Phytochemical screening, antimicrobial and in-vitro cytotoxic activities of several extracts of six medicinal plants (Crossopteryx febrifuga Benth, Khaya anthotheca, Gardenia ternifolia, and Terminalia glaucescens) from central Africa region

Abstract

The qualitative study of secondary metabolites of the root and stem bark of Crossopteryx febrifuga Benth, Khaya anthotheca, Gardenia ternifolia, and Terminalia glaucescens obtained by maceration were estimated by tub reaction. Antimicrobial and cytotoxic activities of the root and stem bark of the same plants obtained by maceration and subcritical water extraction were respectively tested whit antibiogram disc test on Pseudomonas Aeruginosa (ATCC 27853), Enterobacter Hormaechei (ATCC700323), Bacillus Spizizenii (ATCC6633), Enterococcus Casseliflavus (ATCC700327), Staphylococcus aureus subsp. Rosenbach (ATCC 6538), and Salmonella Typhus (ATCC 6539). The Tetrazolium test (MTT test) was realized by using the mouse fibroblast cells (NIH3T3). Extracts based on the bark of Terminalia glaucescens and Crossopteryx febrifuga Benth induce sensitivity to 4/6 bacterial strains, in particular Enterobacter Hormaechei (ATCC700323): 3±2cm; Bacillus Spizizenii (ATCC6633): 5±2 cm; Staphylococcus aureus subsp. Rosenbach (ATCC 6538): 5±2; and Salmonella Typhus (ATCC 6539): 3±2cm.

Extracts based on the bark of Khaya anthotheca and those of the bark of Gardenia ternifolia induce only the sensitivity on 2/6 bacterial strains specially Enterobacter Hormaechei (ATCC700323) and Bacillus Spizizenii (ATCC6633) respectively 4±2cm and 5±2cm. Extracts based on the barks and roots of Khaya anthotheca and those of the roots and barks of Gardenia ternifolia only induce sensitivity on 2/6 bacterial strains tested, in particular the strain of Enterobacter Hormaechei (ATCC700323) and Bacillus Spizizenii (ATCC6633). Only extracts based on the bark of Crossopteryx febrifuga Benth and Terminalia glaucescens show sensitivity against Salmonella Typhus (ATCC 6539).

All the plant extracts tested show very low cytotoxic power. Plant extracts obtained by the maceration method (Hydro Methanol 90%) induce 19% inhibition of cell growth compared to extracts obtained by supercritical water 125°C 60ATM and 125°C80 ATM. The extracts obtained using the supercritical water 125°C 60ATM method are the least offensive on cell proliferation. The most concentrated extracts show the greatest inhibitions. The extracts of plant E show the greatest inhibition at a concentration of 1 g/ml.

Keywords: Crossopteryx febrifuga Benth, Khaya anthotheca, Gardenia ternifolia, Terminalia glaucescens, Antimicrobial activity, cytotoxic effect, phytochemical activities, Maceration, subcritical water, and tetrazolium test,

1- Introduction

The extracts devoted to this study are extracts from plants that grow in tropical and subtropical areas. The literature reports their use in the pharmacopoeia of several African, South American and Asian countries. In the Central African Republic, all these plants are administered to

treat humans and livestock. Very few studies report their effectiveness on the diseases against which they are administered and also their harmlessness on the biological functioning of the organism.

Crossopteryx febrifuga Benth,

Group	Dicot
Family	Rubiaceae
Genus	Crossopteryx
Specie	Crossopteryx febrifuga (Afzel. ex G.Don) Benth.

Table 1: Botanical classification of Crossopteryx febrifuga Benth (Balde et al., 2016)

Figure 1: Photo of Crossopteryx febrifuga Benth branch

It is a shrub, characteristic in appearance with its opposite ramifications, can reach six meters in height. It's oval and opposite leaves are often reddish at their base. It's very fragrant white flowers are grouped in bouquets at the top of the branches. They give rise to round and green fruits, blackish when ripe. This species is present in a disseminated way, in the wooded savannah of Africa **(Jardin-du-monde, 2017).**

Its dry seeds can remain on the tree for 1 to 2 years. Crossopteryx febrifuga Benth (Rubiaceae) is a deciduous tree widely used in African traditional medicine for the treatment of several diseases such as dysentery, diarrhoea, malaria, fever, and inflammatory disorders; Previous work reported analgesic, anti-inflammatory, antipyretic, antimicrobial, hypoglycemic, cytotoxic, antioxidant, antiplasmodial and antitrypanosomal properties of C. febrifuga extracts **(Kayangar et al., 2019).** The powder of its bark is mixed with salt to give cattle in the Central African Republic, but the reels impacts, this while its true impact on health is not known.

K*haya Anthotheca,*

Group	Dicot
Family	Meliaceae
Genus	Khaya
Species	Khaya anthotheca (Welw.) C.DC.
Species	*Khaya anthotheca. Khaya*

Table 2: Botanical classification of Khaya Anthotheca (Chauvet, 2015)

The reddish-brown wood is very popular in carpentry, sculpture, and the manufacture of traditional canoes. The genus Khaya includes 4 species on the African continent. It belongs to the subfamily Swietenoideae (melaceae) and appears to be closely related to Carapa and Swietenia.

Khaya species have strong similarities in flowers and fruits, but the clearest differences is in their leaflets. Due to selective logging, many species of Khaya madagascariens are on the International Union for Conservation of Nature (IUCN) Red List as endangered species in the tropical forests of Africa. **(Maroyi, 2008).** The bitter-tasting bark is widely used in traditional medicine for humans and monkeys. Its decoction or infusion is recommended for the treatment of fever, cough, colds, pneumonia, abdominal pain, vomiting and gonorrhea;

In external use, it is applied to wounds, abrasions and ulcers **(C. J. D. Obboa)**. In **DR Congo**, the leaves are said to be used to make arrow poison (**Maroyi, 2008).**

In Tanzania a root decoction is drunk to treat anaemia, dysentery and rectal prolapse. And then, the bark was formerly used by the Shambaas peoples to dye reddish brown.

