



# IJSRM

INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY

An Official Publication of Human Journals



Human Journals

**Research Article**

January 2018 Vol.:8, Issue:3

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## The Action of Aqueous Extracts of the Bark of the Trunk of *Sclerocarya birrea* on the Proliferation of Hepatic Cells Induced by *Cycas revoluta*



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**Submission:** 19 December 2017

**Accepted:** 29 December 2017

**Published:** 30 January 2018



HUMAN JOURNALS

[www.ijsrm.humanjournals.com](http://www.ijsrm.humanjournals.com)

**Keywords:** *Sclerocarya birrea*, *Cycas revoluta*, proliferation, phytochemical screening, liver

### ABSTRACT

The purpose of this work is to examine the action of aqueous extracts of the bark of the trunk of *Sclerocarya birrea* on the proliferation of liver cells. For this purpose, an aqueous extraction is carried out by maceration with the dried bark powder of the trunk of *Sclerocarya birrea*. Then we carried out the phytochemical screening of the bark of the plant by the method of Houghton and Raman. Finally, the antiproliferative activity is evaluated by assaying the biochemical parameters Alanine Amino Transferase (ALAT), Aspartate Amino Transferase (ASAT) and total proteins in the blood and on the liver broths of the Wistar rats put under experimentation. Proliferation is induced by exposure of rats to *Cycas revoluta* powder through a 5% diet of this powder and a drinking water containing 10 mg/kg of the same powder and the antiproliferative activity is carried out by Administration of a daily dose of 100mg / kg of aqueous extracts of the bark of the trunk of *Sclerocarya birrea*. The results of the phytochemical screening revealed the presence of several chemical compounds: catechic tannins, flavonoids, leucoanthocyanins, anthraquinones, alkaloids, mucilages and phenol. These chemical compounds with various properties are found at various concentrations. The antiproliferative activity is effective and shows a variation in intensity according to the mode and the duration of administration.

## INTRODUCTION

Cancer is a condition characterized by the presence of one (or more) malignant tumor formed from transformation by mutations or genetic instability (cytogenetic abnormalities) of an initially normal cell.

Long regarded as the evil of developed countries, cancer has now become a public health problem in developing countries; Aggravated by the additional difficulty of a much later diagnosis [1].

According to the World Health Organization, one of its characteristics is the rapid proliferation of abnormal cells that can swarm in other organs, forming so-called metastases. It is caused mainly by environmental factors. These carcinogens (carcinogens) may be present in food, water, air, chemicals and sunlight to which people are exposed [2].

Cancer remains one of the great challenges of our time [3].

5.3 million men and 4.7 million women worldwide developed cancer and 6.2 million people died in 2000. Cancer on this date is responsible for 12.5 percent of global adult deaths, more than the proportion of HIV, tuberculosis and malaria deaths combined [4].

Data collected over several years reinforce the idea that cancer will be a major concern in developing countries [5].

While treatments have made great strides and the incidence of some cancers tends to decrease, others are constantly increasing [3].

Cancer can occur on any tissue or cell. We can distinguish several types of cancer, including liver cancer. Like all cancers, liver cancer is due to damage to the deoxyribonucleic acid, which causes the conversion of the normal cell into a cancer cell [6]. These damages to DNA, most often have infectious causes with regard to liver cancers. Globally, it is one of the most frequent cancers [7]. Whatever the cause of cirrhosis, the incidence of degeneration is about 1 to 3% per year. Primary liver cancer can be promoted by chemical carcinogens (aflatoxin in Africa). Aflatoxin is a fungus caused by *Aspergillus flavus* and *Aspergillus parasiticus* found throughout tropical areas (corn, peanut, soybean) [8]. Thus, the population of Benin is particularly exposed to this cancer because its diet is very rich in cereals (but beans) and in cossettes [4].

Plants have always been a useful source of medicine. In the last generation, before the advent of modern allopathic medicine, herbs and plant-derived substances have been the mainstay of traditional medicine in the world [9].

