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# ACTION OF ETHYL EXTRACTS OF ANNONAMURICATA IN THE TRADITIONAL TREATMENT OF CERVICAL CANCER IN THE WISTAR RAT

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***Abstract***
*Our study focused on the action of ethyl extract of Annonamuricata in the traditional treatment of cervical cancer in the Wistarrat. This is an analytical, prospective and control study that took place at the Laboratory of Biomembranes and Cell Signaling of the University of AbomeyCalavi.The purpose of this study is to verify the effects of the ethyl extracts of the leaves of Annonamuricata on the differentiation of cervical cells in the Wistarrat. For this, an ethyl extraction was carried out using dried leaves powder of Annonamuricata and of ethanol at 90 °, likewise the extraction of the leaves of Cycasrevoluta. The activity on differentiation was evaluated by assaying the total protein and alkaline phosphatase parameters on crushed cells of the Wistar rat uterus in which the differentiation of the cervical cells was induced beforehand by exposure to the powder and extracted Ethyls of Cycasrevoluta through a diet. Our sample included a sub-control population of 3 Wistar rats and one sub-population of 6 Wistars.PAL activity in the control sub-population is higher than in the sub-population-case. The PAL / total protein ratio of the sub-control population is significantly higher than in the sub-population case.*
*These results make it possible to demonstrate the action of the extracts of Annonamuricata on the differentiation of the cells of the cervix of the rat.*

**Keywords -** *Annonamuricata, Cycasrevoluta, cervical cancer, extract, differentiation.*

## INTRODUCTION

Non communicable diseases represent an increasing burden on health systems, due to a double demographic and epidemiological transition, in this case in the developing countries. They include cardiovascular diseases, type 2 diabetes, cancer ... and are rising all over the world, including the countries of the south, in terms of incidence and mortality.

Cancers are a major cause of morbidity and mortality in the world. According to estimates by the World Health Organization (WHO), they accounted for 7.6 million deaths in 2008, accounting for 13% of total mortality (lay J. ,2011). Many environmental factors related to lifestyle and industrialization are blamed for the increased incidence of cancers.

Epidemiological data predict a steady increase in cancer mortality. By 2030, 13 to 17 million people will die of cancer each year (Centre International de Recherchesur le Cancer (CICR), 2013.).

In Africa, 600,000 cases of cancer are reported each year and 500,000 people die, according to WHO

Indeed, cancer is a group of various diseases, characterized by abnormal cell proliferation, invading and destroying the surrounding tissues, and can spread to give rise to metastases either in the organ of origin or in organs Different (Ferlay J, 2004.). This dysfunction could be due to the intervention of exogenous factors. These factors include alcohol and tobacco consumption, low consumption of fruits and vegetables, and chronic viral infections. (Ly A. 2007 ) To this end, the most frequently diagnosed cancers in the world are those of lung, breast and colorectal cancer (Bray F, 2013.). One of the most remarkable trends is the increase in the number of cancers among women, which confirms that at the global level priority must be given to measures to prevent and control breast and cervical cancers (WHO. 2013). The latter is the second most common cancer in women with almost 493,000 new cases estimated in 2002 and more than 500,000 in 2007 Curado MP, 2007). This cancer that affects women in their youth can have catastrophic effects with a very high human, social and economic cost. But this disease should not be a death sentence, even in poor countries.

Indeed, Africa has many plants with various therapeutic virtues. These plants have the ability to produce very diverse natural substances. They are recognized as a source of drugs (Bénédicte A, 2012,) (Loïca Z. , 2011.). This is why medicinal plants are used in the management of chronic diseases such as cervical cancer in low-income countries, particularly in Africa (Geoffrey C. Kirby M. ,1996). It is for this purpose that the extracts of the leaves of Annonamuricata are used by the tradithérapeutes to stop the proliferation of the cells of the cervix.Our work will focus on the effects of Annonamuricata on the differentiation of cervical cells.

## General information on Annonamuricata and cycasrevoluta

### Biology of Annonamuricata

To counteract the side effects of anti-cancer treatments, scientific research is carried out on several plants, such as Annonamuricata, used in the traditional African pharmacopoeia (Anil K.,2012.), especially in Benin.