(C. J. D. Obboa). In the Central Africa region the bark is used as an anthelmintic in calves and cattle.

***Gardenia Ternifolia*,**

Figure 2: Photo of a Gardenia Ternifolia tree in its habitat (Central African Savannah)

Group	Dicot
Family	Rubiaceae
Genus	*Gardenia*
Specie	*Gardenia Ternifolia*

Table 3: Botanical classification of Gardenia Ternifolia (Agbodjento, Klotoé, Dramane, Dougnon, & Ategbo, 2019)

It is a flowering plant in the family Rubiaceae native to tropical, subtropical regions of Africa, Australasia and Oceania. Gardenia T. is an evergreen shrub, 1 to 6 meters high. Its short stems (or trunks) support an irregular and open crown. The bark greenish-yellow flaking off in fine irregular gray scales after the passage of fires. Their leaves are grouped in tufts at the end of thick, very short, rigid branches. They are 2 to 10 cm long. The pericarp is thick and fibrous. The fruits remain on the shrubs for much of the year.

An ethno pharmacological study reveals that Gardenia T. is mainly used in the treatment of malaria, hypertension, diabetes, cough, asthma, rheumatism, diarrhea, dental caries, leprosy, hernia, hemorrhoid and cancer **(Agbodjento, et al., 2019)**. In the Central African Republic, the powder of its bark is mixed with salt to be used as cattle feed (Amadou, 2020).It has a antimicrobial properties **(Magassouba et al., 2007)** and used again haemorrhoid lesions, malaria in Ethiopia **(Nureye, Assefa, Nedi, & Engidawork, 2018),** again diabetes in Cameroon **(Guy Bertrand Sabas Nya Njomen, 2008).**

Terminalia glaucescens Planch. Ex Benth.

Figure 3: Terminalia glaucescens Planch tree. Ex Benth in its Natural Habitat (Central African Savannah)

Group	Tracheophyta
Family	Combretaceas
Genus	Terminalia
Specie	Terminalia schimperiana
Species Synonym	*Terminalia glaucescens*

Table 4: Botanical classification of Terminalia glaucescens Planch. Ex Benth (Pakull et al., 2019)

Is a flowering plant in the Combretaceae family and is commonly found in tropical and subtropical regions. The plant is locally abundant and its common names are: bawshe (Hausa), bawshihi (Fullani), Idi Odan (Yoruba), Edo (Igbo). The plant is also reported to have traditional medicinal uses such as antimalarial, treatment of diarrhoea and tooth decay. The potential of aqueous extracts of T. glaucescens stem bark against some pathogenic organisms have been extensively investigated **(Adebayo, 2009)**. It has proven antimicrobial properties and zero cytotoxic effects **(Konan K. Fernique, 2016; Magassouba et al., 2007).**

2- MATERIALS AND METHODES

2.1. Plant's simple collection

Fig 4: Geographical coordinates of the plant sampling area

Plant's simple collection were realized according to the method described by (Hanan Bandar, 2013). Crossopteryx febrifuga Benth barks, Khaya anthotheca bark and roots, Gardenia ternifolia bark and roots, and Terminalia glaucescens bark were collected in the village of Gbiti (4° 40 ′ 55″ WEST, 14° 42 ′ 56″) in west of Central African Republic (CAR) on the border with Cameroon on November 2019. Then, all collected crops were packed in opaque plastic bowls and transported to Turkey. The plants were harvested in November 2019 but extraction was carried out 7 months later in July 2020. The plants were well cleaned and washed with water and then dried in the shade and at room temperature. The roots and stems of the plants. They were cut into small pieces and lowered into powder with an electric mixer. The powders were preserved in clean plastic containers, kept away on -80 ° C from light, heat and moisture until use after seven month later. Maceration and subcritical water extraction methods were used for the extraction.

2.2. Plant's extraction

The different secondary metabolites can be demonstrated by using reactions in tubes or by thin layer chromatography (TLC).

2.2.1. Extraction by maceration (Preparation of methanol extraction)

Maceration method 1g of powdered leaves and stems of those plants were blended with 5ml of Hydro methanol 90% for 24h with agitation at room temperature. After, the extracts were taken and filtered by using a 0.6 Millipore Watt man filter paper **(Salawu et al., 2009).** Then, the extracts were concentrated using a rotary evaporator at 40°C under reduced pressure. Finally, the extracts were weighted and stored at -20°C till their usage in the different tests **(Hanan Bandar, 2013).**

Extraction was carried out by maceration of bark powder or root powder in 90% hydro-methanol.

Each 1g of plant matter was soaked in 5ml Hydro-methanol (90%) (40g/200ml); Left with stirring for 2 days at room temperature to dissolve soluble compounds; The mixture was filtered with a 6 mm number Watt man filter **(Salawu, et al., 2009)**. All the solvent was evaporated by rotary evaporator (recycler) at 40-60 °C and then the remaining solid was extracted 0.2g/ml and 0.5g/ml in Dimethyl Sulfoxide (DMSO). The extracts were used to test for antimicrobial effect and cytotoxic MTT test **(Yeo, et al., 2014).**

2.2.2. Extraction by sub-critic water

Subcritical fluids are fluids that are kept liquid by compression below their critical temperature and are used above their boiling point by pressure. Subcritical fluids are also called hot liquid solvents or pressurized liquid solvents, and the process may also be referred to as pressurized or accelerated solvent extraction. The solvent power of subcritical fluids depends on the temperature used, therefore, pressure is used to help maintain the liquid state of the fluid, while temperature helps to increase the solvent. Subcritical extractions at low temperature and low pressure take more time, but essential oils in the plant can be used to retain terpenes and other sensitive chemicals **(Giray, 2008)**.