Indeed, economic and socio-cultural reasons make more than 80% of the African populations use the traditional medicine which remains an essential element for the effective management of the health of the populations.

Also called Marula, *Sclerocarya birrea* is one of the most sought-after native trees in southern Africa. A plethora of ethnotherapeutic properties and pharmacological actions have been attributed to marula (family: Anacardiaceae) [10]. Bark, root and leaf strains have been used in South Africa and some other African countries to manage a network of human diseases, including malaria, dysentery, headaches, toothache, Back pain and the painful body, infertility, schistosomiasis, epilepsy and diabetes mellitus [11, 12].

This study will, therefore, be carried out to examine the action of aqueous extracts of the trunk bark of *Sclerocarya birrea* on the proliferation of liver cells in order to demonstrate its safety and potential as a possible head for the development of anticancer drugs.

### 1.1.1 Features and description

*Sclerocarya birrea* is a tree 8 to 10 m tall, with a dense rounded crown, with a scaly, silvery-grained bark and alternately imparipinnate leaves (12 to 20 obovate leaflets with whole or toothed margins, often on the same tree); At the extremity of thick branches there are flowers of reddish or greenish colors; The large round fruits of yellow color are numerous [13].

*Sclerocarya birrea* is a dioecious species with terminal breeding inflorescences on female feet, 3 to 5 cm in length and inflorescences in terminal spikes on male feet, 5 to 8 cm long. The female flower is pedicellated (about 1 cm long), reddish or greenish, about 7 mm in diameter. The male flower, subsessile, has 4 pink or greenish petals, about 7 mm in diameter. The fruit is a globular, glabrous, yellow to ripe, thick-skinned drupe, 3 to 3.5 cm long, containing a thick core (Figure 1). Flowering and fruiting occur at the end of the dry season, rather before the appearance of the first leaves. [14]



a)



d)



b)



e)



c)

**Figure 1: Photo of the tree (a) [15], Leaves and fruits (b) [15], Trunk with part removed (c) [16], Fruits (d) [15] from *Sclerocarya birrea* (e)**

### 1.1.2. Systematic classification

The classification of the species *Sclerocarya birrea* is as follows:

Vegetal biology

Under the reign	Eukaryotes
Group	Chlorophyll eukaryotes
Subgroup	Vascular Embryophytes
Branching	Spermatophytes
Under branch	Angiosperme
Class	Dicotyledons
Subclass	Rosidae
Group	Rosidae obdiplostemonous to ovary Super and nectariferous disc
Order	Sapindales
Family	Anacardiaceae
Genus	Sclerocarya
Species	birrea

### 1.1.3. Synonyms, vulgar and local names

• Synonyms:

*Sclerocarya birrea* bears many other names under which it is still designated as *Sclerocarya caffra* Sond. (1850), *Poupartia caffra* (Sond.) H. Perrier (1944), *Spondias birrea* (A. Rich), *Poupartia birrea* (A. Rich) Aubrev. (1950).

- Common Names:

*Sclerocarya birrea* is referred to by several vulgar names as Prunier d'Afrique, *Beer Sclerocarya*, *Plumier jaune* (French), Canhoeiro, Morula (Portuguese), Marula, Cidertree (English), Moroela (Afrikaans), Umgamu (Zulu) [17].

- Noms locaux:

In Benin, *S. birrea* is designated under various vernacular or local names according to the localities: Luley, Moru-Moru, Diney (Dendi), Bunamagbu (Gourmanche), Eddy (Fulani), Damahabu (Wama) [18].

### **Habitat and geographical distribution**

#### **The species is quite common, more or less gregarious, in the Sudanian**

Sahelo-Sudanian [13], growing mainly on sandy soils (Arbonnier, 2002). Yellow Plum is found mainly in sub-Saharan Africa outside the wetland zone, from Mauritania and Senegal to Ethiopia and Eritrea, and south to Namibia, Botswana, Zimbabwe, Mozambique, South Africa and Swaziland. It is present in Madagascar and was introduced to Mauritius and Reunion [14].