Originally from South America, corossol is also grown in other tropical regions. Its tree called Guanabana or Graviola offers exquisite fruits and leaves that would destroy many cancer cells.

According to research conducted at the American University of Purdue, leaves of the sapling tree possess properties that destroy cancer cells (Marie Line D., 2014.).

The hornbill is a shrub or small tree 3 to 10 m high.

The leaves, of a brilliant green, are oblong-lanceolate, of 10-17 × 2-7 cm, the young with ferruginous pubescence below.

The flowers appear on large pedicels (15-20 mm long) opposite the leaves. The 6 petals are yellow, fleshy and thick. The 3 external petals are largely oval at the edges, without being superimposed. It blooms all year round. It tolerates poor soils but cannot withstand low temperatures.

The corossol, fruit of the hornbill is up to 30 cm long and can weigh up to 4 or 5 kg. Its external aspect is of a dark green, its bark pierced with thorns and its white pulpy flesh with black seeds. In Benin, we find the sourscher usually in the south. Being a fruit tree, domesticated, it is often grown in urban areas.

Biology of Cycasrevoluta

The genus Cycas contains about twenty species. This tree, similar to a dwarf palm, originating in Japan, reaches a height of 4m for a width of 3m. It has very slow growth on a robust trunk and an evergreen foliage, dark green, arranged in rosette. It is a dioecious plant whose male or female inflorescences are of no decorative interest. Rustic down to -10 ° C foliage is deteriorated from -3 ° C.

It is a toxic plant. It synthesizes a very toxic glucoside for herbivores (cyclosin) and a neurotoxic amino acid, beta-N-methylamino-L-alanine. Twelve hours after an animal ingests leaves of the tree, the animal begins to vomit, has diarrhea, nose bleeds and other symptoms that can lead to death. (Yves D., 2009.)

**Cycasrevoluta**

## METHOD OF STUDY DRYING OF LEAVES

The fresh leaves of Annonamuricata were harvested in May and September 2013 in a family concession in the town of Cotonou (Akpakpa district). They were identified by the botanist experts of the National Herbarium of the University of Abomey-Calavi, then dried in the shade on a bench at the Laboratory of Biomembranes, and Cell Signaling located at the Faculty of Sciences and Techniques (FAST ) From the University of Abomey-Calavi (UAC), for four weeks for leaves picked in May, and in the oven at 55 ° C at the Laboratory of Biology and Molecular Typing in Microbiology, also at UAS FAST; For leaves picked in September. After drying The dried leaves are ground and powdered using a RETSCH knife mill and stored in glass jars to avoid the installation of polluting microorganisms.

**Preparations of extracts from the leaves of Cycasrevoluta**

100 g of Cycasrevoluta leaf powder weighed using a Sartorius® analytical balance were macerated in 1 L of ethanol for 72 hours with stirring. Then, the macerate is filtered through the hydrophilic fiber cotton. The filtrate obtained is evaporated with the aid of the ROTAVAPOR evaporator at 40 ° C. The recovered extracts were placed in an oven at 45 ° C. for drying. The dry extracts were scraped with the stainless steel spatula and then stored in glass bottles previously labeled.
These extracts will be used to prepare the concentration ranges tested.
The yield is determined by the ratio of the weight of the dry extract after evaporation to the weight of the dry vegetable material used for the extraction multiplied by 100 (Medane A. , 2012).

## PREPARATION OF EXTRACTS FROM LEAVES OF ANNONAMURICATA

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## VERIFICATION OF THE STIMULATORY EFFECT OF DIFFERENTIATION OF CYCASREVOLUTA

For our work, we used 9 female Wistar rats each weighing at least 100g of body weight. We divided the rats into three lots at a rate of three per lot. Then, we performed a sub-chronic gavage for fourteen (14) days, ie two weeks.

| Lot 1 | Lot 2 | Lot 3 |
|---|---|---|
| Granulated + distilled water | Granullated + water + 100mg / kg of Cycasrevoluta extract | Granulated + 5% Cycasrevoluta powder + 10mg / kg of Cycasrevoluta powder diluted in drinking water |

On the 15th day, one rat of each lot was sacrificed and dissected. After dissection, the cervixes of the rat's uterus were removed and crushed. The ground product was centrifuged at 6000 rpm for 5 min. Total proteins and alkaline phosphatase were then assayed on the resulting supernatant.