For all extractions, 1g of herbal extract was filled into aluminum tube.
A plug of wool and sea sand are stuffed at both ends of the aluminum column to serve as a filter. After the extraction column was mounted in the furnace, the inlet needle valve was opened from the pump and the column was pressurized with water. Then the valve is closed and the oven is brought to the desired temperature as quickly as possible. The extraction process has two stages.
By closing the valves, the hydro-static phase takes 30 minutes (Boiling phase) at 125ºC and 60/80 ATM; The inlet and outlet valves are then opened, and 20 minutes of ethanol-dynamic (Collection Phase). Thus, water is pumped from the column at a certain flow rate and the extracts are collected in the bottle **(Giray, 2008).**

Figure 5: Superheated water extraction device and Schematic diagram

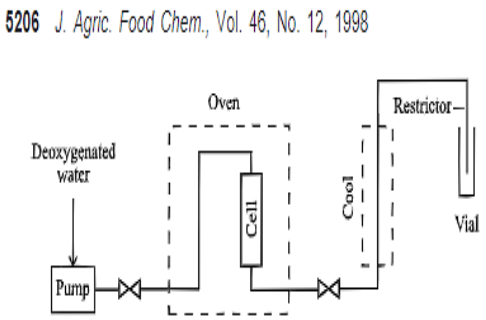


Fig 6. Schematic diagram of the superheated water extraction apparatus

2.3. Phytochemical composition analysis by the tube reaction method of the extracts

2.3.1. Search for alkaloids

Principle: It involves precipitation reactions with general alkaloid developers: Mayer's reagent and Dragendorff's reagent.

Operating mode

Thirstily, Prepare a sulfuric extract from 10g of drug powder and 50ml of H2SO4 diluted to 10%. After macerating for 1 hour, the macerated was filtered and washed with distilled water to obtain 50ml of filtrate. In two test tubes introduce 1ml of filtrate; then add 5 drops of Mayer's reagent to the first and 5 drops of Dragendorff's reagent to the second. In a third tube serving as a control and containing 1ml of a caffeine solution, add 5 drops of Dragendorff's reagent. In the presence of an alkaloid, a precipitate is formed.

2.3.2. Search for poly-phenolic compounds

This will involve identifying the following secondary metabolites: tannins, flavonoids and anthraquinones.

2.3.2.1. Tannins

Tannins are classified into two groups: Catechic tannins and Gallic tannins. There are specific methods for the detection of the two chemical groups.

Procedure:

From 5 ml of a 5% aqueous infused (15 min), add an aqueous solution of iron (III) chloride (1 ml). In the presence of tannins, a greenish or blue-blackish color develops.

2.3.2.2. Catechical tannins

From the 5% infused (5 mL), added concentrated hydrochloric acid (1 mL). The whole was brought to a boil for 15 minutes then filtered.

The formation of a red precipitate soluble in iso-amyl alcohol indicates the presence of catechic tannins.

2.3.2.3. Anthocyanins

Procedure:

From 5ml of infused at 5%, add 5ml of sulfuric Acid (10% H2SO4) then 5ml of Ammoniac (NH4OH) diluted to half. In the presence of anthocyanins, the color is accentuated by acidification and then turns blue-violet in a basic medium.

2.3.2.4. Research on sterols and tri-terpenes

Sterols and triterpenes **(Libermann-Buchard reaction)**.

Preparation of the extract:

From a mass of 1 g, 20 ml of diethyl ether are added. The whole is closed, stirred and left to macerate for 24 hours, then filtered and made up to 20 ml.

Principle: Dissolve the dry ether extract (10 ml) obtained previously in 1 ml of acetic anhydride and add 1 ml of chloroform. Divide the solution between two test tubes, one of which serves as controls. Using a pipette, place 1 ml of sulfuric acid in the bottom of one. A brownish red or purple ring forms at the contact zone of the two liquids, the supernatant layer turns green or purple revealing the presence of sterols and tri-terpenes.

2.3.2.5. Search for Saponosides

Principle:

Their presence is determined quantitatively by calculating the foam index, the degree of dilution of an aqueous decoction giving a persistent foam under the determined conditions.

1) Prepare a 1% decoction of the plant material by boiling for 30 minutes.
2) After cooling and filtration, readjust the volume to 100 ml.
3) In a series of 10 test tubes numbered from 1 to 10, successively distribute 1, 2 …..10 ml of decoction.
4) Adjust the volume of each tube to 10ml with distilled water.
5) Shake each of the tubes is in a horizontal position for 15 seconds.
6) Let each tube rest in a vertical position for 15 minutes then record the height of the persistent foam in cm. If it is close to 1 cm in the X^{th} tube, then the foam index is calculated by the following formula:

$$I = \frac{H_x \times 5}{X} \times 100$$

I: Foam Index; Hx: Height of foam in cm in the X^{th} Tube;
X: Number of the tube where the foam height is equal to 1cm

2.4. Determination of antimicrobial susceptibility

The antibacterial tests of the extracts were used by diffusion method (well diffusion) on agar medium. Each trial was repeated 3 times. Each bacterial strain was seeded in physiological serum at 0.5 McFarland turbidity.