#### **2.2.2.1. Preparation of the aqueous extract of the trunk bark of *Sclerocarya birrea***

To obtain the aqueous extract of the trunk bark of *Sclerocarya birrea*, 88 g of the trunk bark powder was weighed using a Sartorius analytical balance ® and added to 500mL of water, the whole is brought to maceration. The macerate is filtered at the end of each 24h for 72 hours. The deposit is each time macerated until the end of the 72 hours. The filtrate obtained is poured into dishes and then placed in an oven for evaporation at 50 ° C. After drying completely, the dry extracts adjoining the bottom of the dishes are scraped with a stainless steel spatula. The powders obtained are stored in sterile, hermetically closed glass bottles. The extract was made three times in order to obtain a correct and reliable yield.

### **Gavage of rats**

- *Cycas revoluta* powder and aqueous extracts of the bark of *Sclerocarya birrea* should be administered to Wistar rats of about 200g with the same food intake, orally and at various doses for 28 days. These animals do not receive any other medicinal treatment over time.

The rats are randomly assigned to 03 batches of 03 rats (No. 1, No. 2 and No. 3). Batch 1 (control batch) received only distilled water with a simple diet consisting of pellets and tap water. Batch 2 received a feed composed of granules and 5% of *Cycas revoluta* powder and 10 mg/kg of Cycas powder diluted in the drinking water. As for the rats of lot 3, they received a feed composed of granules and 5% of *Cycas revoluta* powder; Of 10mg / kg of Cycas powder and 100mg / kg of aqueous extract of the bark of the trunk of *Sclerocarya birrea* diluted in the drinking water.

Then we weigh each of the rats of each batch in order to find the average weight and calculate the effective dose of extract that will be administered to it. Then rats are given daily and at the same time.

For each series of experiments, the extract will be weighed and dissolved in distilled water in order to obtain the solutions according to the desired concentrations. The volume to be administered per dose was set at  $V = 01 \text{ mL}$ .

- Half of the rats of each batch are sacrificed after 10 days of gavage, in order to dose the different parameters ASAT, ALAT, total proteins on the livers.

- The gavages are continued in the rest of the rats, so Lot 2 received a feed composed of granules and 5% of *Cycas revoluta* powder and 10mg / kg of Cycas powder diluted in drinking water until the 14th day. From the 15th day onwards, these rats received a diet consisting of 100mg / kg body weight of aqueous extracts of the trunk bark of *Sclerocarya birrea* diluted in the drinking water. The rats of lot 3 received from the beginning to the 28th day a feed composed of granules and 5% of *Cycas revoluta* powder; Of 10mg / kg of Cycas powder and 100mg / kg of aqueous extract of the bark of the trunk of *Sclerocarya birrea* diluted in the drinking water. Batch 1 (control batch) received distilled water with a simple diet.

Finally, the rest of the rats are sacrificed 24 hours after the last gavage, in order to take the livers for our various manipulations.

## MATERIALS AND METHODS

### Plant material

It consists of barks of the trunk of *Sclerocarya birrea* and leaves of *Cycas revoluta*. The bark was harvested in May 2016 in the village of Gomez-kparou in the commune of N'dali in northern Benin by a technical team on expedition. They were cut into small pieces and then dried at laboratory temperature for two weeks. The bark was powdered and macerated in water. The leaves of *Cycas revoluta* were also dried at laboratory temperature, then crushed and powdered. The powders obtained were then stored in glass jars in order to avoid the installation of polluting microorganisms.