## PHARMACOLOGICAL ACTIVITY OF THE ETHYL EXTRACTS OF THE LEAVES OF ANNONAMURICATA

After checking the carcinogenic activity of the powder and extracts of the leaves of Cycasrevoluta, the two remaining rats in each batch continued the experiment. The control batch received only distilled water and pellets throughout the experiment. Lot 2, the rats of which had been fed during the first two weeks of the experiment with a diet consisting of granules and 100 mg / kg body weight of Cycasrevoluta extracts by gavage, received 100 mg / kg body weight of Ethyl extracts from the leaves of Annonamuricata by gavage. Finally, lot 3, which the rats had received during the first 2 weeks of the experiment, a diet composed of granules, 5% Cycasrevoluta powder and 10 mg / kg cycas powder diluted in drinking water, were fed with 100 mg / kg weight of ethyl extracts of the leaves of Annonamuricata.
After 14 days, the rats are sacrificed and then dissected. After dissection, the cervixes of the rat's uterus were removed and crushed. The ground product was centrifuged at 6000 rpm for 5 min. Total proteins and alkaline phosphatase (PAL) were then assayed on the resulting supernatant.

**Determination of biochemical parameters**
**Total proteins**
**Principle**
The total protein assay was carried out by the colorimetric method described by Gornall et al in 1949. The peptide bonds of the proteins react with cupric (Cu2 +) ions in alkaline solution to form a colored complex whose absorbance, proportional to the concentration In proteins in the specimen, is measured at 550 nm. The Biuret reagent contains sodium potassium tartrate which complexes cupric ions (Cu2+) and maintains their solubility in alkaline solution.
Mix. Leave for 10 minutes at a temperature of 20 ° C to 25 ° C. Read absorbances at 550nm (530-570) against reagent blank
The calculation is made according to the following rule:
(Abs. Dosage / Abs. Stallion) × concentration of the standard.

**Alkaline phosphatase**
**Principle**
Optimized method based on the recommendations of the DGKC (Food Society of Clinical Chemistry, 1972) and the SCE (Scandinavian Society of Clinical Chemistry).
In an alkaline medium, alkaline phosphatases catalyze the hydrolysis of p-nitrophenylphosphate to p-nitrophenol and phosphate.
The rate of occurrence of p-nitrophenol, followed by the change in absorbance at 405 nm, is proportional to the PAL activity in the specimen.

## STATISTICAL ANALYSIS

The determination of the significance between the batches treated by Annonamuricata and the control lot is made of the Student test. Thus the difference is said to be significant if P isstrictementinférieur à 5% (P < 0.05). Lorsque P estsupérieur à 5% (P > 0.05), la différenceestdite non significative.

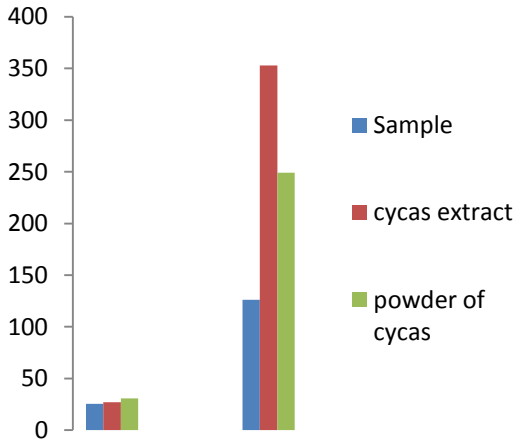


**Figure 1: Variations of total protein parameters and tissue PAL according to a diet containing or Cycas revoluta.**
Compared to control rats receiving only tap water and granules throughout the experiment, a slight variation in total proteins was observed and a significant increase in PAL activity in the sample rats