2.4.1. Preparation of bacterial

Bacterial strains were resuscitated 24 hours before inoculation. Different bacterial species were inoculated in a medium suitable for their physiological conditions. Each bacterium was seeded on solid media by broadcasting. Using a blue tip, wells of approximately 6 mm in diameter were made on agar medium. And 100 microliters of Miller Hilton Agar was poured into the bottom of each well to prevent diffusion of the extracts between the petri dish and the agar. In each well, 200 µl of 20 mg/ml or 50mg/ml of the plant extract to be tested was given **(Aladag, 2016)**.

After 30 minutes of diffusion at room temperature, Petri dishes were incubated for 24 hours at the optimum proliferation temperature of each bacterial strain. The presence or absence of a zone of inhibition was measured.

Code	**Bacterial species**	**Used Medium**	**T (^{O}C)**
I	Pseudomonas Aeruginosa (ATCC 27853),	Tryptic soy agar	37
II	Enterobacter Hormaechei (ATCC700323),	Tryptic soy agar	30
III	Bacillus Spizizenii (ATCC6633),	Brain Heart infusion agar	30
IV	Enterococcus Casseliflavus (ATCC700327),	Brain Heart infusion agar	37
V	Staphylococcus aureus subsp.Rosenbach (ATCC 6538),	Muller Hilton Agar	37
VI	Salmonella Typhus (ATCC 6539)	Muller Hilton Agar	30

Table 5: Preparation of bacterial offenses using

2.4.2. Anti-bio-gram disc preparation

The sensitivity of these four bacterial strains to plant extracts was achieved by the Kirby-Bauer disc diffusion technique according to the protocol described by (Hudzicki., 2009). The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.

2.5. Cell Proliferation Assay.

There are several methods to assess the cytotoxicity of plant extracts: XTT staining method, MTT staining method and neutral red absorption staining method **(Orhan TOKUR & AKSOY, 2017).**

To investigate the cytotoxic potential of the extracts the MTT colorimetric assay was used like it was described by **(Gonzalez et al., 2018)**.

2.5.1. MTT TEST PRINCIP

It is the conversion of Tetrazolium salt into a colored formazan by the mitochondrial activity of living cells. In this test, MTT is reduced to purple formazan by NADH. Formazan crystals do not form in the presence of dead cells. Various tetrazolium compounds can be used to detect living cells. One of them is the MTT compound. MTT is positively charged and can easily penetrate living eukaryotic cells **(Mosmann, 1983)**.

Aim: In order to measure the cytotoxicity by showing the metabolic activity, the mitochondrial activity of the cells is examined and living cells are detected (Hart, 2012). The MTT assay is, today, one of the most widely employed viability assays in biomedical research.

2.5.3. Plants' extract preparation

Two methods were used to perform the extraction. Firstly, maceration was carried out in 5ml of 90% methanol per 1g of substance; After that, by subcritical water method, 1g of dry matter per plant was carried out at 125°C-60 ATM or 125°C-80 ATM;

The extracts were filtered through a 0.6 mm Wattman filter and evaporated at 60ºC in a rotary evaporator. The residue is dissolved in DMSO at 0.1g/ml. the extracts were stored at 6ºC for 24h. The extracts were centrifuged at 2000 rpm for 10 minutes. 2 ml of supernatant was taken and centrifuged at 130 rpm for 10 minutes. The extracts were filtered using a sterile 0.20µm Watt Man nano filter.

To evaluate the cytotoxic effects of these extracts by the MTT method according to the protocol described by Mosmann (1983), two sequential dilutions were performed from C0: 1 g/ml to C1: 0.1 and C2: 0.01 g/ml concentrations.

- 2.3.4. In-vitro Cell Culture
- NIH3T3 mouse embryonic fibroblasts Culture For cytotoxicity tests, NIH3T3 mouse embryonic fibroblasts were obtained from Çukurova University Faculty of Medicine, Department of Medical Biology.
- Cells were cultured in T75 flask;
- Removed media;
- Added 4ml trypsin;
- Centrifuge and refill the supernatant,
- Cell count in THOMA cell;
- 100µl added to each well of 96 pallets;
- In each well, 10,000 cells are grown two days before treatment;
- Incubation at 370C and 5% carbon dioxide for up to 24 hours;
- Change culture medium / 100µl of medium added per well;
- Incubated for 4 hours in a 370C incubator;
- Added 100µl of DMSO per well after removing the culture medium;
- Added 100µl of plant extracts to each well;
- Incubation at 24h to room T°;
- After removing the medium using a micropipette, 20 microliters of MTT solution (5 mg/ml) was added and mixed well. The medium was carefully removed and 100 ml of DMSO was added. At the end of the treatment and incubation period with DMSO solution, the reading was taken at 630 nm with the Biochrome EZ READ 4000 Microplate Reader with reference to the wavelength of 560 nm;

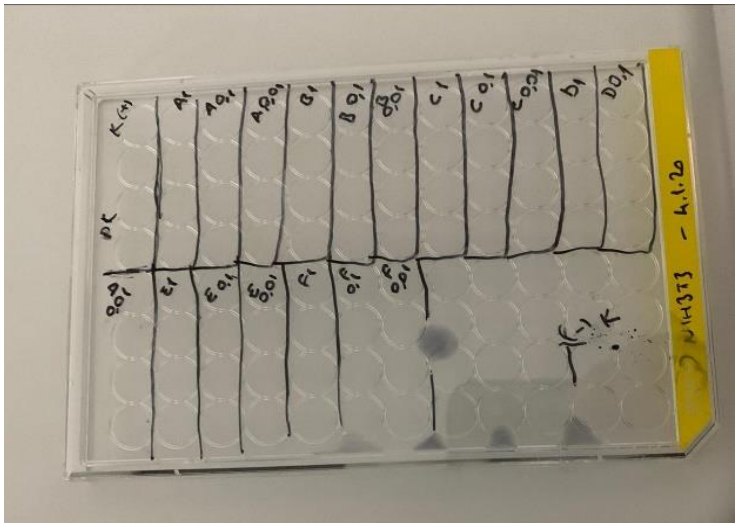


Figure 7: Each sample was diluted in 3 concentrations (1; 0.1 and 0.01 µg/µl) in 96 Micro pallets and repeated in 4 parallels