+: Présent; - : Absent

## RESULTS AND DISCUSSION

### Results

Yield of Extraction

**Table 3: Extraction yield, color and appearance of the extract**

Extrait	Rendements en %	Couleur	Aspect
macérat/eau	$R_1=14,04 \times 100/88$ $R_1= 15,95$ $R_2=14,98 \times 100/88$ $R_2=17,02$ $R_3=15,90 \times 100/88$ $R_3=18,07$ $R= (R_1+R_2+R_3)/3$ $R=(15,95+17,02+18,08)/3$ $R=17,01$	Rouge au vin	En forme de cristal

The yield obtained is:  $[17.01 \pm 1.06]$  %

**Results of Phytochemical Screening of *Sclerocarya birrea***

**Table 4: Results of phytochemical screening of the trunk of *Sclerocarya birrea* trunk**

Métabolites secondaires	<i>Sclerocarya birrea</i>
Phénol	+
Tannins catéchiques	+
Tannins	
Tannins galliques	-
Anthocyanins	-
Flavonoïdes	+
Leuco-anthocyanes	+
Anthraquinones	+
Alcaloïdes	+
Mucilages	+
Stérol et terpènes	-
Coumarines	-
Saponosides	-

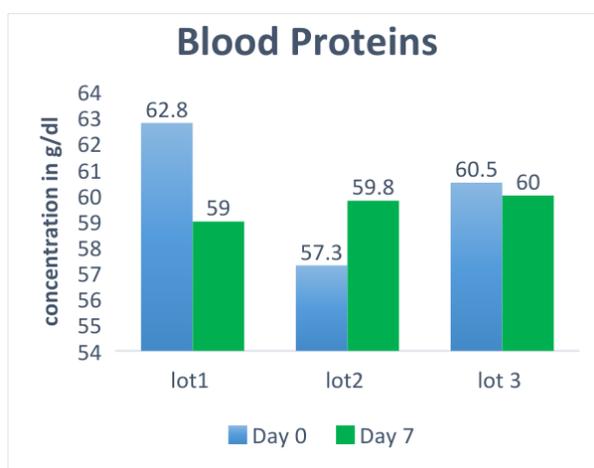
The phytochemical screening of aqueous extracts of the bark of the trunk of *Sclerocarya birrea* reveals the presence of the chemical compounds: catechic tannins, flavonoids, leuco-anthocyanins, anthraquinones, alkaloids, mucilages and phenol.

**Results of biochemical tests**

**Results of blood tests**

**Total proteins**

**Series 1**



**Figure 2: variation of the blood proteins from day 0 to day 7 in rats having undergone 10 days of gavage.**

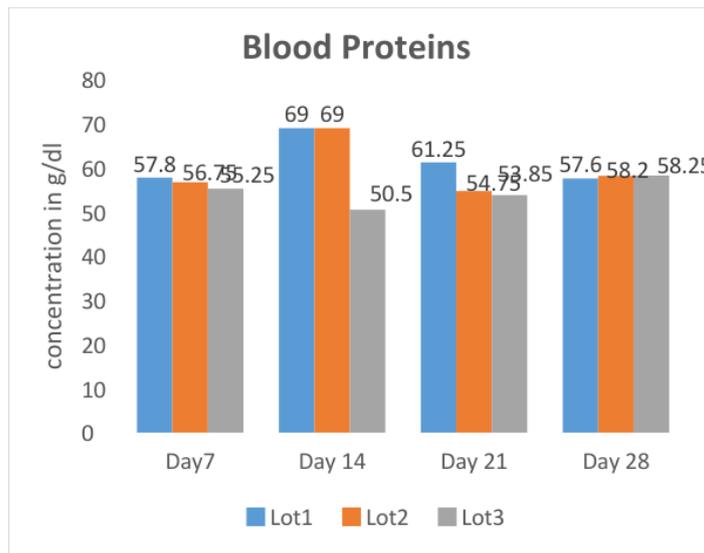
Lot 1 ': control rats

Lot 2 ': rats having received Cycas for 10 days

Lot 3 ': rats having received Cycas and extracted *S. birrea* for 10 days

The graph shows an increase in blood proteins in batch 2 'and a slight decrease in batch 3' after 7 days of gavage.