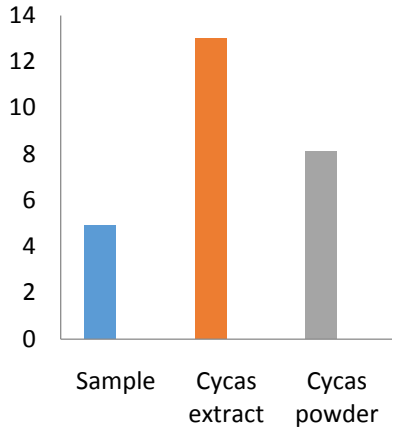


**Figure 2 : variation du rapport PAL / proteins totalestissulairesselon régime alimentairecontenantou non du *Cycasrevoluta.***

Le rapport PAL/Protéines totales des rats ayant reçu l'extrait de *Cycas* est plus élevé que ceux ayant reçu la poudre de *Cycas*.

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**Pharmacological effect of extracts from the leaves of Annonamuricata**

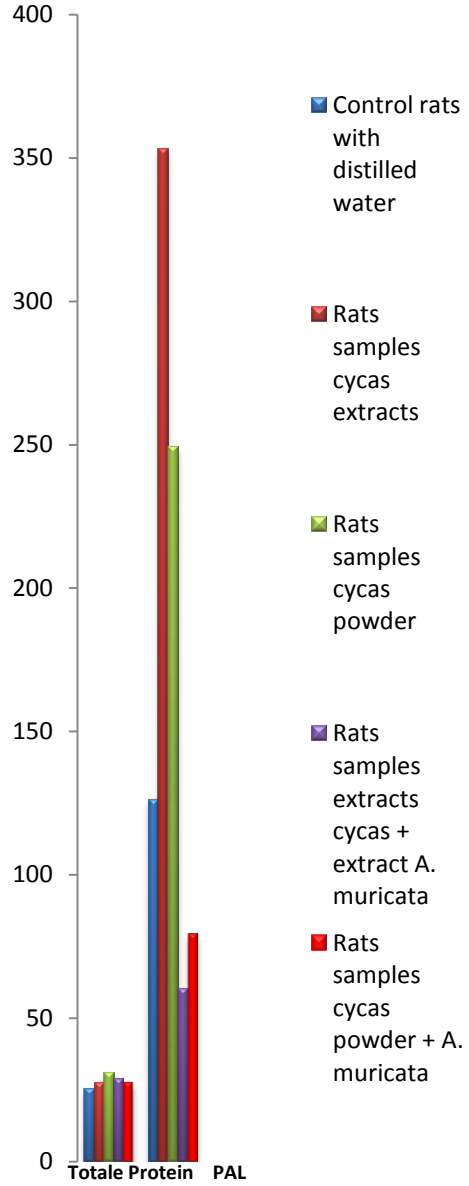


**Figure 3: Changes in total protein and PAL parameters as a function of the diet containing or not containing Cycasrevoluta and the administration of the ethyl extracts of the leaves of Annonamuricata**.

Compared with the rats of the first experiment, it is observed that after the administration of the extracts of the leaves of Annonamuricata a considerable decrease in the PAL act ivity.

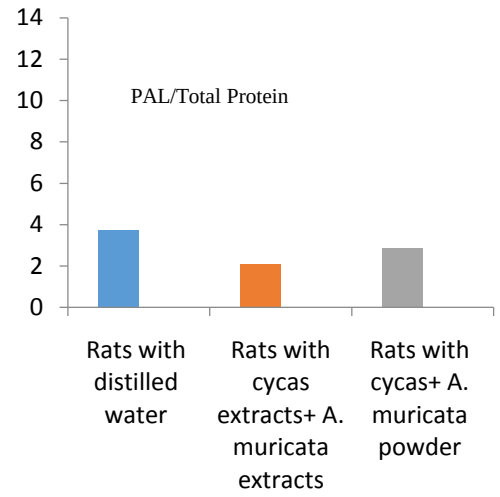


**Figure 4: Variation of total PAL / tissue protein ratio according to the diet containing or not containing Cycasrevoluta and the administration of the ethyl extracts of the leaves of Annonamuricata**.

The PAL / Total Protein ratio of rats given cycas extract and Annonamuricata extract decreased, as well as those receivingCycas powder and Annonamuricata extract.