- Control (+): 4% in 2µl NAOCL 0.04 isopropanol was used
- Control (-): Empty culture medium used

K (+): Positive control

K (-): Negative control

10× 10^3 mouse embryonic fibroblast cells (**NIH3T3)**, suspended in100μL of growth medium, were seeded in 96-wellplates containing100μL of medium in the presence of 1 μL of each tested plant extract, previously dissolved in DMSO (final concentration 1% v/v since no adverse effects on cell growth were observed at this concentration).The extracts were evaluated at a final concentration of 0.1mg/mL. Following the primary screening, those extracts with promising activity on all studied cells were tested at serial dilutions ranging from 0.1 to 0.001mg/mL. The isolated compound was tested at 0.1, 0.01, and 0.001mg/mL. After 24h for, respectively, 20μL of 5mg/mL solution of MTT in sterile PBS was added to each well and further incubated for 2h. Then, the supernatants were removed and replaced with 100μL DMSO to solubilize the resulting purple formazan crystals produced from metabolically viable cells. Absorbance was measured with **Biochrome EZ READ mark 4000 Micro plate Reader** at 560 nm. Four wells were used for each sample assayed and two independent experiments were performed. Untreated and DMSO (1%)-treated cells were used as controls, while DOX (added to reach final concentrations of 0.003 to 40μg/mL) was used as reference. And 2µl of NAOCL 0.04 isopropanol 4% was used like positive control.

2.5.4. Results interpretation

The percentage of cytotoxic activity was determined by the following formula (Barnabe, 2017):

$$\text{Cytotoxicity (\%)} = 1\frac{(\textbf{optical density of treated cells} - \textbf{optical density DMSO})}{(\textbf{opticaldensity of control cells} - \textbf{opticaldensity DMSO})} \times 100.$$

If a value of 120% is obtained, this indicates that cell growth is 20% greater in the sample. Conversely, a value of 75% indicates less cell development of around 25%. This means that the higher the value of the sample is than the value of the white control, its presence does not hinder cell multiplication and growth. So the sample is less toxic. In addition, the value of the sample is lower than the value of the white control (100%), its presence proportionally hinders cell multiplication and growth. So the sample is relatively toxic. **(Lindhagen, Nygren, & Larsson, 2008; Pomothy et al., 2016; Yilmaz et al., 2020).**

3. RESULTS

3.1. Screening phytochimique

The extracts of plants B, C and F present the same chemical components with a high abundance of alkaloids, tannins, anthocyanin's saponosides and relative abundance of sterols and tri-terpenes.

The saponins compounds are very abundance on all plants' extracts. The extracts of plants A and D show a negative results in sterols and tri-terpenes known to have an antioxidant, antiviral and antifungal properties.

3.1.1. Result of alkaloids and poly-phenolic compounds

Samples	Alkaloids	Tannins	Anthocyanin	Saponosides		Sterols and tri-terpenes
				Presence	Foam index	
A	+	++	+	+++	487,71	-
B	+++	++++	+++	+++	1116,68	++
C	+++	+++	+++	+++	1152,77	++
D	+	+	+	+++	508,29	-
E	+	++	++	+++	2215	++
F	+++	+++	+	+++	1108,33	+

Table 6: Alkaloids and Poly-phenolic compounds

Indication: +++: Very abundant; +: Presence -: Absence

3.1.2. Calculation method

3.1.2.1. Formula

$$I = \left(\frac{5.Hx}{X}\right).100$$

I: Foam Index; Hx: Height of foam in cm in the X^{th} Tube;

X: Number of the tube where the foam height is equal to 1cm

3.1.2.2. Foam height

Samples	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
A	-	-	-	1,8	2,7	3,8	4	4,7	5	5,2
B	-	1,5	2,1	3,3	4	4,7	5,1	5,2	6	6,3
C		1	2,3	3,6	4,7	5,1	5,5	6	6,3	7
D	-	-	1,7	2,2	2,4	2,8	3	3,5	3,9	4,9
E	2,2	2,8	3,3	4	4,5	5	5,2	5,9	4,8	6,6
F	-	2	2,5	3,2	4	4,5	5,1	5,6	5,8	6,8

Table 7: saponosides extract foam level

3.1.2.3. Foam Index

Samples	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Foam Index (Median)
A	-	-	-	225	337,5	475	500	587	625	650	**487,71**
B	-	875	525	825	1000	1175	1275	1300	1500	1575	**1116,68**
C	-	250	575	900	1175	1275	1375	1500	1575	1750	**1152,77**
D	-	-	283	367	400	467	500	583	650	817	**508,29**
E	1100	1400	1650	2000	2250	2500	2600	2950	2400	3300	**2215**
F	-	500	625	800	1000	1225	1275	1400	1450	1700	**1108,33**