### Series 2



**Figure 3: variation of blood proteins from day 7 to day 28 in rats having undergone 4 weeks of gavage.**

Lot 1: control rats

Lot 2: Cycas rats for 2 weeks then extracted *S. birrea* 2 weeks

Lot 3: Cycas rats and *S. birrea* extract for 28 days

After 14 days of gavage, a rise in proteins is observed in batches 2 and 3. The breeding is much more pronounced in batch 2. After 21 days, the proteins decrease and increase again after 28 days of gavage.

### ASAT Serum

#### - Series1

Après 7 jour de gavage, on remarque une diminution du taux d'ASAT au niveau des lots 2' et 3' contrairement chez le lot 1'.

#### - Series 2

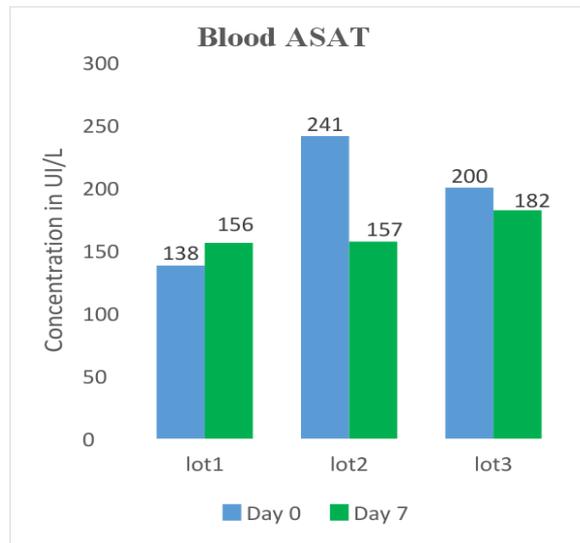


Figure 4: Variation of serum ASAT from day 0 to day 7 in rats having undergone 10 days of gavage.

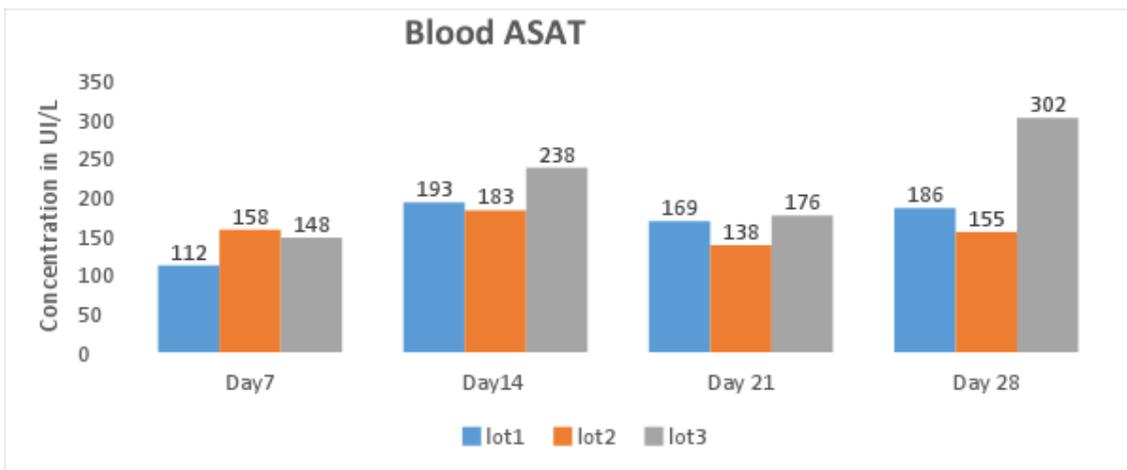


Figure 5: Variation of serum ASAT from the 7th day to the 28th day in rats having undergone 4 weeks of gavage.