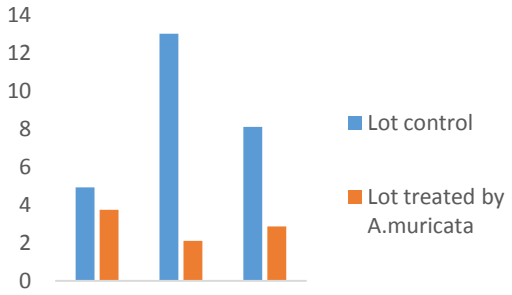


**Figure 5: Comparison of total PAL / protein ratio between control lot and lot treated with Annonamuricata**

A fall in the total PAL / protein ratio was observed in rats treated with Annonamuricata.

## DISCUSSION

According to the WHO in 2006, cancer is a term used to refer to autonomic and anarchic malignant proliferation of cells.
They are a major cause of morbidity and mortality in the world.
In Africa, 600,000 cases of cancer are reported each year and 500,000 people die, according to WHO . This may be due to the high cost of conventional treatment methods such as chemotherapy, radiotherapy, and surgery (Kintzios E, 2006.). To remedy this, they therefore resort to traditional medicine based on the use of medicinal plants. In an extended study the anticancer properties of 187 plants were evaluated Biba V,. 2014.) includingAnnonamuricata.
Biba V, 2014.) states that Annonamuricata is a powerful anti-cancer of the family Annonaceae. It is in this perspective that we used the leaves of Annonamuricata
At the end of our study, we carried out an ethyl extraction using the powder of the leaves of Annonamuricata and 90 ° ethanol. We obtained a yield of 10.5%, which we judge of means compared to the yield obtained by EkaPrasati et al. In 2012 (EkaP,2012) which is 14.86%. This difference could be explained by the difference in degree of ethanol used, which is 70 ° in their case.
We also carried out an ethyl extraction of the leaves of Cycasrevoluta. We had an average return of 10.2%. Cycasrevoluta would contain Cycasin (methyl azoxymethanol) which is known to induce cancer.
Since researchers have become aware of the effective carcinogenic properties of methyl azoxymethanol, these agents have been used to create reliable cancer animal models. This is why we used the powders and extracts from the leaves of Cycasrevoluta.
We therefore first checked the carcinogenicity of the powder and the extract of the leaves of Cycasrevoluta on the proliferation of cells of the cervix. For this, the rats received a diet containing either the Cycas extract or the Cycas powder and the total protein and PAL parameters were assayed. It should be noted that the PAL parameter was chosen because being a cellular enzyme found at different levels of the cell reflects the condition of the tissue and the increase in its sign activity, tissue suffering.
There was a slight increase in total proteins and a significant increase in PAL activity. These results are consistent with those

obtained by Aivodji N. in 2014 Aivodji , 2014) which state that Cycas stimulates the increase of total proteins.
The PAL / total protein ratio accounts for the stage of differentiation (Kouadio K. , 2006). Our results show that with the extract of Cycas, the differentiation is stronger than with the powder of the leaves of Cycas.
However, studies in Nigeria by Okolie N. P. et al. In 2013 showed an anticancer effect of the ethyl extracts of the leaves of Annonamuricata in the colorectal cancer induced by Cycascircinalis which is a plant of the same family as Cycasrevoluta.
Our results show that after the administration of the extracts of the leaves of Annonamuricata, a decrease in PAL activity is observed. Similarly, the PAL / total protein ratio of rats receiving Cycas and Annonamuricata extracts decreased significantly as did the PAL / total protein ratio of those receiving Cycas powder and extracts from the leaves of Annonamuricata. From our results it results that the extracts of the leaves of Annonamuricata could stop the differentiation of the cervical cells of the Wistar rat.

## CONCLUSION

The results of our study show that the powder and extracts of Cycasrevoluta significantly stimulate the differentiation of the cervical cells of the rat and the extracts of the leaves of Annonamuricata diminishes the differentiation. These results have in many respects promoters for the fight against cervical cancer suggest research on the mechanisms of action of these extracts.

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