Table 8: Saponosides foam index

3.2. Antimicrobial test

Plants→		*Crossopteryx febrifuga Benth* (Stem bark)		*Khaya anthotheca* (Stem bark)		*Khaya anthotheca* (Root)		*Gardenia ternifolia* ((Stem bark)		*Gardenia ternifolia* (Root)		*Terminalia glaucescens* (Stem bark)		CONTROLS CN10
Bacteria's cod↓		20mg/ml	50mg/ml	20mg/ml	50mg/ml	20mg/ml	50mg/ml	20mg/ml	50mg/ml	20mg/ml	50mg/ml	20mg/ml	50mg/ml	10 mg
*Maceration	I	0	0	0	0	0	0	0	0	0	0	0	0	9±2
	II	5±2	7±2	4±2	5±2	1±1	2±1	5±2	6±2	2±1	3±2	3±2	3±2	5±2
	III	8±2	6±2	0	0	2±1	4±2	2±1	5±2	0	0	5±2	6±2	10±2
	IV	0	0	0	0	0	0	0	0	0	0	0	0	3±1
	V	5±2	3±2	0	4±2	0	0	0	0	0	0	5±2	5±2	7±2
	VI	5±2	0	2±2		0	0	0	0	0	0	3±2	0	5±2
**Sub critic water 125°C and 60 ATM	I	0		0		0		0		0		0		9±2
	II	1±1		1±1		0		1±1		0		1±1		4±2
	III	3±2		2±2		0		0		1±1		4±2		10±2
	IV	0		0		0		0		0		0		8±2
	V	5±2		0		0		0		0		0		5±2
	VI	0		0		0		0		0		0		5±2
***Sub critic water 125°C and 80 ATM	I	0		0		0		0		0		0		9±2
	II	1±1		1±1		0		2±1		0		1±1		4±2
	III	2±1		1±1		1±1		2±1		1±1		4±2		10±2
	IV	0		0		0		0		0		0		6±3
	V	0		0		0		0		0		5±2		5±2
	VI	3±2		0		0		0		0		2±2		5±2

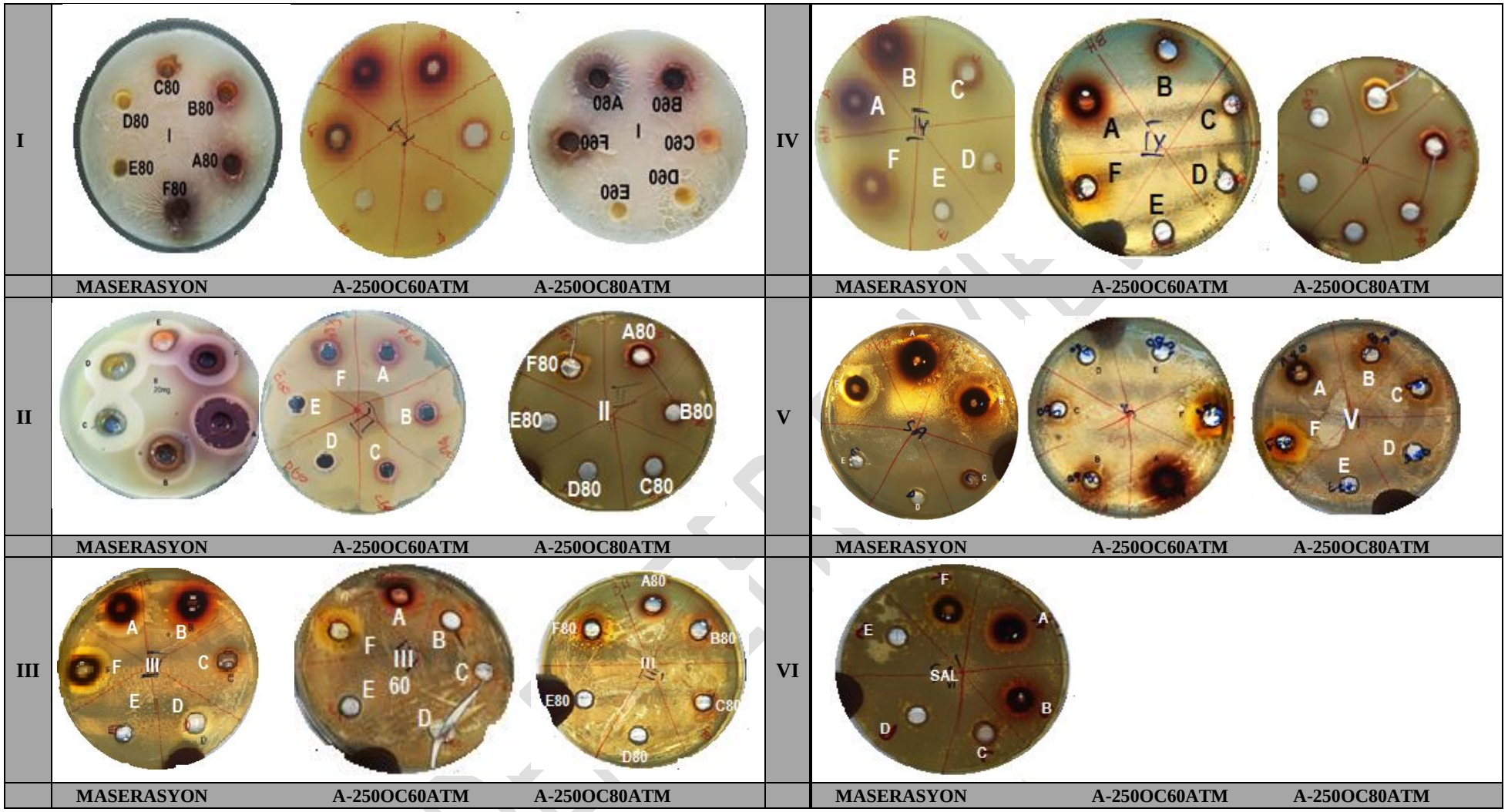
Table 9: Results of antimicrobial tests performed with the dark diffusion method at 0.2g/ml (200 µl) and 0.5 g/ml (500 µl).

* First extraction: It was carried out by softening the plants in H2O-Met solution (90%) with stirring for 48 hours at an amount of 5g/ml.