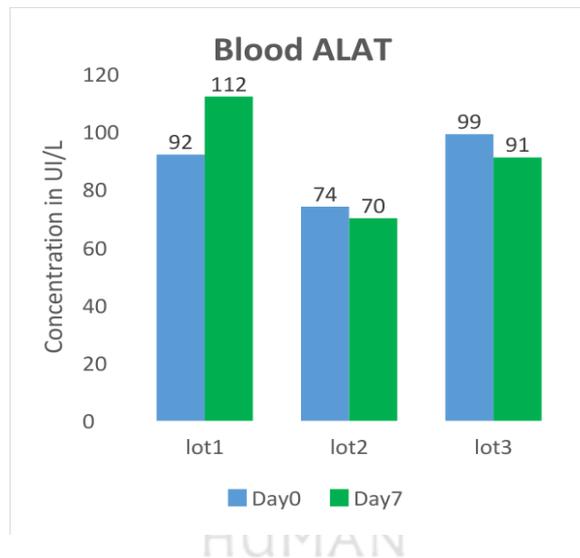
The blood ASAT increased on day 14 at the 3lot level, decreased on day 21 and increased again on day 28.

Serum ALAT

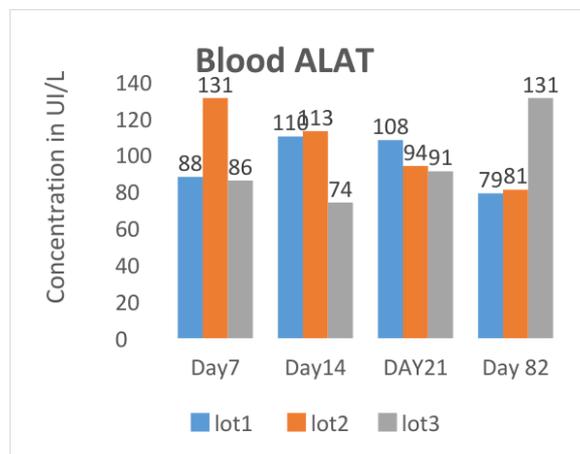
**Series 1**

ALAT decreased in lots 2 'and 3' while it increased in lot 1 '.

**Series 2**



**Figure 6: Variation of serum ALAT from day 0 to day 7 in rats having undergone 10 days of gavage.**



**Figure 7: Variation of serum ALAT from the 7<sup>th</sup> day to the 28<sup>th</sup> day in rats having undergone 4 weeks of gavage.**

Throughout the period of force-feeding, ALAT had a decreasing pattern in lot 2. In lot 3, it decreased to the 14<sup>th</sup> day, believed in the 28<sup>th</sup> day.

### Results of assays on the tissue

**Table 1: Result of the assays carried out on the liver of rats having undergone 10 days of gavage.**

#### Series 1

	lot 1	lot 2	lot 3
<b>Protéines (g/dL)</b>	17,75	16,85	15,95
<b>ASAT (UI/L)</b>	1301	1739	1913
<b>ALAT (UI/L)</b>	2622	2611	2669

An increase in the level of the three parameters was observed in rats given *Cycas revoluta* alone for 10 days (lot 2 ') compared to that of the control (lot 1). This value decreased slightly in rats receiving *Cycas revoluta* and *Sclerocarya birrea* for 10 days.

**Table 2: Results of the assays performed on the liver of rats having undergone 28 days of gavage.**

#### Series 2

	lot 1'	lot 2'	lot 3'
<b>Protéines (g/dL)</b>	15,6	17,2	16
<b>ASAT (UI/L)</b>	1674	1804	1643
<b>ALAT (UI/L)</b>	2652	2692	2628

The total protein level decreased slightly in lot 2 (rats taking *Cycas revoluta* for 14 days and *Sclerocarya birrea* from day 15 to day 28) compared to lot 1 (control). This decrease is much more pronounced in lot 3 (rats having received *Cycas revoluta* and *Sclerocarya birrea* for 28 days). ASAT increased in lot 2 and lot 3. As for ALAT, it decreased in lot 2 and increased in lot

## DISCUSSION

The dry barks of the trunk of *Sclerocarya birrea* contain active substances which are soluble in water and therefore extractable by the latter. At the end of the extraction, we obtained a

yield of  $[17.01 \pm 1.06]$  %. This seems normal as several studies have shown the rate of return of (16%) [20; 21] by the aqueous maceration extraction method. On the other hand, our performance is quite high compared to that (9.1%) obtained by Amos SOSSOUNON [19]. This difference could be explained by the diversity of extraction methods.

