bioactive compounds present in the tea and mainly responsible for its antiproliferative activity.

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Conflict of Interest

The authors declare no conflicts of interest for this study.

Data Availability

The datas analyzed during the current study are available from the corresponding author upon reasonable request.

Author's Contribution

Fokou Elie, Mbacham Wilfred and Mercy Tah-Monunde conceived the study, edited and reviewed the manuscript; Mono Anne Sophie prepared the proposal and conducted laboratory analysis under the guidance of Djouhou Michelle and drafted the manuscript. Djouhou Michelle and Maptououm Laure were involved in data analysis and reviewing of the manuscript. All authors read and approved the final manuscript.

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