** The second extraction was obtained by sub critic water extraction at 125oC-60 ATM;

*** The second extraction was obtained by sub critic water extraction at 125oC 80ATM.

Code I: Pseudomonas Aeruginosa (ATCC 27853); Code II: Enterobacter Hormaechei (ATCC700323); Code III: Bacillus Spizizenii (ATCC6633);



Code IV: Enterococcus Casseliflavus (ATCC700327); Code V: Staphylococcus aureus subsp.Rosenbach (ATCC 6538); Code IV: Salmonella Typhus (ATCC 6539) ; CN10: Gentamicin 10mg (Bio analysis)

Fig 8: Results of antimicrobial tests performed with dark diffusion method at 0.2g/ml (200µl) and 0.5g/ml (500µl).
(A): Crossopteryx febrifuga Benth (Stem bark); (B): Khaya anthotheca (Stem bark); (C): Khaya anthotheca (Root); (D): Gardenia ternifolia (Stem bark); (E): Gardenia ternifolia (Root); (F): Terminalia glaucescens (Stem bark)

3.3. MTT TESTI

	Control	A1	A0,1	A0,01	B1	B0,1	B0,01	C1	C0,1	C0,01	D1	D0,1	D0,01	E1	E0,1	E0,01	F1	F0,1	F0,01	K+(NaOCl)	Sum	Average	%
Maceration	100	65	97	99	71	94	88	45	82	80	12	72	84	8	61	73	49	77	80	93	1239	65	-35
125c /60ATM	100	84	143	150	96	108	123	76	101	149	29	136	137	1	101	108	94	102	143	163	1882	99	-1
125C 80ATM	100	111	114	125	77	119	126	29	63	75	55	64	108	6	128	98	66	104	133	134	1602	84	-16

Table 10: Inhibition value in % of the different extracts at concentrations 1; 0.1 and 0.01 µg/ µl on the proliferation of fibroblast cells in the embryo of an NIH3T3 Mouse

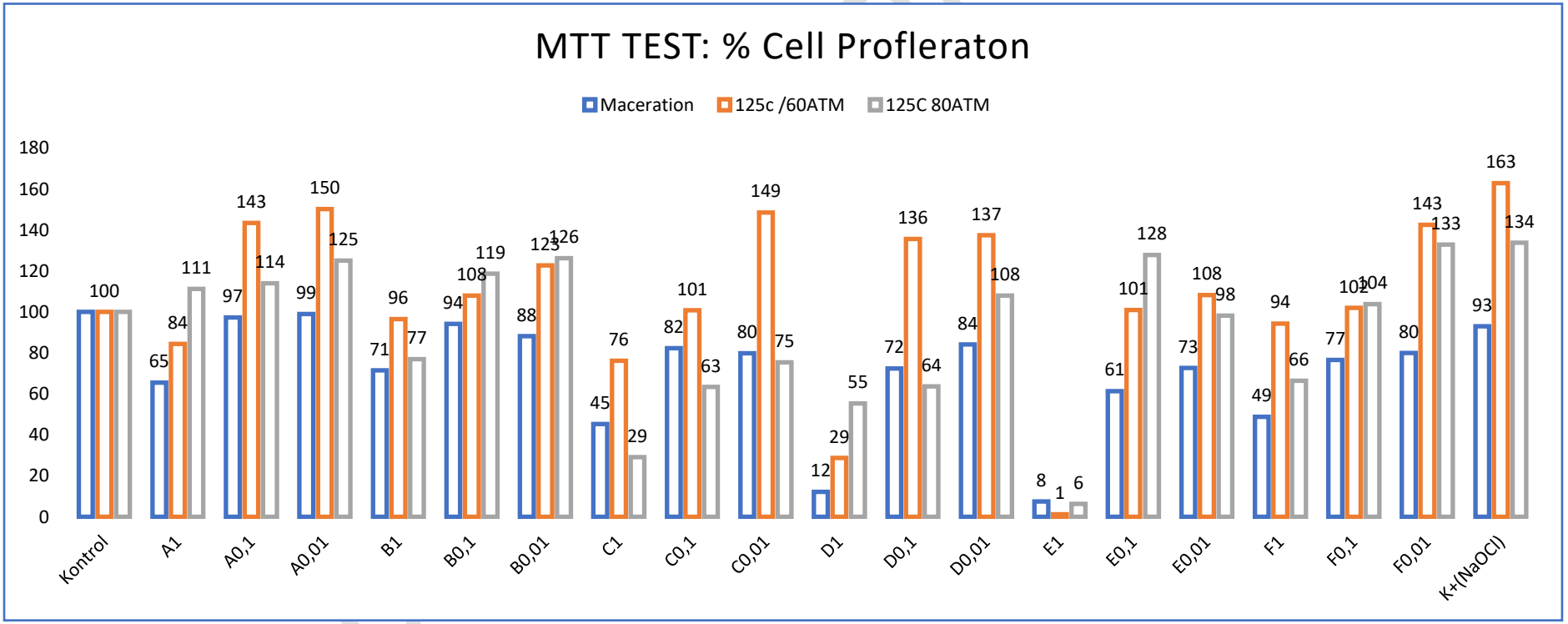


Figure 9: Inhibition in % of the different extracts on the proliferation of fibroblast cells from the embryo of an NIH3T3 mouse.