The phytochemical screening carried out by the method of Houghton and Raman (1998) allowed us to detect the following compounds: catechic tannins, flavonoids, leucoanthocyanins, anthraquinones, alkaloids, mucilages and phenol. The presence of these chemical groups largely confirms the results obtained by Amadou Adiza [16] who was unable to detect mucilages in the bark of the trunk of *Sclerocarya birrea*. This could be due to the origin of the plant used or the nature of the solvents.

The alkaloids of periwinkle are part of anticancer agents (anti-fusorales) [22]. They could be the molecule with antiproliferative action because being contained in the bark of *Sclerocarya birrea* (positive alkaloids). Indeed, the periwinkle alkaloids act on the mitotic spindle by inhibiting the polymerization of the tubulin dimers, thus preventing the formation and growth of the microtubules. The cells being blocked in metaphase, the cell division will be stopped.

Our study revealed an increase in the level of ASAT, ALAT and total protein in rats consuming Cycas powder for 10 days compared to the control group. As total proteins have increased from normal, this leads us to say that there has been abundant and abnormal expression (transcription and translation) of certain genes. The increase in transaminases may reflect a multiplication of liver cells (cell proliferation) because these enzymes are cytosolic proteins of the liver cells. This only confirms the carcinogenicity of *Cycas revoluta*. In fact, *Cycas revoluta* contains cycasin (aglycone methyl azoxymethane), which is known to induce liver cancer and many others [23]. After ingestion of a meal containing the Cycas powder, the intestinal bacteria hydrolyze the glucoside bond of the cycasin to release the aglycone, methyl azoxymethane [6]. At the level of the population has undergone 28 days of gavage, we note a decrease of the total proteins in the two batches. This could translate an anti-transcriptional or anti-translational action of our extract. Note that the decrease is much more pronounced in rats of lot 3 (rats having received *Cycas revoluta* and *S. birrea* for 28 days). AST and ALT levels decreased in rats in lot 2 (rats consuming Cycas for 14 days and *S. birrea* rats from day 15 to day 28), whereas those in lot 3 increased. This could mean that the proteins that decreased in lot 3 were neither ASAT nor ALAT. The decrease observed in lot 2 leads us to say that the aqueous extract of *S. birrea* decreased the abnormal multiplication of the

hepatocytes induced by the powder of the leaves of *Cycas*. Our results are in agreement with those obtained by Okolie N'gozi P. *et al.* (2013) hence the hypothesis of an antiproliferative activity is confirmed.

Finally, the difference in the effects of the extracts of leaves of *Annona muricata* according to whether they were administered simultaneously with the powder of *Cycas* or after the powder of *Cycas* could be explained by the fact that the acetogenins which would be the active anticancer compounds of our extracts have for mode of action to eliminate already induced cancer cells [6].

Thus the extracts would be more effective when administered after inductions of proliferation by *Cycas* powder.

This weakness of efficacy in the case of the simultaneous administration of our extracts with the *Cycas* powder could also be explained by the fact that the active compounds contained in these two plants could be antagonist molecules having the same receptors on the cells and thus trigger routes of contrary signals. Thus, simultaneity in the administration of the two plants could induce competition, hence inefficiency.

## CONCLUSION

Plants relieve many Africans in health because of their high availability and accessibility. In Benin, about 80% of the population uses traditional medicine for health purposes. Liver cancer is becoming increasingly widespread in Benin, studies on the search for new molecules able to constitute more accessible and less expensive treatments for

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