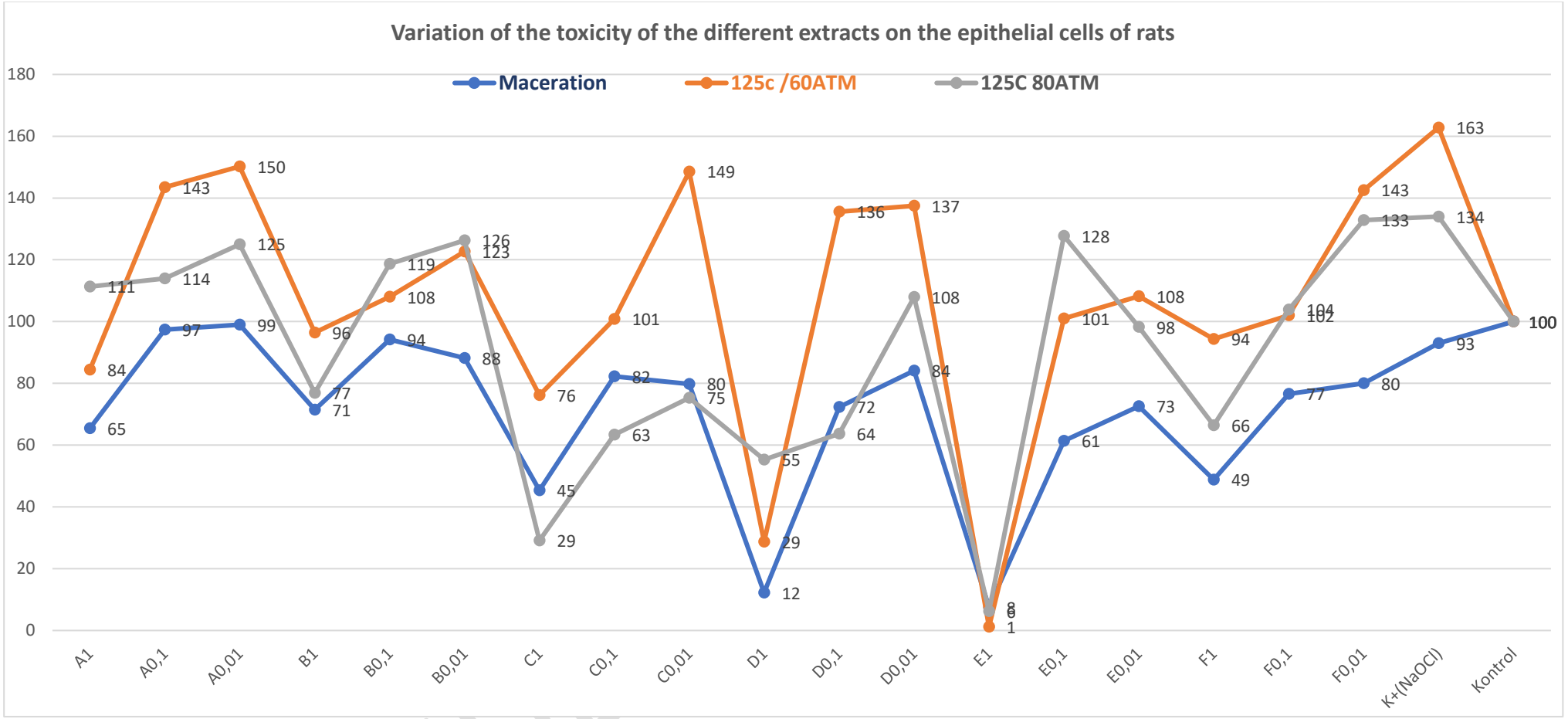


Figure 10: Inhibition in % of the different extracts on the proliferation of fibroblast cells from the embryo of an NIH3T3 mouse treated with Tetrazolium by the MTT method.
A): Crossopteryx febrifuga Benth (Stem bark); (B): Khaya anthotheca (Stem bark); (C): Khaya anthotheca (Root); (D): Gardenia ternifolia (Stem bark); (E): Gardenia ternifolia (Root); (F): Terminalia glaucescens (Stem bark)

The extracts of the plants obtained by the maceration method (Hydro Methanol 90 %) induce inhibition of fairly large cell growth (-36 %) that the extracts obtained by the subcritical Water 125 ° C80 ATM and 125 ° C 60ATM. The extracts obtained based on the Water 125 ° C 60ATM supercritical method are the least offensive on cell proliferation. The most concentrated extracts show the most important inhibitions. At a concentration of 1G/ml the

extracts from the roots of Gardenia Ternifolia (E) show the most important inhibition than the other extracts. On the other hand, the bark of Khaya Anthotheca (plant B) reveal a clearly low cytotoxicity (27% lower than witnesses).

CONCLUSION

Extracts based on the barks of Terminalia glaucescens and Crossopteryx febrifuga Benth show very moderate cytotoxicity and strong antibacterial potential. Enterobacter Hormaechei, ATCC700323 (Code II) and Bacillus Spizizenii ATCC6633 (Code III) show a relatively high sensitivity on the concentrations of all the tested extracts. Bacillus Spizizenii ATCC6633 (Code III) and Salmonella (Code IV) show sensitivity to extracts from the bark of Crossopteryx febrifuga Benth and Terminalia glaucescens. Pseudomonas Aeruginosa, ATCC 27853 (Code I) and Enterococcus Casseliflavus ATCC700327 (Code IV) are not susceptible to any of the plant extracts tested; Only the extracts based on the barks of Crossopteryx febrifuga Benth (A) and Terminalia glaucescens (F) show sensitivity against five out of six bacterial strains tested, including salmonella typhus.

The extraction by maceration method appears to induce more toxicity than the extracts obtained by sub critic water extraction. Extracts by maceration reveal a higher cytotoxicity 36% with white control; The extracts obtained by the subcritical water methods at 125°C and 60 ATM reveal a moderate cytotoxicity of -1% with the blank; The extracts by supercritical 125°C 80 ATM reveal a lower cytotoxicity -16% with the blank; Extraction by subcritical water at low pressure is less inducing oxidation.

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